

TQ Analyst Software User Guide



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1 Introduction

Welcome to Thermo Scientific TQ Analyst™ software. TQ Analyst is a powerful yet versatile software package for developing analytical *methods* for spectroscopic applications, including mid-infrared, near-infrared, far-infrared, and Raman. The software offers a complete selection of qualitative and quantitative analytical techniques, an easy-to-use graphical interface and a broad range of on-line support.

The Professional Edition contains all of the algorithms that are typically used for calculating *component* concentrations and *classifying* spectra based on a set of *standards*. You can also set up a method that simply measures spectral features and reports the measured values. If you purchased TQ Analyst EZ Edition, you have full access to the software features for basic experiments, such as quality control and quantitative analyses. Once a method is developed, it can easily be run from a Thermo Scientific spectral analysis application, such as OMNIC® or RESULT™.

With TQ Analyst, you don't have to be an expert to create accurate analytical methods. The software's *Explain help window* provides information instantly on any feature in the software. Our Help package includes tools for basic training in method development and a set of example methods. You can also take advantage of the software's "suggest" wizards and performance index to get answers to common questions, such as,

What type of analysis will work best for this application?

How many standards should I use and with what concentrations?

Which regions of the spectrum will work best for this analysis?

How many factors should I use for a PLS analysis?

Will a correction curve improve my results?

TQ Analyst Professional Edition

We've eliminated the guesswork from method design. If you're wondering which settings will give you the best answers, ask the TQ Analyst wizards for help!

The Professional Edition of TQ Analyst provides a broad selection of *quantitative* and *classification analyses*. You can also set up a method that simply measures spectral features and reports the measured values. Once a method is developed, it can easily be run from a Thermo Scientific spectral analysis application, such as OMNIC or RESULT.

You can use the Professional Edition to create the following method types.

For quantitative analysis:

- Simple Beer's Law
- Classical Least Squares (standard version)
- Stepwise Multiple Linear Regression (SMLR)
- Partial Least Squares (PLS)
- Principal Component Regression (PCR)

For sample classification:

- Similarity Match
- Distance Match
- Discriminant Analysis
- Search Standards
- QC Compare search

For basic measurements:

- Peak locations
- *Peak heights or areas*

- Peak ratios
- Peak width at half maximum

You can also measure random *noise* in a given *region* and find where a *peak* is reduced to 1%, 2%, 5% or 10% of its maximum height.

TQ Analyst EZ Edition

The EZ Edition of TQ Analyst includes the features you need to create and run the common quantitative and classification experiments. You can also set up a method that simply measures spectral features and reports the measured values. Once a method is developed, it can easily be run from a Thermo Scientific spectral analysis application, such as OMNIC or RESULT.

You can use the EZ Edition to create the following method types.

For quantitative analysis:

- Simple Beer's Law
- Classical Least Squares (basic version)

For sample classification:

- Similarity Match
- Search Standards
- QC Compare search

For basic measurements:

- Peak locations
- *Peak heights* or *areas*
- Peak ratios
- Peak width at half maximum

You can also measure random *noise* in a given *region* and find where a *peak* is reduced to 1%, 2%, 5% or 10% of its maximum height.

Note If you purchased the EZ Edition, some of the features described in this manual are not available in your software. In some cases a described button does not appear in the software. In other cases certain options do not appear in the list of available options for a *parameter* or group. Read and follow the instructions for the features that are available. ▲

Here are some other things you should be aware of when using the EZ Edition:

- The *toolbar* is always displayed when you use the EZ Edition. It provides a convenient way to *calibrate* and run *methods* and to open and close the *Explain help window*.
- The full *menu bar* is available in the EZ Edition. No menu *commands* have been altered or omitted.
- The choices for quantitative *methods* available in the EZ Edition are limited to Simple Beer's Law and a simplified version of classical least squares. They provide a basic approach to single or multi-component experiments that are based on *peak height* or peak area measurements.

If each *component* in your method has an associated spectral *peak* that changes proportionally with *concentration*, use the Simple Beer's Law analysis type. If some of the component *bands* overlap, choose classical least squares.

- The classical least squares analysis type available in the EZ Edition is somewhat different from the one in the full version of TQ Analyst. The full version lets you associate peak heights and peak areas as well as larger regions with each component in a classical least squares *method*. The EZ Edition allows you to select peak heights and peak areas but not regions.

- The Discriminant Analysis and Distance Match classification techniques are not available in the EZ Edition of TQ Analyst.
- The Suggest Analysis Type wizard is not available in the EZ Edition.

How to use this manual

This manual provides an overview of TQ Analyst software operation. It also serves as a training tool for creating *quantitative*, *qualitative* (classification) and *spectral measurement methods*.

The contents of the individual chapters are summarized below:

- The first chapter, “Introduction,” tells you how to get started and how to use the software’s extensive on-line help system.
- Chapter two “Principles of TQ Analyst” explains some basic analytical terms and concepts and describes the software’s integrated approach for designing analytical experiments.
- The third chapter, “Using TQ Analyst Software,” explains how to interact with the features in the *TQ Analyst window*.
- Chapters 4, 5 and 6 provide details about working with method and data files, TQ Analyst windows and *standards*.
- Chapter 7 covers processing spectral data for all types of analyses.
- Chapters 8, 9, and 10 take you step-by-step through the process of developing methods for *quantitative analysis*, *qualitative analysis* (classification) and spectral measurement.
- Chapter 11 explains how to set up sample reports for all types of methods and add security features to your method files.
- Chapter 12 tells how to update the calibration data for quantitative and *classification methods*.
- Chapter 13 “Method Diagnostics” itemizes the diagnostic routines available in TQ Analyst and details their use.

- Read chapter 14 “Troubleshooting Tips” for helpful hints when operating the software.

At the end of the manual is an index that will help you locate information about specific software features.

The manual is intended to be used as a training tool; it is not a comprehensive guide to every feature in the software. If you want detailed information about a specific software feature, refer to the on-line help. See the section titled “On-line Help” in this chapter for a tour of the software’s extensive help system.

Examples used in this manual

Many of the methods and spectra used to create the illustrations in this manual are included with the TQ Analyst software. You may wish to use these example methods and spectra as you learn about the software. The example methods and spectra are contained in method and spectral data *files* in the EXAMPLES directory within the QUANT directory. Each example method also comes with a step-by-step tutorial and real data that you can work with while following the tutorial lessons (see the Example Methods item in the TQ Analyst help menu.)

The example method files are read only. If you want to use one as a starting point for a new method, open the example method and save it with a new *file name*.

Conventions used in this manual

This manual includes safety precautions and other important information presented in the following format:

Note Notes contain helpful supplementary information. ▢▲

Notice Follow instructions labeled “Notice” to avoid damaging the system hardware or losing data. ▢▲

Caution

Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. ▲□

Any text that you are required to type at the command prompt is underlined in this manual. The Enter key is shown as <Enter>. Here is an example:

```
C:\PROGRAM FILES\THERMO SCIENTIFIC\  
TQ ANALYST\TQA.EXE <Enter>
```

Words or phrases that appear in *italic* in this manual are included in the glossary.

Questions or concerns

In case of emergency, follow the procedures established by your facility. If you have questions or concerns about safety or need assistance with operation, repairs or replacement parts, you can contact our sales or service representative in your area or use the information at the beginning of this document to contact us.

Learning the software

TQ Analyst software is intended to serve a broad audience of users, from the novice chemist to the advanced chemometrician, whose job is to create *methods* for *sample* analysis. The software's tabbed layout, integrated wizards, context sensitive on-line help and artificial intelligence are designed to simplify this task and help ensure quality results with little rework.

In all of the software screens, the simplest techniques and algorithms are listed first and the software displays only the features that relate to the selected algorithm or technique. Thus, the novice user is not confused by software features he or she doesn't want or need and the experienced user has instant access to advanced features in the software.

In addition to this printed user's guide, we provide the following on-line tools to help you learn how to use the software.

TQ Analyst on-line tour - A quick demonstration of the main features in TQ Analyst software.

TQ Analyst example methods - Brief tours of working methods for *quantitative analysis*, sample classification, and *spectral measurement*

TQ Analyst is a standard Windows[®] application. The program can be installed with or without the training guides, such as the TQ Analyst tour and the example methods.

Where to start

Depending on your knowledge and experience, we recommend the following approach to learning the software:

If you . . .

Then start with . . .

are less familiar with Windows applications

chapters 3 through 6 (or the TQ Analyst Tour in the Help menu) for an introduction to the software.

are new to analytical concepts and method design

chapter 2 "Principles of TQ Analyst" for an overview of the methods you can create

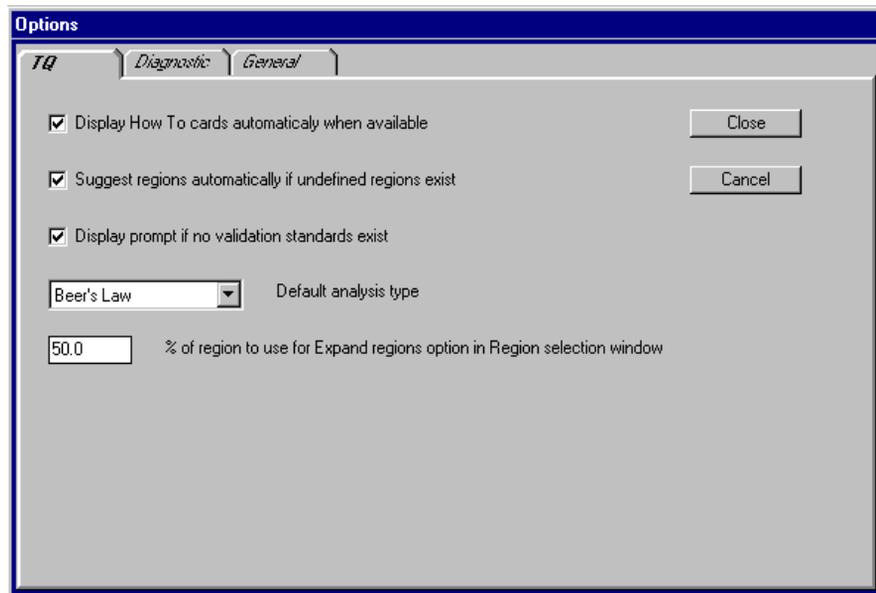
are familiar with Windows applications and other method development software

chapter 8, 9 or 10 (or the example methods in the Help menu) to learn how to create quantitative, qualitative (classification) or spectral measurement methods

Customizing TQ Analyst

You can customize the way TQ Analyst displays certain windows and dialog boxes by setting options. You can also use options to set *parameters* that apply to all of the TQ Analyst method types, such as the algorithm for the *performance index*.

Use the Options command in the TQ Analyst Edit menu to display the options dialog box.



There are three groups of options you can set:

TQ Analyst options Use the options in this group to define *default settings* for TQ Analyst software and control when certain messages and help screens are displayed.

Diagnostic options The Diagnostic options allow you to select the algorithm TQ Analyst will use to calculate the *performance index* and to indicate whether the software should display legends for the

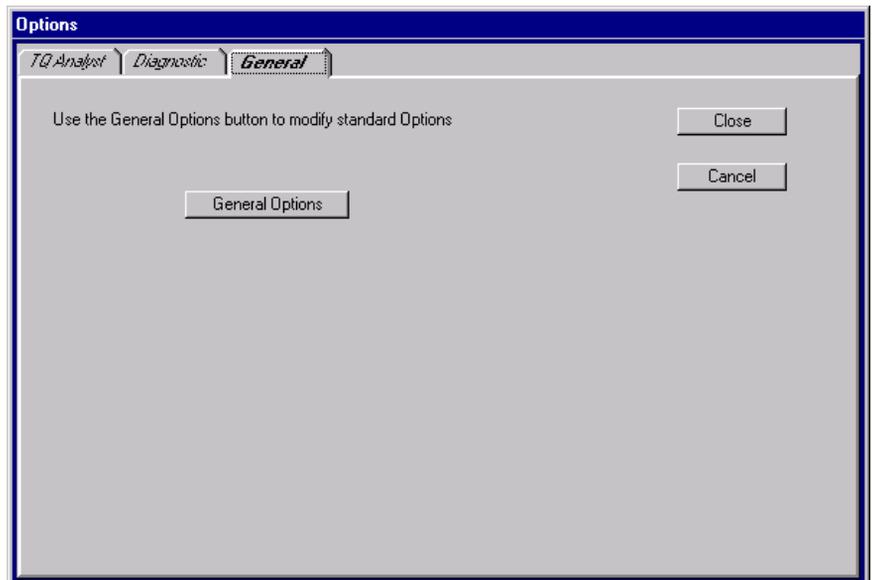
diagnostic plots. The Display Legends *check box* allows you to turn off the legends when they cover data points that are critical to your diagnostic analysis.

General options

This large group of options allows you to define how TQ Analyst performs many operations, including collecting, viewing, processing, saving, and printing windows, spectral and other data within TQ Analyst software. Many of these general options are also available in other Thermo Scientific spectral analysis software, such as OMNIC or RESULT.

To set the options, click the *tab* that corresponds with the group of options you want to set.

The General tab provides a link to the general options. To see the General options, click the General tab and then click the General Options button.



When you are finished viewing or setting options, click OK to close the General Options dialog box. Then click Close to close the TQ Analyst Options box.

On-line help

The TQ Analyst Help package provides many kinds of on-line support, including a comprehensive list of Help topics with standard Windows search features, plus context-sensitive help and several wizards. We also provide an on-line tour of the unique features in TQ Analyst and a set of example methods.

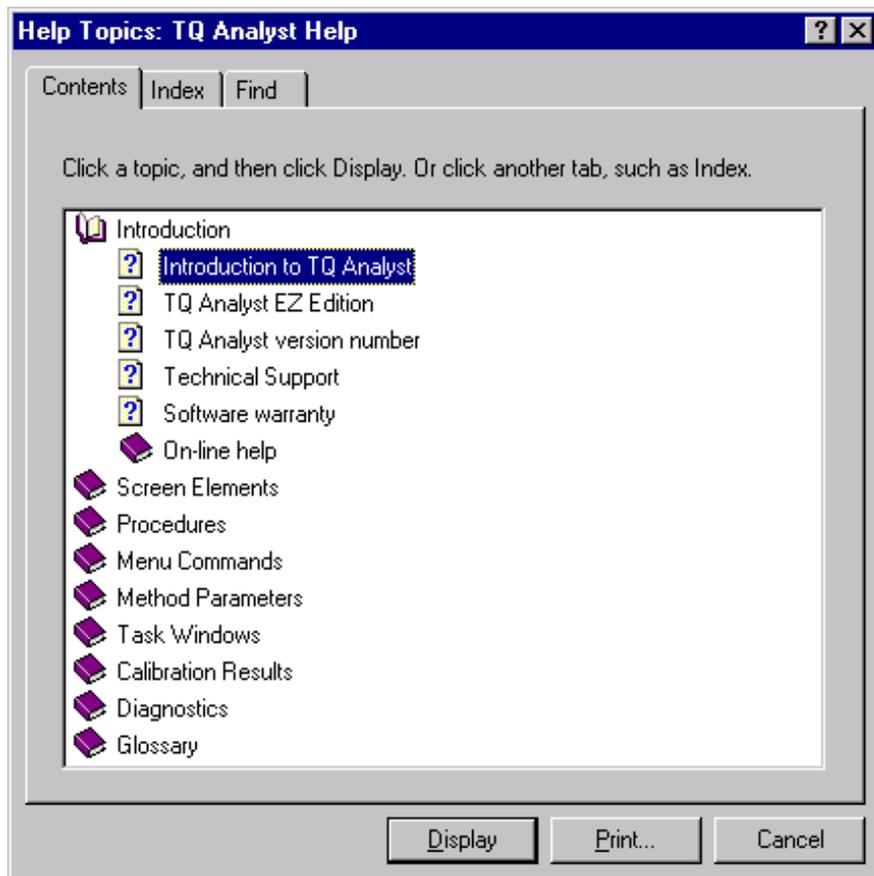
This section details the Help features and explains where to find them. The Help package can help you quickly learn how to use TQ Analyst and take advantage of its powerful features for method development and diagnostics.

Help menu

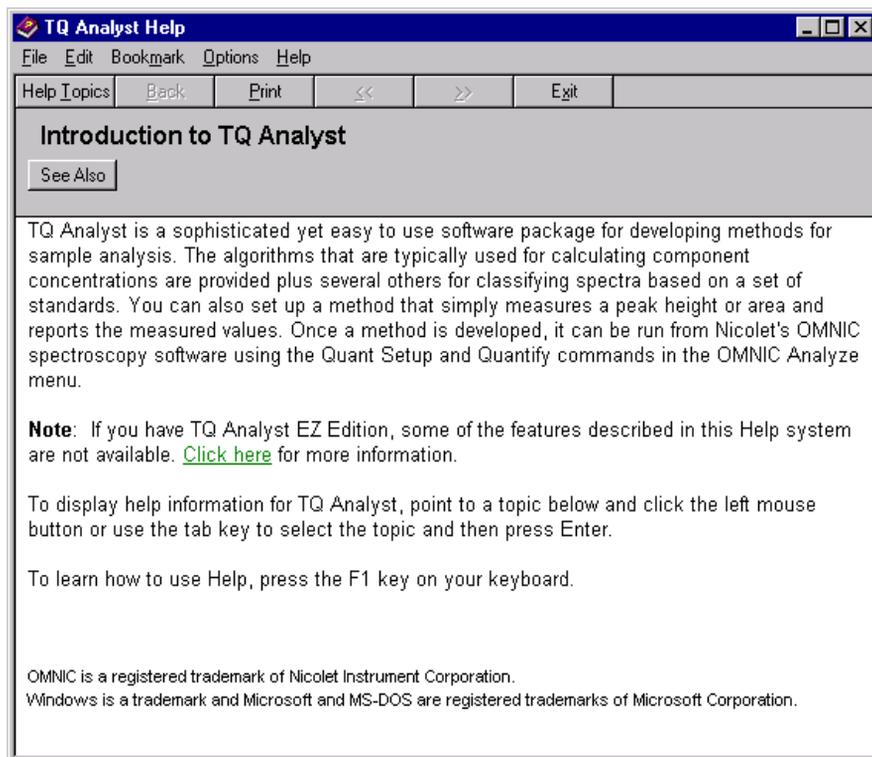
The Help menu is the last menu in the TQ Analyst *menu bar*. It lists several categories of information that you can select to find the information you need. The categories are described in the following table.

<i>For this information...</i>	<i>Choose this category...</i>
List of main Help topics. Also search and keyword index.	TQ Analyst Help Topics
A quick demonstration of the main features in TQ Analyst software.	TQ Analyst Tour
Brief tours of working methods for <i>quantitative analysis</i> , sample classification, and <i>spectral measurement</i> .	Example Methods
Information on how to reach Thermo Fisher Scientific's hot line for technical support.	Technical Support
The version number for your TQ Analyst software.	About TQ Analyst

When you choose TQ Analyst Help Topics, a Help window appears presenting information about the software.



As you request information within Help, the information is presented one window at a time. Each window includes all the standard Windows Help buttons for finding information.



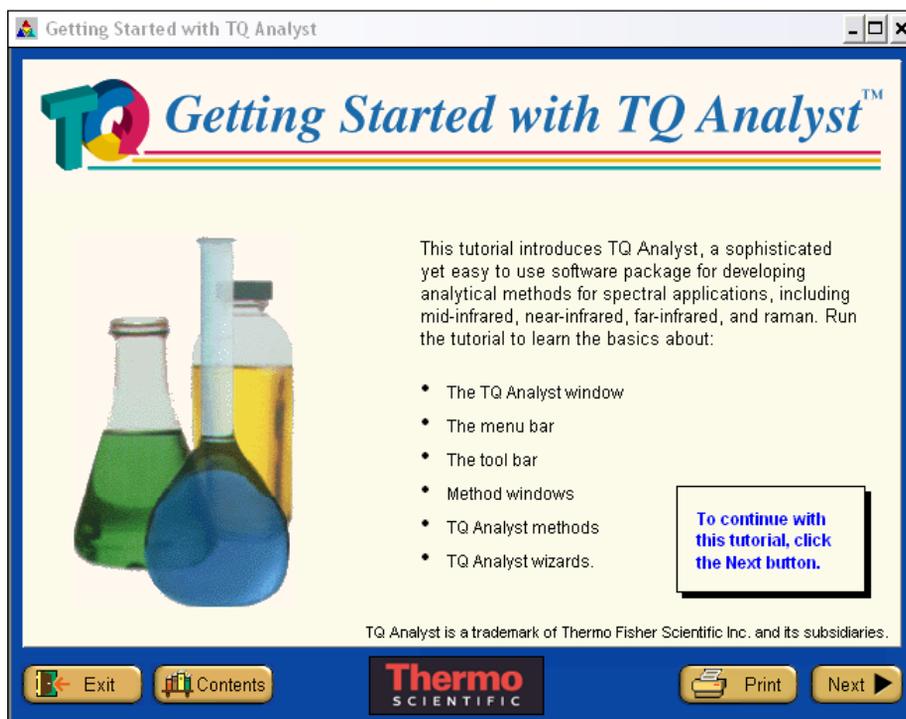
The Help window in TQ Analyst works essentially the same as other Help software found in Windows applications. If you have not used Windows Help before, see your Windows documentation for complete information. You can also choose Using Help to see information on using the Help features. When you are finished reading help information, close the Help window by choosing Exit from the File menu.

If you choose Find or Index, a search window appears.



Use the Find window to run a full text search of all the on-line help for the word or phrase you enter. The topics that contain the most occurrences of the search text will be listed first. The Index search window allows you to search the on-line help for all of the topics that are associated with the text you enter.

The TQ Analyst Tour and Example Methods present an interactive help window. Follow the instructions in the help window to complete the tour or view an example method.



Context-sensitive help

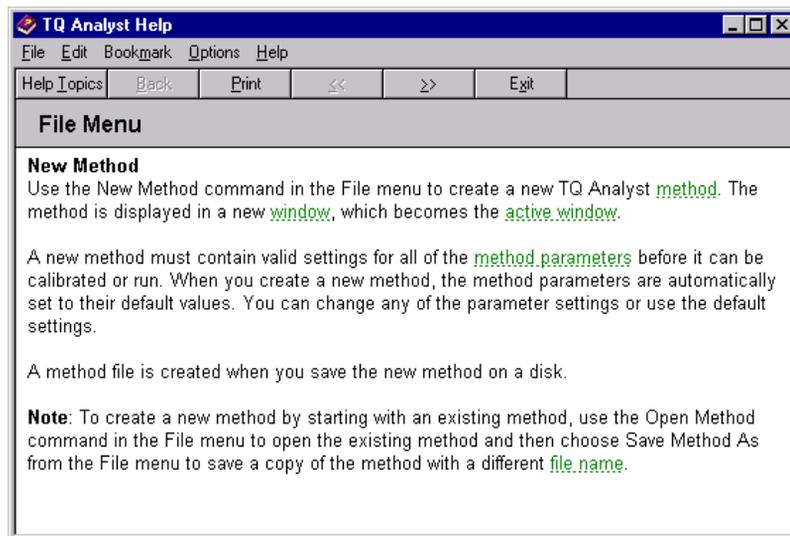
While using TQ Analyst, you won't waste time searching for information about how a software feature works or when it should be used. Context-sensitive help lets you see information instantly about any feature in the software. Three kinds of context-sensitive help are available in TQ Analyst: F1 Help, Explain Help and How To Help.

F1 help

For help information about a *command* in a TQ Analyst *menu*, use key combinations to select the command and then press the F1 key on your keyboard.

To open a menu using the keyboard, hold down the Alt key and then press the underlined letter in the *menu name* on the screen. For example, to open the File menu, hold down the Alt key and then type F.

Use the up and down arrow keys on the keyboard to select a command in the open menu. Press the F1 key on the keyboard to display help information on the selected command.



When you are finished reading the Help information, close the Help window by choosing Exit from the File menu.

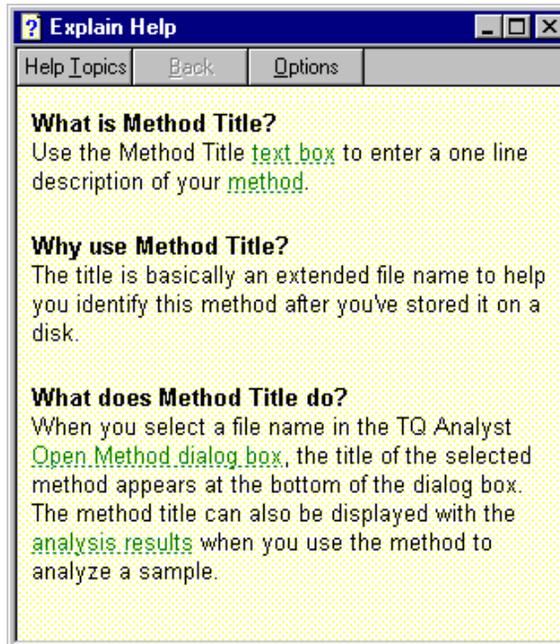
Explain help

You can display information about the features in TQ Analyst software by opening the *Explain help window* and then selecting the feature you want to read about. To open the Explain help window, click the *Explain button* on the *toolbar*.



The Explain help window always contains information about the currently selected feature. The feature can be a button, a parameter, or a graphical window or plot.

Three characteristics of the feature are typically described in the Explain help window: a definition of the feature, a description of how the feature works, and tips on when the feature should be used. The illustration below shows the kind of information you can expect to see in the Explain help window.



To display help information for another feature, simply use the left mouse button to click the feature. The information provided in the Explain help window is updated each time a new feature is selected. The Help window updates without changing the status of the feature you clicked.

Keep the Explain window open as you work if you think you may need information about other features in a *TQ Analyst window*. If the Explain window is in your way, you can easily move it by clicking and dragging its title bar or close it by clicking the Close button in the Explain help window.

How To help

Whenever a new type of window is displayed in TQ Analyst software, a help card is available to explain step-by-step how to complete the tasks presented in the window. How To help cards are available for the following windows:

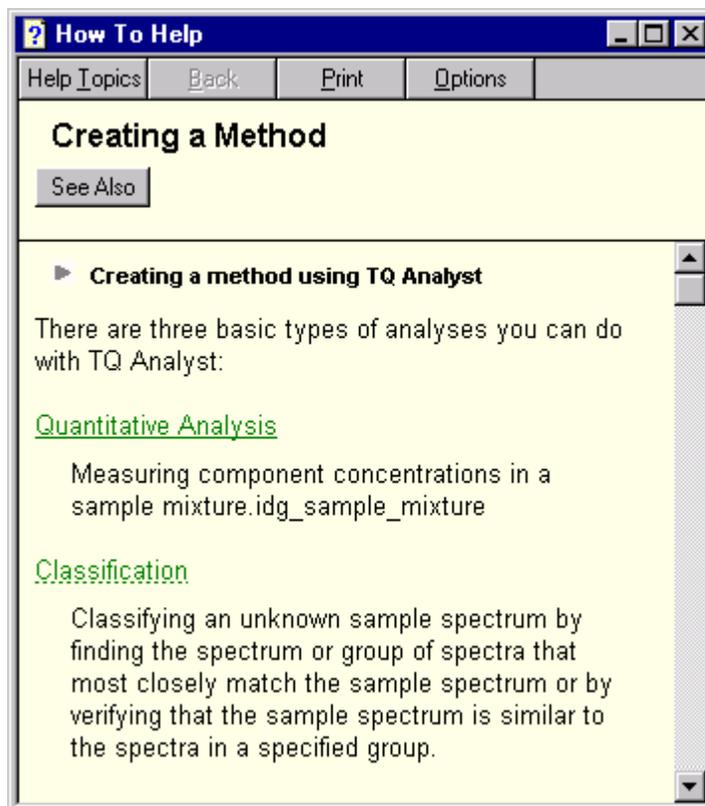
- Method windows

- Region Selection task window
- Correction task window

To display the How To help card for a window, click the How To button displayed in the window.



The following illustration shows the How To help card for a *method window*.



The help card stays visible until you close it, even while you interact with other features in the software. To close a How To help card, click the Close button at the top of the How To Help window.

Note When you install TQ Analyst on your computer, the software is configured to display the How To Help window whenever a procedure is available for the current task. You can turn this feature on and off from the TQ Analyst *tab* in the Options dialog box (choose Options from the Edit menu and then click the TQ Analyst tab). ▲

Wizards TQ Analyst offers a number of wizards to help you complete each method development task. From choosing the proper settings for *method parameters* to setting up your experimental design, the wizards can help make even your first attempt at developing a method a success.

The following table gives brief descriptions of the wizards available in TQ Analyst.

<i>Location</i>	<i>Wizard</i>	<i>Function</i>
Description tab	Suggest Analysis Type	Recommends a setting for the Analysis Type parameter.
Pathlength tab*	Suggest Pathlength Type	Recommends a setting for the Pathlength Type parameter.
Components tab*	Assess Feasibility	Determines whether there is sufficient variability in the <i>sample</i> data that correlates with differences in sample composition. (Tells you whether your method is feasible.)
Standards tab*	Suggest Standards	Fills in the Standards table with the names and concentrations of the <i>standards</i> you should prepare and collect.
	Evaluate Standards	Determines whether the standards that are listed in the Standards table were prepared correctly and whether additional standards are needed.
Regions tab	Suggest Regions	Chooses appropriate <i>spectral regions</i> for the analysis.

*Available for quantitative methods only.

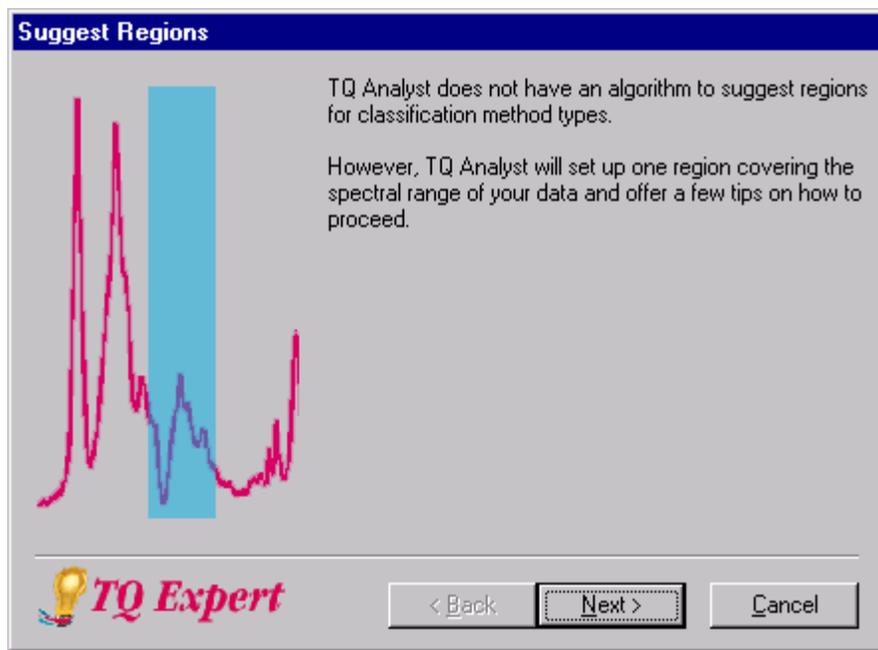
Continued on next page

<i>Location</i>	<i>Wizard</i>	<i>Function</i>
Other tab*	Suggest Factors	Chooses appropriate <i>factors</i> or <i>principal components</i> (PCs) in a partial least squares or principal component regression method. Use the Edit Factors button to view or edit the chosen factors or PCs.
Corrections tab*	Suggest Corrections	Determines whether a correction curve will improve the accuracy of a quantitative method and, if so, which settings for the correction parameters are appropriate for each component.

*Available for quantitative methods only.

To start a wizard, click the appropriate button on the specified *tab*. For example, to start the Regions wizard, click the Suggest Regions button on the Regions tab.

When you start a wizard, an interactive window appears.



Follow the instructions in the window. When you are finished, click the Next button to display the next screen. You can stop the wizard at any time by clicking the Cancel button in the interactive window. Click the Finished button in the last screen to complete the wizard.

The wizards are intended to be used in the order shown in the table above. However, some of the wizards' recommendations will be limited if they can't access certain information. See the sections on using wizards in the individual chapters on creating quantitative and classification methods for more details.

The results from the Suggest Analysis Type, Suggest Pathlength Type and Suggest Standards wizards will be displayed on the corresponding tab. The remaining wizards display their results in a special window or tab. The regions that are recommended by the Suggest Regions wizard,

for example, are displayed in the Region Selection task window and the results from the Assess Feasibility wizard appear on the Feasibility tab.

The wizards use the spectral and other data you provide and their built-in expertise in multivariate analysis to determine appropriate settings for the *method parameters* or to suggest how you should proceed. You may use the wizard's recommendations, alter them, or overwrite them completely.

We suggest using the wizards' recommended settings as a starting point. Then add your knowledge of spectroscopy and the chemical system you are measuring to determine the optimum settings.

2 Principles of TQ Analyst

TQ Analyst is a software package for developing calibrated methods for either *quantitative analysis* or classification of spectral data.

Quantitative methods calculate the *concentrations* of one or more *components* in a *sample mixture*. They can tell you how much of a component is present in an unknown *sample*.

Classification methods identify features in a *sample spectrum* and compare them to predefined groups (classes) of spectra. The *class* that provides the closest *match* is reported along with a measure of similarity. Classification methods are typically used for quality control analyses and prefilters for quantitative experiments. They can help you identify an unknown material or measure its purity.

You can also set up a method that simply measures spectral features and reports the measured values. These “measure only” methods can be used to determine *peak heights* or areas, peak ratios, peak locations and even the *noise* in a given *spectral region*.

TQ Analyst is designed to help you develop successful analytical methods quickly and easily. This chapter explains the basic concepts of chemical spectroscopy and the quantitative, classification and *spectral measurement* analytical techniques provided in TQ Analyst. If you understand these concepts and are familiar with the techniques included in TQ Analyst, skip this chapter and review the example methods instead. They demonstrate how to create methods using a typical data set and explain how to interpret the calibration results. To run an example method, choose Example Methods from the TQ Analyst Help menu. Then click the method name.

Chemical spectroscopy

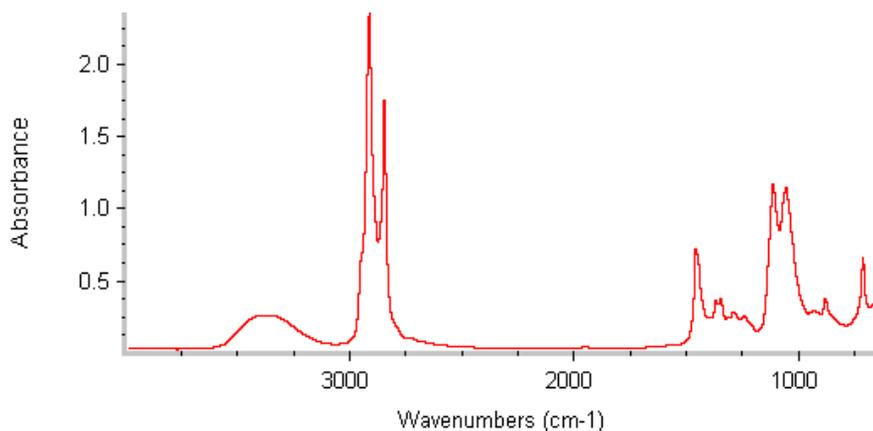
Spectrometers measure the interaction of radiation with experimental *samples*. They record the frequencies or *wavelength* at which the sample absorbs radiation and the intensities of the absorptions. *Frequency*, or wavelength, and intensity of sample absorption are depicted in a two-dimensional plot called a *spectrum*.

Intensity is reported in terms of *absorbance*, the amount of light absorbed by a sample, or by % *transmittance*, the amount of light which passes through it. Percent transmittance is related to absorbance through the equation

$$A = -\text{Log}_{10}(\%T/100\%)$$

where A is absorbance

Frequency is usually reported in terms of *wavenumbers* (cm^{-1}); wavelength is typically reported in nanometers (nm). For this discussion, we will use the term “frequency” to refer to either frequency or wavelength.



Infrared Spectrum

Determining the absorption frequencies allows identification of a sample's chemical makeup, since chemical functional groups are known to absorb light at specific frequencies. By comparing the spectrum of an unknown sample, such as a polymer film, to the spectra of known materials (*standards*), the spectrometer can quickly find the spectra that most closely match the unknown. Identification of what makes up a sample is called *qualitative analysis*.

We may also be interested in finding out how much of a component is present in a sample. For example, we may need to know the amount of an antioxidant additive that is present in a polymer film.

The intensity of an absorption is related to the *concentration* of the *component* in the sample. A *calibration model* relates concentration changes to absorbance changes. Using a calibration model, the absorbance of an unknown sample can be used to calculate component concentration. We refer to the determination of component concentrations in a *sample mixture* as *quantitative analysis*.

Quantitative techniques

We often know the *components* in a *sample mixture* but need to find their *concentrations*. We refer to the determination of component *concentrations* in a sample mixture as *quantitative analysis*.

TQ Analyst provides a wide range of quantitative techniques. All of them are based on the following basic premise:

The intensities of the peaks in the absorbance spectrum of a sample are directly related to the amount of sample present.

In other words, as the concentrations of the components in a sample mixture increase, so do the spectral *peaks* related to each component. This relationship is true at any *frequency* in the *spectrum*.

Calibration models for quantitative methods

The simplest quantitative technique is based on a relationship called Beer's Law (*Beer-Lambert-Bouguer law*). Beer's law states that not only is peak intensity related to sample concentration, but the relationship is linear as shown in the following equation::

$$A = a b c$$

where: A = absorbance measured at a given frequency

a = absorptivity of the component at the measured frequency
(constant for a given component and frequency)

b = pathlength of the component

c = concentration of the component

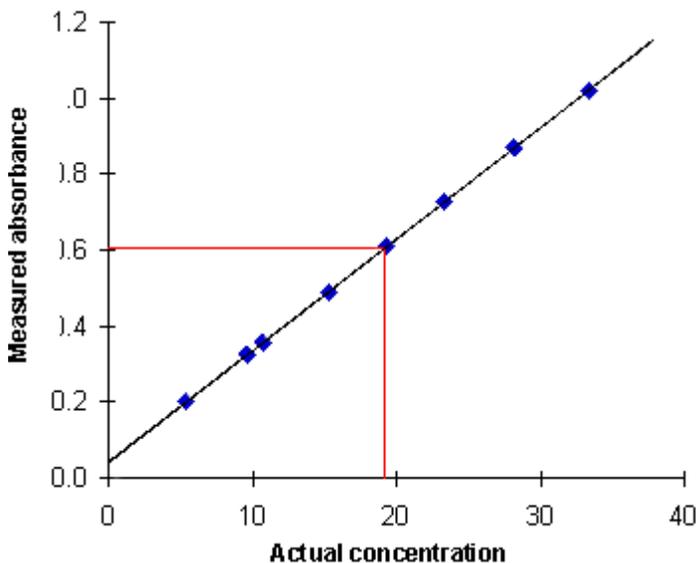
As we mentioned earlier, the intensity of an absorption is related to the *concentration* of the *component* in the *sample*. A *calibration model* relates concentration changes to *absorbance* changes. Using a calibration model, the absorbance of an unknown sample can be used to calculate component concentration.

For compounds that do not tend to interact, such as gases, the calibration model may consist of a single *standard*. (For *quantitative analysis*, standards are samples which have known concentrations of each component the method will be used to measure.) This works best when the concentrations of the component of interest in the unknown samples are close to the concentration of the chosen standard.

For liquid and solid samples and gases that vary widely in concentration, the calibration model should be based on several standards that contain different amounts of the components you want to measure. When multiple standards are used to create a calibration model, this group of standards are defined as the "*calibration set*." The spectra of the standards are collected and used to determine the absorbance value of each component peak at several different concentrations.

Simplest calibration model

In the simplest *calibration model*, the concentration data and the absorbance measurements from the *calibration standards* allow us to generate a plot of absorbance versus *concentration* for each *component* to be measured. The simplest way to determine absorbance is by measuring the height or area of a single *peak*. The best fit of this data is called a “*calibration curve*,” or “working curve.”



Calibration Curve

If you measure the same *peak* in the *spectrum* of a *sample* of unknown concentration and compare the measured absorbance to the calibration curve, you can determine the concentration of the component in the unknown sample.

Assuming that the spectra of the *standards* and the unknown sample are collected at the same *pathlength*, the equation used to calculate component concentration is the equation of a straight line:

$$A = m \cdot C + b$$

where: A = absorbance at a given frequency

m = slope of the calibration curve

C = concentration of the component

b = Y-axis intercept of the calibration curve

If we rearrange this equation to solve for sample concentration, C, the new equation looks like this:

$$C = (A - b) / m$$

Multivariate calibration models

There are many other quantitative techniques besides Beer's law which can be used to generate a *calibration model*. However, all of the quantitative techniques provided in TQ Analyst are based on the same basic premise: that *peak* intensity is related to *concentration*. The techniques differ only in the way they relate spectral information to *component* concentration.

For multivariate techniques, such as classical least squares, partial least squares and multiple linear regression, calibration produces a model that is more sophisticated than a simple *calibration curve*. However, the multivariate calibration model serves the same purpose as the calibration curve; relating *spectral measurements* to component concentration. We'll explain more about multivariate quantitative techniques later in this chapter.

Choosing a calibration technique

The various techniques available in TQ Analyst for *quantifying* unknown *samples* using absorption spectroscopy differ mainly in the way they relate spectral information to *component* concentrations.

A list of the calibration techniques available in TQ Analyst software is provided below.

- Simple Beer's law
- Classical Least Squares (CLS)
- Stepwise Multiple Linear Regression (SMLR)
- Partial Least Squares (PLS), and
- Principle Component Regression (PCR).

All of these calibration techniques use the spectral and component concentration information from the *standards* to create a calibration model. Techniques that are based on simpler calibration models are listed first, while those with more complex models appear later in the list.

It is best to select the simplest technique that is capable of modeling the absorbance versus *concentration* response of the samples you want to analyze. If you're not sure which technique to use, let the TQ Analyst wizard recommend one. You can always switch to another technique later if the results from the first attempt are unsatisfactory or try several techniques and choose the one that calculates component concentrations with the most accuracy.

Note If you switch the analysis type between two options in the same group, for example if you change from Simple Beer's Law to CLS, the information you entered and the settings you chose for the previous analysis type will be saved. ▲

The following sections explain each of the calibration techniques available in TQ Analyst and suggest applications for their use.

Simple Beer's Law calibration technique

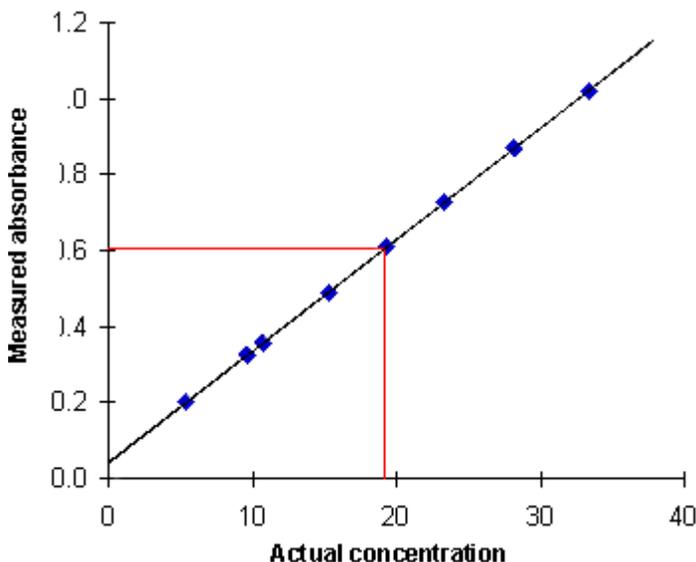
This calibration technique uses the classic *Beer-Lambert-Bouguer law* (absorbance increases proportionally with *concentration*) to create a *calibration model*.

To achieve accurate quantitative results from a Simple Beer's Law method, the following conditions must be met:

- The spectra of the *standards* have one unique spectral *peak* for each *component* you want to measure. (It is best if each component produces only one peak in the *spectrum*.)
- The component peaks can be calculated using simple *peak height* or peak area measurements.
- You have at least as many standards as there are components.
- Each component is included in at least two different standards and at two different concentrations.
- If the *samples* are mixtures, the standards contain all of the components that will be present in the samples.

The software uses the concentration data and the measured absorbance values from the standards to create the calibration model. If the method is configured to use the default (nonzero) setting for the *intercept*, the calibration model is the best fit straight line between the data points in the absorbance versus concentration plot.

This simple calibration model is often referred to as a “*calibration curve*,” or “*working curve*.” A separate calibration curve is created for each *component*.



Calibration Curve for Component A

If you are measuring gas phase *components*, a single pure standard for each component may be sufficient, depending on the calibration range you want to model. Molecules in a gas sample, especially molecules that contain six or less atoms, are generally far apart and don’t tend to interact.

When measuring most solids and liquids, include each component in at least two different standards and at two different concentrations. If you expect the concentrations of a *component* in the unknown samples to vary over a wide range, you may need additional standards to measure that component accurately.

It is best if the concentrations of the components in the standards “bracket” the concentrations you expect to measure in the unknown samples. In other words, the sample with the lowest concentration of a given component should not be below the concentration of its lowest standard. In the same way, the sample with the highest component concentration should not be above its highest standard. If the samples are mixtures, the standards should contain all of the *components* that will be present in the samples.

Be sure to include one or two additional standards that can be used to validate your calibration model. The *concentration* of each component in the *validation standards* should fall within the specified *analysis range*.

The Simple Beer’s law calibration model works well for solving basic analytical problems in which the following statements are true:

- Component peaks overlap a little or not at all.
- Spectral *baselines* are stable.
- Relationships between absorbance and concentration are linear or nearly linear.
- There are little or no chemical interactions between components (chemical interactions will cause component peaks to shift from sample to sample).
- *Sample matrix* is well understood (you don’t expect additional components to show up in the unknown samples).

Examples of materials which can usually be quantified using a Simple Beer’s law method include ethyl alcohol in water and gasoline in oil.

Advantages of Simple Beer’s law methods:

- Mathematics are easy to understand.
- Few standards are required.

- Easy to apply knowledge of sample chemistry and experience in spectroscopy.

Disadvantages of Simple Beer's law methods:

- Not effective when component peaks overlap significantly.

Accurate only in the concentration range for which the *calibration curve* is linear. The linear range depends on the material to be analyzed as well as the *sampling technique*, *analysis region* and the type of *detector* used for the analysis.

Classical least squares calibration technique

The Classical Least Squares (CLS) calibration technique expresses measured *absorbance* as the sum of the absorbance contribution from each of the *components* being measured. In other words, the *calibration model* assumes the component absorptions extend beyond a single *peak* and can look at many *regions* of the spectra to find relationships between absorbance and *concentration*. This kind of model works well for differentiating components that produce overlapping *bands* in the spectral data. Mathematically, a CLS analysis is a simultaneous equation application of Beer's Law.

The CLS calibration model can be based on spectral *peak height* or area measurements or the use of many data points in a spectral range. You must include at least one peak or region for each component you want to measure. Multiple peaks or regions are often used for each component. You can also associate more than one component with each region.

To achieve accurate quantitative results from a CLS method, the following conditions must apply:

- You can identify at least one *analysis region* for each component.
- The method includes at least as many *calibration standards* as there are components being measured.

- The concentrations of the components in the *standards* vary independently. (Whenever possible, avoid using serial dilutions of one standard to prepare combinations of standards.)
- There are little or no chemical interactions between components (component peaks don't tend to shift from *sample* to sample).
- *Sample matrix* is well understood (you don't expect additional components to show up unexpectedly in the unknown samples).
- Relationships between absorbance and concentration are linear or nearly linear.

The CLS method type is useful for solving analytical problems that are more complex than the problems that can be solved by Simple Beer's Law. For example, you can use a CLS method under the following conditions:

- Component peaks or regions overlap significantly (isolated component peaks may or may not exist).

Spectral *baselines* are variable.

Examples of materials which can be quantified using a CLS method include gases and other samples with little or no intermolecular interactions.

Advantages of CLS methods:

- Able to *calibrate* using component peaks or regions that overlap significantly.
- Do not require as many standards as a PLS or PCR analysis.
- More accurate than Simple Beer's Law because all of the data points in the *analysis regions* are used.

Disadvantages of CLS methods:

- Cannot handle unexpected impurities.

Stepwise Multiple Linear Regression calibration technique

Multiple linear regression, also known as Inverse Least Squares or P-Matrix, is one of the most common modeling approaches for *quantitative analysis*. The stepwise multiple linear regression (SMLR) technique expresses concentration as a function of the absorbance at multiple frequencies. This is the opposite (inverse) of the classical least squares, or K-Matrix, technique where absorbance is a function of concentration. Select the SMLR technique when you are interested in measuring the concentrations of a few *components* in a multicomponent mixture.

If you use the *default settings* for the SMLR technique, the software selects the *analysis regions* automatically. TQ Analyst uses a stepwise algorithm to select regions for each component. It begins by selecting the first region (frequency) for the first component. Additional frequencies that provide complementary information may be used to achieve the best possible prediction of the *standards* (the second region typically accounts for the sample matrix). When the software is finished selecting regions for the first component, it begins selecting them for the second component and so on until corresponding frequencies have been chosen for all components in the method.

You can specify multiple components in an SMLR method. The SMLR *calibration model* can be based on spectral *peak heights* or the average peak height in a region. Multiple peaks or regions are typically used for each component (*default settings* select 3 regions) and more than one component can be associated with a region.

Note The second region may be used as a denominator peak. This can be useful for minimizing *pathlength* differences in the standards and *samples*. See “Setting Up a Denominator Peak or Region for an SMLR Method” in the chapter on “Creating a Quantitative Method” for more information. ▲

The number of regions used is limited by the number of *calibration standards* in the method. We recommend using at least 3 standards for each component in an SMLR method. Ideally, the number of standards should be $3n + 1$, where n is the number of components in the method.

You cannot edit the regions in an SMLR method. However, you can specify the starting and ending point of the *frequency* range the software will use to find regions and the limits of the first region used to measure each component. You can also expand or limit the number of regions used to measure each component and specify the widths of the regions and the minimum gap between regions.

Keep in mind, however, that a multivariate calibration model such as SMLR can become *overfit* as more regions are added to the model and the method's prediction accuracy may degrade. For example, let's say you are creating a method to analyze a vitamin compound consisting of vitamin C, lactose and other materials. As you add regions to the method, the calibration model may begin using some spectral features of lactose to predict concentrations of vitamin C. If changes occur in the lactose content of the samples, the calibration errors may increase. This happens because the model is less able to detect and correctly identify changes in the spectra which are not related to concentration changes in the *sample mixture*.

Each component is *calibrated* independently. This is an advantage over the CLS technique and is the reason why SMLR can handle unexpected changes in the sample mixture.

SMLR works well in the following conditions:

- You can identify at least one *analysis region* for each component.
- There is little or no overlap between component peaks.
- The components of interest absorb linearly with concentration.

- You have at least 3 standards for each component and one additional standard (plus *validation standards* if you use them).
- Spectral *baselines* are fairly stable (you can try using mean centering or applying a processing operation if they're not).
- There are regions that show little or no chemical interactions between components (chemical interactions will cause component peaks to shift from sample to sample). You can try using wider regions to accommodate some shifting of sample peaks.

Since SMLR expresses concentration as a function of the absorbance at multiple frequencies, it is not necessary to account for every component or interference in the sample mixture.

SMLR may be used to analyze a broad range of sample types, especially multicomponent mixtures for which the measured components are well defined. If the components of interest dominate the spectral variation in the standards, the SMLR technique will work even when the *sample matrix* contains other components that are not well defined.

Because near-infrared *peaks* are broad, they can appear to overlap. SMLR will work with near-infrared spectra as long as there is at least one *spectral region* that varies linearly with concentration for each measured component. SMLR has been used extensively in near-infrared applications but it also works well with other forms of spectroscopy.

If the samples contain a few well-defined components and none of the components interact or have overlapping peaks or if the samples do not absorb linearly with concentration, we recommend trying Beer's Law rather than SMLR for the analysis. If you are not satisfied with the results from a Beer's Law analysis, then try SMLR. If the samples and/or the spectral response is complex or if the *noise* level on the spectral data is high, use the PLS or PCR analysis type rather than SMLR.

Advantages of SMLR methods:

- Can handle unexpected impurities in the samples.
- Don't require as many standards as a PLS or PCR analysis.
- Can handle intercorrelations
- More accurate than Simple Beer's Law because more of the data points in the *analysis regions* are used.

Disadvantages of SMLR methods:

- Cannot handle component peaks that overlap severely.
- Components must absorb linearly with concentration.

Partial least squares calibration technique

Our partial least squares (PLS) calibration technique is based on the standard partial least squares (PLS1) algorithm. This is a statistical approach to *quantitative analysis*.

The PLS1 algorithm examines the specified *region* or regions of the calibration spectra to determine which areas vary statistically as a function of *component* concentration. The PLS *calibration model* is developed in one operation using the spectral and concentration information from the *standards*.

To achieve accurate quantitative results from a PLS method, the following conditions must apply:

- You have at least 3 *calibration standards* for each component being measured and one additional standard, plus *validation standards* if you use them. (10n + 1 standards are recommended, where n equals the number of components.)
- The standards are mixtures that contain all of the components that you expect to find in the unknown *samples*.

- The concentrations of the components in the standards vary independently. (You can't use serial dilutions of one standard to prepare combinations of standards.)
- The spectral variation present in the standards accurately represents the variation expected in the unknown samples.

The PLS method type is useful for solving complex analytical problems for which one or more of the following statements may be true:

- Large number of components.
- Component peaks or regions overlap severely.
- Spectral *baselines* are variable.
- Chemical interactions between components cause peaks in the *mixture spectrum* to shift or broaden.
- *Sample matrix* may not be well understood (unknown materials or impurities may crop up in the unknown samples).

Examples of materials which can usually be quantified using a PLS method include detergents in water and boron and phosphorus in glass. PLS also works nicely for analyzing sample properties rather than the concentrations of specific chemicals. For example, you can use PLS to look for physical characteristics of the sample, such as color, hardness or viscosity, if those characteristics produce a spectral response.

Advantages of PLS methods:

- Able to *calibrate* using component peaks or regions that overlap severely.
- Can handle unknown components or impurities that might absorb in the *analysis regions*.
- More robust than Simple Beer's Law because all of the data points in the analysis regions are used.

- User does not need to specify individual regions for each component.
- Software allows you to optimize the number of *factors* for each measured component.
- Software offers a full suite of diagnostic tools for evaluating PLS methods.

Disadvantages of PLS methods:

- Require lots of standards.
- Mathematics are difficult to understand.

Principal component regression calibration technique

Principal Component Regression (PCR) is another statistical *quantitative analysis* technique. Similar to the partial least squares (PLS) analysis, the PCR algorithm examines the specified *region* or regions of the calibration spectra to determine which areas are varying statistically as a function of *component* concentration.

But rather than developing the calibration model in one operation, like PLS, PCR calibration is a two-step process. First, the spectral information is used to calculate the principal component spectra. Then, the principal component spectra and the component concentration information are used to create the calibration model. All component *concentration values* are calculated simultaneously.

The same conditions that are needed for a successful PLS analysis are also required for PCR. For example, to achieve accurate quantitative results from a PCR method the following statements must be true:

- at least 3 *calibration standards* for each component being measured and one additional standard, plus *validation standards*, if you use them. (10n + 1 standards are recommended, where n equals the number of components.)

- The *standards* are mixtures that contain all of the components that you expect to find in the unknown *samples*.
- The concentrations of the components in the standards vary independently. (You can't use serial dilutions of one standard to prepare combinations of standards.)

In general, you should try the PLS analysis type before using PCR. The two techniques are similar. However, the PLS *calibration model* is developed in one operation using spectral and concentration information from the standards while PCR creates a calibration model in two steps. Step 1 uses the spectral data to determine spectral variation; step 2 correlates spectral variation with concentration. Since the regression step is done after the principle components are selected, the principle components aren't weighted toward producing concentration information. Because of this difference, PLS results may be more accurate than the results from a PCR analysis.

On the other hand, PCR is a well known and widely understood technique for statistical analysis. We included the PCR analysis option in our software in case you need to reproduce a PCR method that was created in another application or you simply prefer PCR over PLS.

Advantages of PCR methods:

- Able to *calibrate* using component peaks or regions that overlap severely.
- Can handle unknown components or impurities that might absorb in the *analysis regions*.
- More robust than Simple Beer's Law because all of the data points in the analysis regions are used.
- User does not need to specify individual regions for each component.

Qualitative (classification) techniques

Disadvantages of PCR methods:

- Require lots of standards.
- Mathematics are difficult to understand.
- Since the regression step is done after the principle components are selected, the principle components aren't weighted toward producing concentration information as they are in a PLS method.
- TQ Analyst does not offer as many diagnostic routines for PCR as it does for PLS.

TQ Analyst offers a variety of techniques for comparing and identifying unknown *samples* based on the spectra of known materials. These “classification” techniques are all qualitative measurements because they can tell you what’s in a sample (or which *class* it belongs to), but not how much of a *component* the sample contains. Qualitative techniques can also tell you how closely a sample matches a group of known materials so you can distinguish “good” samples from “bad” ones.

TQ Analyst provides the following *classification method* types:

- Similarity Match
- Distance Match
- Discriminant Analysis
- Search Standards
- QC Compare Search

All of the classification methods compare the spectrum of an unknown sample with the spectra of one or more groups (classes) of known materials (standards). If you want to use one class of standards for the comparison, you must create a Similarity Match or Distance Match method. When several classes of standards are needed, a Distance

Match, Discriminant Analysis, or QC Compare search method should be used. If you want to compare the unknown to many kinds of materials, a Search Standards method is ideal.

A Search Standards method uses only one standard to define each class (TQ Analyst considers each standard as a separate class). Similarity Match methods need at least one standard in each class but allow multiple standards per class. Distance Match, Discriminant Analysis, and QC Compare search methods use statistical algorithms to determine similarity and require at least two standards to define each class.

The various classification methods also differ in the kind of information they provide about an unknown sample. For example, a Similarity Match method compares the unknown sample to a single class of standards and reports a *match value*, which is a measure of similarity, while a Discriminant Analysis method can compare the unknown to several classes and give a “distance from class” value for each class.

Classification methods are typically used for quality control analyses and prefilters for quantitative experiments. For example, once a sample has been “classified,” you can run it through a quantitative method that has been optimized for that type of sample.

The classes may be quite different or very similar. For example, you may set up a classification method where each class represents a material that is different from the other classes. The results can help you sort or identify an unknown material. The classes in another method may differ only in the concentration of a single component and the results can be used to sort materials based on dosage.

The next two sections discuss *calibration models* for classification methods and provide tips on selecting an appropriate classification technique.

Calibration models for classification methods

Classification methods do not build *calibration models* in the same sense that quantitative methods do. However, you must *calibrate* a classification method before the software will allow you to use it to analyze a *sample*.

Only Discriminant Analysis and Distance Match methods display a Calibration Results window. The calibration results show how well the method performs by *classifying* the method *standards*. This information can help you identify standards that may have been assigned to the wrong *class* or standards that are simply too different from the rest of the standards in their class. See the item called “Calibration Results for Classification Methods” in the TQ Analyst Help Topics for more information about calibrating Discriminant Analysis and Distance Match methods.

Similarity Match methods also do some calculations during the calibration step. Search Standards and QC Compare search methods do not require formal calibration. However, even for these methods the software uses the “calibration” step to test the method for consistency and experimental design. So be sure to click the Calibrate button on the TQ Analyst *toolbar* when you think your *classification method* is ready to be implemented and TQ Analyst will let you know if anything looks out of order.

For more information on calibrating a classification method, see the section titled “Calibrating the method” in the chapter on “Creating a Classification Method” in this document.

Choosing a classification technique

There are a number of factors to consider when *classifying samples* using absorption spectroscopy. The most important considerations are the number of different materials the algorithm must distinguish, the number of *standards* available to define each known material and the quality of the spectral information available for the standards and unknown samples. Each of these factors plays an important role in helping you determine the proper experimental design.

The first step in classifying samples is to define the analytical problem, that is,

- How much information do you have about the unknown samples?
- What do you want to compare them to?
- What information is needed from the comparison?

Are you simply trying to verify the purity of a known sample or determine whether it is compound a, b, or c or are you trying to identify a material that is completely unknown?

TQ Analyst provides the following techniques for sample classification. The basic concepts behind these classification techniques are summarized below.

<i>If you . . .</i>	<i>Then use . . .</i>
are interested in monitoring the purity of an incoming material or the product of some process	Similarity Match Distance Match
want to screen incoming materials to determine if they are compound a, b, or c	Discriminant Analysis Distance Match QC Compare search
are trying to identify a material that you know little or nothing about	Search Standards

If you're not sure which technique to use, let the TQ Analyst wizard recommend one. You can always switch to another technique later if the results from the first attempt are unsatisfactory or try several techniques and choose the one that classifies your samples with the most accuracy.

If you switch the analysis type between two options in the same group, for example if you change from Similarity Match to Discriminant Analysis, the information you entered and the settings you chose for the previous analysis type will be saved.

The following sections explain each of the classification techniques available in TQ Analyst and suggest applications for their use.

Similarity Match classification technique

Similarity Match is a spectral classification technique that indicates how closely an unknown material matches a known material. Multiple *standards* may be used to set up a Similarity Match method. The software places all of the standards in a single class. Multiple *regions* of the *spectrum* may be used for the comparison.

Similarity Match methods are typically used to monitor the purity of incoming materials. This type of method works well when all of the standards represent different lots of the same material. A Similarity Match method may also be used to compare a mixture of compounds with the spectra of the individual *components* by using the pure component spectra as the method standards. Similarity Match methods are also good for prequalifying materials that will be used for quantitative measurements.

The Similarity Match algorithm is set up to measure similarity based on a *residual spectrum*. When the method is *calibrated*, the software performs a Gram-Schmidt analysis of the standards. The resulting orthogonal model represents the spectral information provided by all of the data points in all of the standards. During *sample* analysis, the

software uses the Gram-Schmidt model to remove any spectral information represented by the method standards from the spectrum of the unknown sample. The result is called a residual spectrum. The software then compares the information in the *sample spectrum* with the information in the residual spectrum and calculates a “*match value*.” The match value represents the unexplained variation in the spectrum of the unknown sample. See the section on “Similarity Match” in Appendix C: Algorithms Used in TQ Analyst Software” for more information.

Notice The Match Type parameter on the Other *tab* determines the format for reporting the match value. If Match Type is set to “Find Similarities,” the match values will be scaled from 0 to 100 where 100 is a perfect match (little spectral information in the residual spectrum). If Match Type is set to “Find Residual Differences,” the match values will be scaled from 100 to 0 where 0 is a perfect match. ▲

By specifying a limit for the match value, you can monitor the quality of the samples and flag samples that are below (or above) the specified limit. You may also set up a *pass/fail indicator* on the sample reports. See “Preparing a Method for Sample Analysis” at the end of this document for information on specifying limits and pass/fail indicators for TQ Analyst methods.

Distance Match classification technique

The Distance Match classification technique can be used to determine how closely an unknown material matches two or more classes of known materials by calculating a *conformity spectrum* for each *class* and measuring its distance from the class average. This technique is typically used to screen incoming materials, for example, to determine how closely they match compound a, b, or c or to determine “degrees of difference” between known and unknown materials. The Distance Match technique works well for distinguishing materials that are closely related.

We recommend using at least five *standards* to define each class (two standards are required in order to perform the variance analysis during calibration). Multiple *regions* of the *spectrum* may be used for the comparison.

During calibration, the software calculates an average spectrum and a standard deviation spectrum for each class.

When you use the method to *classify* the spectrum of an unknown *sample*, the software subtracts the average spectrum from the *unknown sample spectrum* to create a *residual spectrum* and divides by the corresponding standard deviation spectrum to create a *conformity spectrum* for each class. (The subtraction step shows the differences between the two spectra; the ratio tells you the significance of that difference.) Then it calculates the percentage of frequencies in the residual spectrum that exceed the Distance Match threshold in standard deviation units (typically 5). The Distance Match threshold is located on the Other *tab*.

Notice The result of the comparison is called the “*match value*,” which is an indicator of the quality of the match. The match values for Distance Match methods will range from 100 to 0, where 0 (percent frequencies exceeding the threshold) is a perfect match. ▲s

If one class is defined in the method, the software reports the match value for that class. If multiple classes are used, the software reports the class name and match value for the specified number of classes. The best matched class (the one with the lowest match number) is listed first.

By specifying a limit for the match value, you can monitor the quality of the samples and flag samples that are below (or above) the specified limit. You may also set up a *pass/fail indicator* on the sample reports. See “Preparing a Method for Sample Analysis” at the end of this

document for information on specifying limits and pass/fail indicators for TQ Analyst methods.

The Distance Match algorithm works well for differentiating materials that contain different amounts of the same *components*. In this type of analysis, the spectra of the standards in each class are very similar. The main difference may occur only in the intensities of the peaks in a few key regions. For example, a lab might set up a Distance Match method to check the dosage of drugs used in a double-blinded clinical study to confirm that no mix-ups occurred in the packaging. In this case, the analyst might set up classes that define different levels of an active ingredient, such as salicylic acid. The method could then be used to test incoming drugs to see which category they match and to what degree.

You can also use Distance Match to detect slight changes in incoming raw materials or production samples by comparing them to previous samples of the same type. We refer to this procedure as “qualification.” It can be an effective technique for statistical quality control.

Discriminant Analysis classification technique

The Discriminant Analysis classification technique can be used to determine the *class* or classes of known materials which are most similar to an unknown material by computing the unknown’s distance from each class center in *Mahalanobis distance* units. The discriminant analysis technique is typically used to screen incoming materials to determine if they are compound a, b, or c.

The method usually specifies two or more classes of known materials. Multiple standards are typically used to describe each class. You can use multiple *regions* of the *spectrum* for the analysis.

During calibration, the software computes an average spectrum and then generates a distribution model by estimating the variance at each *frequency* in the *analysis range*. You can select whether a unique

distribution model is developed for each class or one model is used for all classes (see Within Class Variance on the Other *tab*). If one model is used for all classes, the software subtracts the class average from each standard and then creates a single *variance spectrum* using information from all of the classes. If you elect to use a unique distribution model for each class, the software subtracts the class average from each standard and uses only the information from a given class to create a unique variance spectrum for that class. This is similar to the technique known as *SIMCA* (Soft Independent Modeling of Class Analogies).

In order to calculate the statistics properly, the single model requires at least one class that contains two or more standards. If you select the option that creates a unique distribution model for each class, every class must contain at least two standards. Both models work best with several standards per class.

When you use the method to analyze an unknown sample, the software performs a *principal component* analysis on the *unknown sample spectrum* and on the variance spectrum (or spectra) to determine *score values*. The score plots are used to produce *Mahalanobis distance* values, which in turn are used to rank the classes.

Note The Discriminant Analysis technique uses the spectral information from all of the method standards to create the distribution model or models. As a result, adding or deleting standards may have a significant impact on the method's calibration model, especially when using the pooled distribution model. If you are using a unique distribution model for each class, you may add or delete standards from one class without affecting the other classes in the method. ▲

Notice The result of a discriminant analysis is the name of the class or classes that are most similar to the spectrum of the unknown sample. The Mahalanobis distance between the unknown sample and each reported class can also be reported. The closer each *distance value* is to zero, the better is the match. ▲

For most experiments, a Discriminant Analysis method will perform best when the software creates a unique distribution model for each class. However, if you have fewer than five standards to define each class and the spectra that make up the various classes are very similar, we recommend using a pooled distribution model to differentiate the classes.

The Discriminant Analysis technique works well when you are trying to analyze samples in which the composition may be very different but they all contain a common ingredient. For example, you can use a Discriminant Analysis method to analyze street drugs, such as cocaine or heroin, that are mixed with various materials. In this case, the standards used to define each class all contain the drug of interest but the other components in the standards (cutting agents, etc.) are very different. Since this kind of experiment guarantees lots of variation in the standards, make sure the method is set up to calculate a unique distribution model for each class.

QC Compare search classification technique

The QC Compare search classification technique can be used to verify the composition of an unknown material that is known to belong to one of several classes. In these applications, the analyst is interested in identifying which known material each *sample* is most like. The QC Compare search algorithm compares the intensities at each *frequency* of the *unknown sample spectrum* with the individual spectra in each *class* in order to find the spectra and class that most closely match the unknown.

A QC Compare search method must specify at least two classes of known materials. Multiple *standards* may be used to describe each class. Multiple *regions* of the *spectrum* may be used for the analysis.

The result of a QC Compare search is the single best *match* from each reported class. The classes are listed in order of importance; the class that contains the best matched spectrum is listed first.

Notice The library *index number* of each reported standard is given as well as a *match value* between 0 and 100. The match value tells you how well the standard matches the unknown. A match value of 100 indicates a perfect match. ▲

Note If you are interested in identifying unknown materials by their near-infrared spectra, we recommend using QC Compare search rather than Search Standards because you can use several spectra to define each known material the unknown will be compared with. ▲

Search Standards classification technique

You can use the Search Standards classification technique to look at a wide range of known materials (*standards*) to determine which of them are most similar to an unknown material. The Search Standards algorithm compares the intensities at each *frequency* of the *unknown sample spectrum* with the *spectrum* of each standard and finds the spectra that most closely match the unknown. The Search Standards technique is often used to identify a material that is completely unknown.

You can use only one standard to describe each known material in a Search Standards method. The software considers each standard in the method as a separate “class.” The method must specify at least two standards; in most cases, many standards are used. Multiple *regions* of the spectrum may be used for the comparison.

You may choose between five *search algorithms* when creating a Search Standards method. We recommend using the Correlation search algorithm.

Notice When you use a Search Standards method to analyze the spectrum of an unknown *sample*, the *index number* and title of each reported standard are given as well as a *match value* between 0 and 100. The match value tells you how well the standard matches the unknown. A match value of 100 indicates a perfect match. ▲

If you are familiar with Thermo Scientific OMNIC spectral analysis software, the Search Standards classification technique is the same as the OMNIC Search feature.

Spectral measurement techniques

You can use TQ Analyst to set up methods that simply measure spectral features and report the measured values. These “measure only” methods can be used to determine *peak heights* or areas, peak locations and even the *noise* in a given *spectral region*.

A *spectral measurement method* can be configured to do any of the following:

- Measure the height or area of a peak.
- Measure the ratio of two spectral peaks.
- Measure the random *noise* in a given region.
- Measure the width at half maximum of the largest peak in a region.
- Locate the largest peak in a specified region.
- Find where a peak is reduced to 1%, 2%, 5% or 10% of its maximum height.

You can specify up to 50 measurements in a spectral measurement method.

Note To set up a method that calculates the ratio of two spectral peaks, set the TQ Analyst Pathlength option to Peak Ratio. The numerator peak must be listed with the other measurements in the Measure Only method. The Peak Ratio options let you define the denominator peak. See the Explain Help in your TQ Analyst software for more information about setting Pathlength options. See the section titles “Selecting the Analysis Regions” in this chapter for instructions for using the Peak Ratio pathlength option to define a denominator peak. ▲

Methods that report *peak heights or areas* are typically used to consistently measure a peak or area in a series of spectra. The absorbance values could then be used to plot a *calibration curve* by hand or on a computer that can calculate spectral plots.

An optional linear function can be applied to the absorbance values from each measured peak for simple mathematical corrections. Each function is defined by its *slope* and *intercept* values. If a *linear correction* is specified for a given peak, the corrected absorbance value is reported instead of the measured value.

People use methods that measure *spectral noise* to test spectrometer performance or to validate a specific analysis. You can configure a method to measure either *RMS noise* or *peak-to-peak noise* in a specified region.

Some people need to consistently measure the *width of a peak* in a spectral region or simply report the peak’s exact *location*. These measurements are often useful for *validation* purposes or to detect changes in peak shape. Peak width values and peak locations are reported in the X-axis unit of the corresponding *spectrum*.

Others simply want to report the location where a peak is reduced to a certain percentage of its maximum value. Percent-of-maximum

measurements are typically used for instrument validation. For example, you can use them to report the X-axis location where the energy in a *single-beam spectrum* is reduced to a level that is no longer useful.

Calibration models for Measurement Only methods

Measurement methods do not require calibration as no *calibration model* is needed. These methods are intended for measuring or reporting information about spectral data, rather than *classifying* spectra or calculating *component* concentrations. However, TQ Analyst uses the “calibration” step to test the method for consistency and experimental design. So be sure to click the Calibrate button when you think the method is ready to be implemented and TQ Analyst will let you know if anything looks out of order.

How pathlength affects quantitative and classification techniques

Sample *pathlength*, defined as the distance that a beam of incident energy travels within a *sample*, affects both quantitative and qualitative (classification) analyses. When analyzing samples that are in the solid state, “sample pathlength” is often referred to as “sample thickness.” The pathlength of a liquid sample is defined by the distance between the internal walls of the holder used to place the liquid in the energy beam.

Note Pathlength is important to a spectral measurement analysis only when the measurement data are used for quantitative or classification purposes. ▲

In our discussions so far, sample pathlength, represented by the term “b” in the Beer’s Law equation, was assumed to be constant.

$$A = a b c$$

where: A = absorbance measured at a given frequency

a = absorptivity of the component at the measured frequency
(constant for a given component and frequency)

b = pathlength of the component

c = concentration of the component

Beer’s Law Equation

This allowed us to drop the pathlength term from the equation so we could focus the discussion on the relationship between *absorbance* and concentration. In reality, sample pathlength isn’t always constant. If the pathlengths of the *standards* and the unknown samples are different, the *calibration model* must account for the differences. This is true for any quantitative method.

TQ Analyst provides a number of options for handling variations in sample pathlength for quantitative methods. All of these pathlength options are described in the sections that follow.

Sample pathlength is important to qualitative (classification) analyses for the same reasons. This is especially true for Distance Match and Discriminant Analysis methods when the method must discriminate between various materials that contain different amounts of a key compound. If the pathlengths of the standards and the unknown samples are different and component concentrations are not important to your classification analysis, the method should include a pathlength correction to account for the pathlength differences. For example, if you are classifying samples of the same material but with different

particle sizes, apply a pathlength correction only if particle size effects are not something you want the method to track. A pathlength correction may also allow you to tighten class distribution for spectra that have significant anomalies, due to scattering or some other problem.

The pathlength options that are provided for *classification methods* (Peak Ratio, Multiplicative Signal Correction, and Standard Normal Variate) all correct the spectral data rather than the calculated *concentration values*, which would be useless for a classification method since no concentration values are reported. See the sections that follow for details on the Peak Ratio, Multiplicative Signal Correction and Standard Normal Variate pathlength options.

Choosing a pathlength option

TQ Analyst provides a range of techniques for handling variations in sample *pathlength*. The following is a complete list of the pathlength options available in TQ Analyst software:

- Constant
- Known
- Predict
- Internal Reference
- Peak Ratio
- Multiplicative Signal Correction (MSC)
- Standard normal variate (SNV)

Detailed descriptions of the pathlength options are provided at the end of this section.

The software offers more pathlength options for the multivariate quantitative techniques (CLS, SMLR, PLS and PCR) than for Simple Beer's Law because a multivariate *calibration model* is better able to

identify subtle changes in the spectral data that are due to differences in sample pathlength. If sample pathlength is unknown or difficult for you to measure, a multivariate analysis may be required to accurately analyze your *samples*.

If you are creating a classification method, the software provides the pathlength options that rescale the *spectrum* (or do nothing) rather than correct the calculated *concentration values*, including:

- Constant
- Peak Ratio
- Multiplicative Signal Correction (MSC)
- Standard Normal Variate (SNV)

Pathlength corrections that operate on the calculated *concentration values* would be useless for a *classification method* since no concentration values are available.

Note No pathlength options are available for the Search Standards and QC Compare search classification techniques. ▲

In most cases, your *sampling technique* will dictate the correct pathlength option (frequently Constant or Known). If the pathlengths of your samples aren't constant or known, we recommend setting the pathlength option to "Undecided." Using this option will allow the TQ Analyst Pathlength wizard to recommend a pathlength setting for your method.

Note The Pathlength wizard is available only for quantitative methods. ▲

To use the Pathlength wizard, set the Pathlength Type to "Undecided" and then click the Suggest Pathlength Type button on the Pathlength *tab*. Keep in mind, however, that the method must be fairly complete before the Pathlength wizard can recommend a pathlength setting. For

example, you must define the *components*, collect and identify the *standards* and select the *spectral regions* to be used for the analysis before the wizard can recommend a pathlength setting.

If you select the Undecided pathlength option and then use the Suggest Regions wizard to recommend spectral regions for your method, the Regions wizard will recommend a pathlength option as part of the region selection process. The Pathlength wizard performs a statistical analysis of the spectral data from the standards to find the spectral *peak* or region that correlates best with variations in sample pathlength.

You may also try several pathlength types and then choose the one that produces the best results. Here are some brief guidelines:

<u><i>If sample pathlengths are...</i></u>	<u><i>Use this pathlength type...</i></u>
Always the same	Constant
Variable and known (or easily measured)	Known
Variable and unknown (or not easily measured)	Predict Internal Reference MSC SNV

If you are creating a classification method that contains standards collected at different pathlengths and you can find a peak that varies only with pathlength, use the Peak Ratio pathlength option. The Peak Ratio pathlength correction is especially useful for Distance Match and Discriminant Analysis methods when the method must discriminate between various materials that contain different amounts of a key *component*. Peak Ratio scales the spectrum based on the *pathlength peak*, leaving the concentration differences intact.

If the pathlengths of the standards and the unknown samples are different but you can't find a peak that varies only with pathlength, use the MSC or SNV pathlength correction.

If the pathlengths of the standards and the unknown samples are all the same, use the Constant pathlength option.

The remaining sections of this chapter list the pathlength options available in TQ Analyst and explain how they work and when each of them should be used.

Constant pathlength option

Select the Constant pathlength option only if you can collect the spectra of the *standards* and every unknown *sample* the method will analyze at exactly the same *pathlength*. You do not need to know the pathlength value. It is only important that the pathlengths are all the same.

When the pathlengths of the standards and unknown samples are exactly the same, the pathlength term “b” can be dropped from the Beer's Law equation, and the analysis focused on the relationship between absorbance and concentration.

$$A = a b c$$

where: A = absorbance measured at a given frequency

a = absorptivity of the component at the measured frequency
(constant for a given component and frequency)

b = pathlength of the component

c = concentration of the component

Beer's Law Equation

Select the Constant pathlength type if you are using a sampling accessory that has a fixed pathlength, such as a liquid ATR accessory or fixed path gas cell.

If you know the exact pathlength of your sampling accessory (some accessories have a label that tells you the measured pathlength), we recommend using the Known pathlength option even though pathlength is constant. Then, if the cell you are using breaks and must be replaced, you can enter the pathlength of the new cell and continue analyzing samples without rerunning the standards.

The following sampling accessories may have a constant pathlength:

- Uncalibrated liquid transmission cells.
- Liquid ATR accessories, such as a Circle cell or Horizontal ATR.
- Diffuse reflectance accessories.

Known pathlength option

Select the known pathlength option if you know or can easily measure the *pathlengths* of the *standards* and any *samples* you want to analyze with your method. The pathlengths of the standards and the unknown samples can be different but the pathlength values must be known.

When the Known pathlength option is selected, you must enter a pathlength value for each standard before the method can be *calibrated*. You must also specify the pathlength of every unknown sample that you use the method to analyze.

Select the Known pathlength type in the following cases:

- You are analyzing samples that have different but known thicknesses, such as a series of molded polymer films.
- You will be using a variety of sampling accessories that have a fixed pathlength or a pathlength that can be accurately set.

- The pathlengths of the standards and samples are the same but the pathlength could change, for example if the cell you are using breaks and must be replaced. Then you can enter the pathlength of the new cell and continue analyzing samples without rerunning the standards.

The following types of samples and sampling accessories may have measurable pathlength values:

- Molded polymer films.
- Variable path gas cells.
- Variable path liquid cells.

Predict pathlength option

If you know or can easily measure the *pathlengths* of the *standards* but don't know the pathlengths of the unknown *samples*, try using the Predict pathlength option. Predict uses the pathlength information in the *calibrated* method to predict the pathlength of each unknown sample.

The Predict pathlength option works only when there is sufficient variation in the *calibration model* that correlates with differences in pathlength. Since pathlength is treated as an additional *component* to be quantified, at least one additional *calibration standard* is required.

When the Predict pathlength option is selected, you must enter a pathlength value for each standard before the method can be *calibrated*. When the method is used to analyze an unknown sample, the software uses the calibration model to predict the sample pathlength. The predicted pathlength value is used to calculate component concentrations in the unknown sample. The sample results will show the predicted pathlength value along with the component concentrations.

People often use the Predict pathlength option when the pathlengths of the standards and samples can be measured but the process is time consuming or expensive. The pathlengths of films and polymers are often calculated this way.

Note The Predict pathlength option in TQ Analyst is the same as the “Internal known” pathlength option in Thermo Scientific QuantIR software. ▲

Internal Reference pathlength option ($A = b * c$)

The internal reference pathlength option can be used when the following conditions apply:

- You are creating a *CLS* or *Simple Beer’s Law* method,
- The *pathlengths* of your *standards* and unknown *samples* vary,
- Sample pathlength is difficult or impossible to measure accurately,
- You can identify a spectral *peak* or *region* that varies only with pathlength.

The Internal Reference pathlength setting uses a specified peak to compensate for differences in sample pathlength. When using the internal reference pathlength option, you must specify an *absorbance peak* that is indicative of sample pathlength. This can be a peak from a known amount of a *component* that is added to the *calibration standards* and unknown samples or it can be a peak that is due to the matrix material. The selected peak must respond only to pathlength; it cannot contain absorptions from other components or impurities in the *sample mixture*.

Examples of sample types whose pathlength values can sometimes be determined this way include samples mixed with KBr and pressed into a pellet (added pathlength component) and molded polymer films (matrix peak).

If you need help finding a *pathlength peak* for a quantitative method, let the TQ Analyst wizard find one for you. There are several ways you can do this, depending on how far along you are developing your method. For example, if you simply want to investigate the Internal Reference pathlength option, use the Suggest Pathlength Wizard to recommend a pathlength region. The Pathlength wizard performs a statistical analysis of the spectral data from the standards to find the spectral peak or region that correlates best with variations in sample pathlength.

Keep in mind, however, that the method must be fairly complete before the Pathlength Wizard can make a recommendation. For example, you must define the *components*, collect and identify the standards and select the *spectral regions* to be used for the analysis before the wizard can recommend a pathlength region.

If you leave the Pathlength Type set to Internal Reference and then use the Suggest Regions wizard to recommend *analysis regions* for your method, the Regions wizard will recommend a *pathlength peak* or region along with the analysis regions.

We prefer using the Internal Reference pathlength option rather than Peak Ratio because it provides information about the measurement error. A method based on Peak Ratio, $A / b = C$, calculates just concentration, C. But this calculation is based on the pathlength corrected analytical measurement, A / b . The calibrated method cannot tell you anything about measurement error because this error is masked by ratioing the raw data before presenting it to the method.

Note If you need to use a pathlength peak to compensate for changing pathlengths, use either Internal Reference or Peak Ratio. See the section titled “Comparison of Internal Reference and Peak Ratio Pathlength Types” in Appendix B for details on how these two pathlength options differ. ▲

Note The Internal Reference pathlength option in TQ Analyst is the same as the “Internal standard” pathlength option in Thermo Scientific QuantIR software. ▲

Peak Ratio pathlength option (A / b = c)

The Peak Ratio pathlength setting allows you to use a specified *peak* to compensate for differences in sample *pathlength*. The Peak Ratio pathlength type works only when the sample spectra contain a peak that varies in proportion to pathlength. This pathlength option works for *PLS*, *PCR* and *SMLR* methods.

When using the Peak Ratio pathlength option, you must specify an *absorbance peak* that is indicative of sample pathlength. This can be a peak from a known amount of a *component* that is added to the *standards* and unknown *samples* or it can be a peak that is due to the *sample matrix*. The selected peak or spectral region must respond only to pathlength; it cannot contain absorptions from other components or impurities in the *sample mixture*.

If you need help finding a *pathlength peak* for a quantitative method, let the TQ Analyst wizard find one for you. There are several ways you can do this, depending on how far along you are developing your method. For example, if you simply want to investigate the Internal Reference pathlength option, use the Suggest Pathlength Wizard to recommend a pathlength region. The Pathlength wizard performs a statistical analysis of the spectral data from the standards to find the spectral peak or *region* that correlates best with variations in pathlength. Keep in mind, however, that the method must be fairly complete before the Pathlength Wizard can make a recommendation. For example, you must define the *components*, collect and identify the standards and select the *spectral regions* to be used for the analysis before the wizard can recommend a pathlength region. If you leave the Pathlength Type set to Peak Ratio and then use the Suggest Regions wizard to recommend *analysis regions* for your method, the Regions wizard will recommend a *pathlength peak* or region along with the analysis regions.

When the Peak Ratio pathlength option is used, the software divides all of the data points in all of the analysis regions for each standard by the pathlength reference peak before the method is *calibrated*. Because the correction is applied before calibration, the peak ratio pathlength correction is not described by the *calibration model*. This technique tends to mask the measurement error of the raw data and can skew the sample results.

Note If you need to use a pathlength peak to compensate for changing pathlengths, use either Internal Reference or Peak Ratio. See the section titled “Comparison of Internal Reference and Peak Ratio Pathlength Types” in Appendix B for details on how these two pathlength options differ. ▲

Note You can also use the Peak Ratio pathlength type to specify the denominator peak in a spectral measurement method when taking the ratio of two measured peaks. See the section titled “Selecting the Analysis Regions” in the chapter on “Creating a Spectral Measurement Method” for instructions. ▲

Multiplicative Signal Correction pathlength option

The multiplicative signal correction (MSC) pathlength option is useful under the following conditions:

- The *pathlengths* of your *standards* and unknown *samples* vary,
- Sample pathlength is difficult or impossible to measure accurately,
- You can't find a spectral *peak* or *region* that varies only with pathlength.

The MSC pathlength setting uses a mathematical function to compensate for differences in pathlength. MSC works when there is a multiplicative contribution to the spectral signal that correlates with pathlength. It is assumed that the relationship between pathlength and its contribution to the signal absorbance is perfectly linear. MSC is

designed to work with spectra that also have a linear response to concentration, that is, spectra which are in absorbance, $\log(1/R)$ or Kubelka-Munk units.

This allows you to analyze samples with different pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength. For example, the pathlengths of oils smeared on a metal surface can often be calculated this way. The MSC pathlength treatment may also be used to compensate for variations in sample thickness that are caused by particle size and scattering. These variations often occur in diffuse reflectance measurements, especially when measured in the near-infrared range.

When the MSC pathlength type is selected, no additional pathlength information is required. Pathlength is treated as a multiplicative contribution to the spectral signal. The calculated pathlength function corrects the multiplicative contribution of the scattering due to pathlength. The software applies the same function to the standards and the unknown samples.

Standard Normal Variate pathlength option

The standard normal variate (SNV) pathlength option is useful under the following conditions:

- The effective *pathlengths* of the *standards* and unknown *samples* vary,
- Sample pathlength is difficult or impossible to measure accurately,
- You can't find a spectral *peak* or *region* that varies only with pathlength,
- Scattering, due to differences in particle size for example, produced significant variation in the spectra of your standards.

The SNV pathlength setting scales the spectral data in order to compensate for differences in sample pathlength. This allows you to

analyze samples with different effective pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength. The spectra of the standards and any unknown samples the method is used to analyze are all scaled independently.

SNV works when there is a multiplicative contribution to the spectral signal, due to scattering or some other effect, that correlates with pathlength. It is assumed that the relationship between pathlength and its contribution to the signal absorbance is perfectly linear. SNV is designed to work with spectra that also have a linear response to concentration, that is, spectra which are in absorbance, $\log(1/R)$ or Kubelka-Munk units.

You can define the region used for the SNV pathlength correction. You may want to limit the region used for correcting pathlengths if one or both ends of your spectral data tend to be quite noisy. If the *noise* level is fairly even across the spectra of the standards or samples, we recommend using the entire *spectrum* for the pathlength correction (*default setting*). For information on how to define the pathlength region for the SNV pathlength type, click the Pathlength *tab* and select the SNV pathlength option. Then click the *Explain button* on the TQ Analyst *toolbar* to open the *Explain help window* and click the Region for SNV Pathlength feature in the software.

If you are creating an SMLR method, the SNV pathlength region can be combined with a quadratic removed baseline type for corrections and *detrending*. The SNV pathlength type removes the effects of scattering from the specified region and the quadratic removed baseline type removes additional baseline curvature.

The SNV pathlength option allows you to analyze samples with different pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength and you cannot find a peak or region in the sample spectra that varies only with pathlength. The SNV technique may also be used to compensate for variations in

sample thickness that are caused by particle size and scattering. For example, the near-infrared diffuse reflectance spectra of two ground tablets displayed in log (1/R) units can appear quite different even though the tablets are made from the same materials. The differences are due to scattering effects from variations in particle size. The SNV pathlength correction can remove the effects of scattering from the spectral data so that chemical differences can be observed.

SNV can also be used to “normalize” spectra in order to minimize the effects of scaling and offsets. For example, if you want to use a group of spectra of the same material that were measured at different pathlengths to define a class in a discriminant analysis method, you can use SNV to normalize the spectra so the pathlength variations do not contribute to the classification process.

When the SNV pathlength type is selected, no additional pathlength information is required during sample analysis. Pathlength is treated as a multiplicative contribution to the spectral signal. For each standard in the method, the software computes the mean and *standard deviation* of the baseline region. Then, for each point or points in the *spectral region*, it subtracts the mean value and divides by the standard deviation. This places all of the standards on the same scale. The SNV pathlength correction is automatically applied to any unknown samples you use the method to analyze.

The SNV pathlength correction is similar to the Multiplicative Signal Correction (MSC) pathlength option. However, MSC calculates an ideal spectrum from the *calibration standards* and uses it to correct the data, while the SNV correction removes the effects of scattering by normalizing the spectra individually. We recommend using SNV instead of MSC if you think the spectra of the unknown samples you want to measure may have different scattering characteristics than the calibration spectra, or if you are using a classification technique that allows multiple classes (for example, Discriminant Analysis, Search Standards, or QC Compare search).

Note The Pathlength wizard will not suggest the SNV pathlength option. ▲

3 Using TQ Analyst Software

Read this chapter to learn about the unique features of your TQ Analyst method development software.

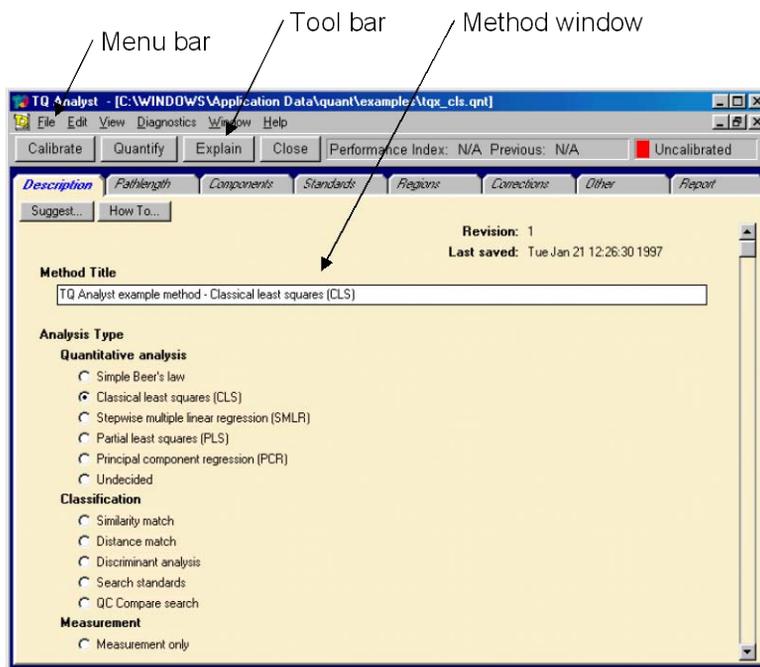
The following software features are introduced in this chapter:

- TQ Analyst main window
- Menu bar
- Toolbar
- Method windows
- Tabs
- Scroll bars
- Action buttons
- Method parameters

You should understand how to use these features before attempting to create methods using TQ Analyst.

TQ Analyst window

While you are using TQ Analyst, the TQ Analyst window is displayed on the screen.

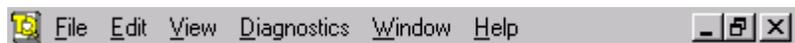


TQ Analyst Window

You can maximize, minimize and resize the TQ Analyst window just as you can other application windows that run with Microsoft® Windows® software. See your Microsoft Windows documentation for information on these standard Windows features.

The TQ Analyst window is divided into three distinct parts: the *menu bar*, the *toolbar* and the *method windows*. You may also see other types of windows, such as *task windows* and *spectral windows*. All of these features are described in the sections that follow.

Menu bar The menu bar contains the TQ Analyst *menu names*.



The *menus* provide useful *commands* for working with method files and spectral data. You will also find a *menu* of diagnostic commands which can be used to evaluate a method as you develop it.

The Help menu allows you to access the software's extensive on-line help system any time you need information. The Help menu provides links to a comprehensive list of help topics, organized by tasks, plus a tour of the software and a set of example methods.

You can choose commands in TQ Analyst menus just as in other Windows applications: by using the mouse or by typing key combinations on the keyboard. The key combinations are shown in the menus.

Toolbar The *toolbar* allows you to perform the following operations with the current method:



- The Calibrate button calibrates the method.
- The Quantify button allows you to use the method to analyze an *unknown sample spectrum*.
- The Explain button opens the *Explain help window* for context-sensitive help with any feature of the software.
- The Close button closes the active method or other window, such as a *task window* or *spectral window*.

To use a feature on the toolbar, click the appropriate button.

The first time you *calibrate* a method, the software calculates a *performance index* which appears next to the Close button on the toolbar. The performance index is a measure of how accurately a calibrated method can *quantify* or *classify* the *validation standards*.

If you change the method and then recalibrate, the toolbar shows the performance index from the previous calibration as well as the index for the new calibration.

A screenshot of a toolbar element showing the text "Performance Index: 92.2 Previous: N/A". The text is displayed in a light gray font on a slightly darker gray background.

Since method development is usually an iterative process, this allows you to determine whether the changes you make to optimize a method actually improve its performance.

Note Validation standards are required to calculate the performance index. If your method has no validation standards, the symbol “N/A,” meaning “not available,” is displayed instead of a performance index. ▲

The indicator to the right of the performance index tells whether the selected method is *calibrated*. If the method has never been calibrated, the bar is red and the message reads “uncalibrated.” After the first calibration, the bar turns green and the “calibrated” message appears.

A screenshot of a toolbar element showing a green square indicator followed by the text "Calibrated". The text is displayed in a light gray font on a slightly darker gray background.

If you then change something in the method that affects calibration, the calibration indicator turns red, letting you know that the method is no longer calibrated. Once you recalibrate, the indicator changes back to green.

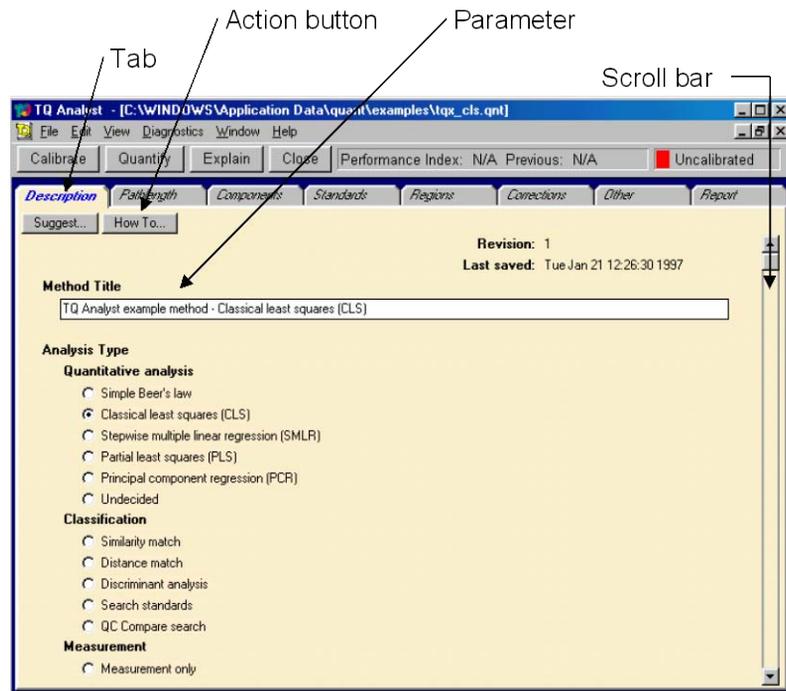
Note The calibration indicator updates when the method is saved on the computer's hard disk. ▲

Method windows

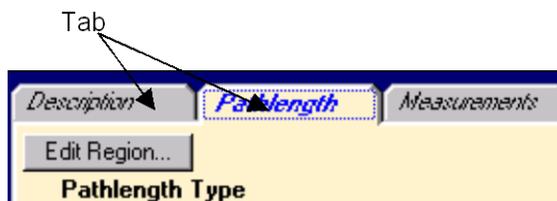
Method windows can be displayed in the main portion of the *TQ Analyst window*. A method window contains all the features needed to create methods for sample analysis.

You can have many method windows open at the same time. To make an open method window active, choose its name in the Window menu.

The main features of a method window include the *tabs*, *scroll bar*, action buttons and *parameters*.



Tabs The *method parameters* specify how your method will work. Method parameters are grouped by function. Each group of *parameters* is contained on a file card which has a tab that is always visible at the top of the *method window*. To display a group of parameters, click the corresponding tab. The file card containing the parameters is brought to the front.

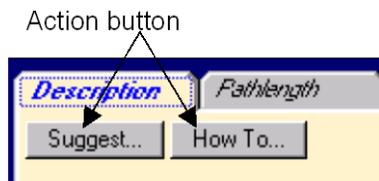


The parameters on a tabbed card work together to specify important features of your TQ Analyst methods. For example, the parameters on the Standards tab identify the spectral data files which will be used to create a method *calibration model* or classification scheme.

The tabs are arranged in an order that is convenient for creating a TQ Analyst method. If you select the tabs in sequence from left to right, starting with the Description tab, they will lead you step by step through the method development process.

Scroll bars A *scroll bar* will appear along the right side or bottom of the selected *tab* when the tab contains more features than will fit in the window. Click the arrow at either end of a scroll bar to move the tab's contents up and down or right and left in order to bring additional features into view. You can also operate the scroll bar by clicking and dragging its scroll box.

Action buttons When you select a *tab*, one or more buttons may appear below the tab name. These buttons, called “action buttons,” allow you to perform tasks related to the selected tab. For example, the “Suggest” button shown in the previous illustration may be used to help you set the Analysis Type parameter on the Description tab.



Method parameters The method parameters specify how the method will work. There are several distinct types of parameters, including *text boxes*, *readouts*, *option buttons*, check boxes, list boxes, and tables.

Text boxes

Text boxes allow you to enter information that you want to save with a method, such as the *method title* or the unit used to measure sample *pathlength*. You can also use a text box to specify a value, such as pathlength or component concentration.

Text boxes have a white background, which indicates that their contents can be changed.

Method Title

TQ Analyst example method - Classical least squares (CLS)

You can select a text box by using the mouse to click in the box or by clicking the window and then pressing the Tab key until the desired text box is highlighted. Then type the text you want to enter.

Readouts

A readout displays information or values provided by TQ Analyst, such as the version number of a method or the method's *performance index*. Readout fields have a colored background, which indicates that their contents cannot be changed.

PI: 97.5 Previous: 97.7

Option buttons

Some parameters, such as the Analysis Type and Pathlength Type, provide a group of option buttons and allow you to select only one of the available options.

Quantitative analysis

- Simple Beer's law
- Classical least squares (CLS)
- Stepwise multiple linear regression (SMLR)
- Partial least squares (PLS)
- Principal component regression (PCR)
- Undecided

You can select an option from a group of option buttons by using the mouse to click the corresponding button. If you want to use the keyboard to select an option, tab to the option and then press the space bar.

Check boxes

Check boxes allow you to turn parameters on and off.

General Information

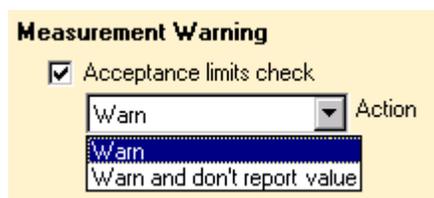
- Method title

You can operate check boxes using a mouse or the keyboard. To turn a check box on or off using a mouse, click the check box. The parameter is on when an X appears in the box and off when the box is blank.

To turn a check box on or off from the keyboard, tab to the check box and then press the space bar.

List boxes

List boxes allow you to select an item from a list of available options. To see the options in a list box, use the mouse to click the arrow next to the list box. Then click an item to select it.



To select an item in a list box using the keyboard, tab to the list box and press Enter. Then use the arrow keys to select an item and press Enter again.

Tables

Tables help you keep data and specifications organized. For example, the Standards table identifies the spectral data files which will be used to create a *calibration model* or classification scheme.

Tables also allow easy access to the data once it has been entered.

Index	Display	Spectrum Title	Usage	Glucose Wt %
1		pls example; standard 1	Calibration ▼	1.94
2		pls example; standard 2	Calibration ▼	1.26
3		pls example; standard 3	Validation ▼	2.59
4		pls example; standard 4	Calibration ▼	0.36
5		pls example; standard 5	Validation ▼	1.55
6		pls example; standard 6	Calibration ▼	1.13
7		pls example; standard 7	Validation ▼	2.25
8		pls example; standard 8	Validation ▼	2.22
9		pls example; standard 9	Calibration ▼	1.69
10		pls example; standard 10	Calibration ▼	1.36

The fields in a table may be text boxes, list boxes, or buttons.

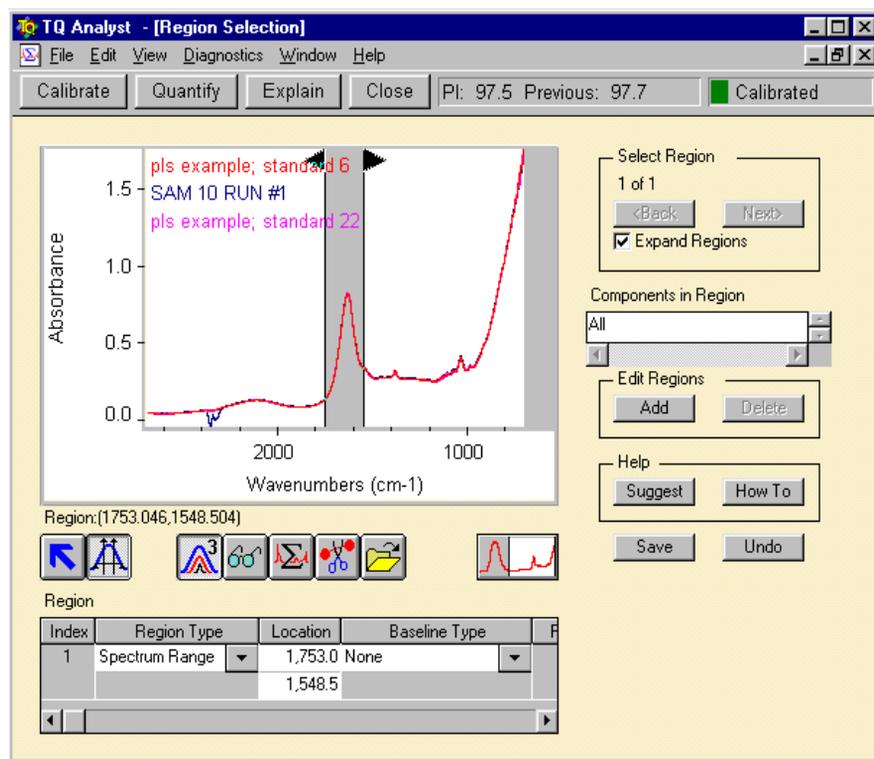
You can select a text field by using the mouse to click in the field or by using the tab or arrow keys on the keyboard. When the field is selected, use the keyboard to fill in the text or enter a number. Use the mouse to select another cell or press the Enter key on the keyboard to select the next cell.

To see the options in a list box that is part of a table, use the mouse to click the arrow next to the list box. Then click an item to select it.

To use a button that is part of a table, such as the Display buttons in the Standards table, click the button with the mouse. To turn a button on and off from the keyboard, tab to the button and press the space bar.

Task windows

Special *task windows* may be displayed for certain operations. Task windows often contain tables and plots of spectral and method data to help you make a selection or complete a procedure. For example, the Region Selection task window shown in the illustration below allows you to choose the limits and *baseline* of the *analysis regions* for the current method and to specify how the region and baseline will be measured.



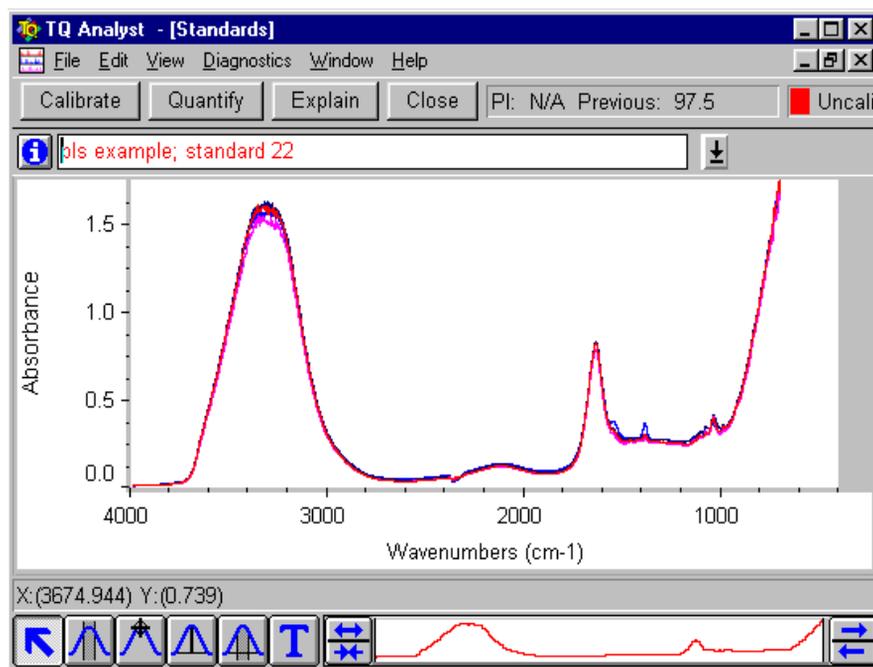
Example of Task Window

Task windows appear on top of the current *method window*. To close a task window, click the Close button on the TQ Analyst *toolbar*.

Spectral windows

TQ Analyst allows you to open and display spectral data files and work with displayed spectra. A *spectral window* is opened automatically when you open a *spectrum* or display the selected *standards* in your method. Many of the diagnostic results are also displayed in a spectral window.

The illustration below shows a spectral window with the spectra of several standards displayed.



Example of Spectral Window

You may have several spectral windows open at the same time. Spectral windows appear on top of the current *method window*. Use the Window menu to see the windows that are currently open or to change the *active window*. To close the active spectral window, click the Close button on the TQ Analyst *toolbar*.

4 Managing TQ Analyst Files

The TQ Analyst File menu commands and *toolbar* allow you to work with method and spectral data files. Read this chapter to learn how to use the File menu and toolbar commands to create, open, save, print and run methods from TQ Analyst and to save a TQ Analyst *spectrum* in a file on your disk. This chapter also explains how to open and run a completed TQ Analyst method from other applications and to move method files from one computer to another.

Creating a new method

Create a new method when you want to use the software's *default settings* as a template for the new method.

Note If you want to create a new method that is based on an existing method, use the Open Method command in the File menu. See “Opening An Existing Method” in the next section for more information. ▲

To create a new method based on the software's template:

Choose New Method from the File menu.

The *method parameters* appear in a new *method window*, which automatically becomes the *active window*. The *method file name* is displayed in the title bar.

A new method must contain valid settings for all of the method parameters before it can be *calibrated* or run. When you create a new method, the method parameters are automatically set to their default values. You can change any of the parameter settings or use the *default*

settings. See the chapters on creating TQ Analyst methods for more information.

As with any software application, be sure to save your work while creating a method or editing the *method parameters*. For information on saving a method, see “Saving a Method” or “Saving a method with a new file name” in this chapter.

Note To create a new method by starting with an existing method, use the Open Method command in the File menu to open the existing method and then choose Save Method As from the File menu to save a copy of the method with a different file name. ▲

Opening an existing method

Open an existing method when you want to view or edit the method or use it as a template for a new method. Many methods can be open at the same time.

To open an existing method:

- 1. Choose the Open Method command from the File menu.**

The Open Method dialog box is displayed.

- 1. Select the file you want to open from the list of available files.**

If the method you want to open is not listed, select a different drive or directory from the Drives or Directories list boxes or select another file format. When you select a *file name*, the method’s title appears in the Method Title box.

TQ Analyst allows you to open methods that were saved using TQ Analyst (*.QNT) or methods that are in another Thermo Scientific format, or “file type,” such as QuantIR or Quick IR® +

(Basic Quant). Both of these applications use the *.QMT and *.MSR extensions. To list all the files in the indicated directory, select All Files (*.*) from the Files of Type list box.

Note If you want to open a QuantIR or Quick IR method from TQ Analyst, make sure the method file and its associated spectral data files are stored in the same directory before using the Open Method command. ▲

2. Choose OK.

3. If a dialog box appears prompting you to enter a password, type the correct password for the selected method and then choose OK.

The person who created the method and set up the password requirement determines what password must be used to reopen the method.

The *method parameters* appear in a new *method window*, which automatically becomes the *active window*. The *method file name* is displayed in the title bar.

Note If you open a QuantIR or Quick IR[®]+ method from TQ Analyst, you must use the Save Method As command in the TQ Analyst File menu and enter a new file name in order to save the method on a disk. The original QuantIR or Quick IR+ method will not be changed. ▲

Saving a method

It is always a good practice to save your work while creating a method or editing the *method parameters*.

Note If you want to change the *file name* of the method, use the Save Method As command. ▲

To save a method:

1. Select the method you want to save by clicking the method window or choosing the method name in the Window menu.

2. Choose Save Method from the File menu.

If the method you are saving has a file name, the method is saved on the selected disk using the same name.

If the method does not have a file name, the Save Method As dialog box appears.

3. If the Save Method As dialog box appears, type a file name and select the directory and disk where you want the method saved.

4. When you are finished, choose OK.

The *readout* for Last Saved Date on the Description tab will be updated to show the current date and time. The Revision readout will be incremented by one.

Saving a method with a new file name

Save the current method with a new *file name* when you want to modify it but also want to keep a copy of the original method.

Three files are created when you save a method with a new file name. All three files will use the base file name you enter. The method specifications are saved in the file “methodfilename.QNT.” The spectral data for the *standards* specified in the method are stored in two associated library files (“methodfilename.LBD” and “methodfilename.LBT”).

To save a method with a new file name:

- 1. Select the method you want to save by clicking the method window or choosing the method name in the Window menu.**

- 2. Choose the Save Method As command from the File menu.**

The Save Method As dialog box appears.

- 3. Type a file name for the method in the File Name text box.**

The software automatically adds the *extension* “.QNT” to the end of the file name you enter.

- 4. Select the directory and disk where you want the method saved.**

- 5. Choose OK.**

Saving a method with a minimum file size

If you run any diagnostic routines on a method after the method is *calibrated* and then save the method on a disk, the diagnostic results will be saved along with the *method parameters* and calibration results. To save a method with a minimum file size, save the method immediately after you calibrate it.

Some of the *sample checking* features also increase the size of the method file. If you don't plan to use the spectrum checks, such as the *full spectrum check* or the *measurement region spectrum check*, make sure the *check boxes* for these features are off (see Report tab). The check box for Fit Value (see Report tab) should also be off unless you want the *full spectrum fit values* or the *measurement region fit values* reported with the *analysis results*. See the chapter titled "Preparing a Method For Sample Analysis" for more information on sample checking and fit values.

Opening spectra

Use the Open Spectrum command in the File menu to retrieve one or more spectra or a group of spectra stored in spectral data files on a disk. When you open a *spectrum*, TQ Analyst displays the spectrum in the active *spectral window* or in a new spectral window if no spectral window currently exists.

Note Open Spectrum only displays the data in the selected spectral file. If you want to open a spectrum and add the spectrum to the Standards table, use the Open Standard button on the Standards tab instead of the Open Spectrum command. ▲

You can use Open Spectrum to open spectra that were saved using any Thermo Scientific software or spectra that are in another format, or "file type," such as GRAMS386 and CSV. The *file name extensions* for the common file types are shown in the following table.

<i>File Type</i>	<i>Extension</i>
Spectra	.SPA
Group of spectra	.SPG
JCAMP-DX	.JDX
PCIR	.IRD, .IFG
Nicolet SX/DX	.SPC, .NIC
Comma-separated text	.CSV
Perkin-Elmer	.SP
Mattson	.IGM, .ABS, .DRT, .SBM, .RAS
Spectacle	.IRS, .SDA, .UVD
GRAMS386	.SPC, .GLD

*Nicolet SX and DX files both use the Nicolet SX/DX file type.

The available file types are shown in a list box in the Open dialog box.

Note You can list all the files in the indicated directory by selecting All Files (*.*) from the Files of Type list box in the Open dialog box. ▲

If you want to import spectra collected in another spectroscopic analysis program that we do not support, use that program to save the spectra in one of the formats listed above before attempting to import them into TQ Analyst.

Saving a spectrum

If you want to save a *spectrum* you collected in TQ Analyst as a Thermo Scientific spectral data file, use the View Standards button on the Standards tab to display the spectrum in a *spectral window*. Then select the spectrum you want to save and use the Save Spectrum As command in the TQ Analyst File menu to save the selected spectrum. The spectrum will be saved as a Thermo Scientific spectral file (*.SPA extension). When two or more spectra are selected in the spectral window, they will be saved as a Thermo Scientific spectral group (*.SPG extension).

You can also save spectra that are displayed in a TQ Analyst *task window*, such as the Region Selection or Corrections task window, by selecting the spectrum or spectra you want to save and then choosing Save Spectrum As from the File menu.

Protecting a method

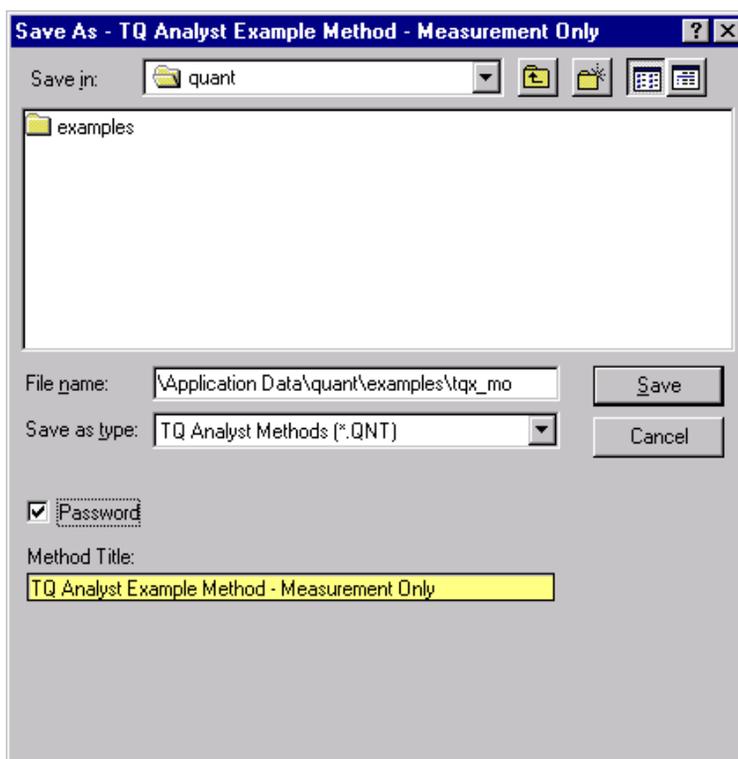
You can activate a security system to keep the *method parameters* and calibration spectra safe from inadvertent or deliberate changes. A protected method can be opened in TQ Analyst only when the correct password is entered. You don't need to enter a password to analyze a *sample* with a protected method, whether you are running the method from TQ Analyst or from another Thermo Scientific application, such as OMNIC or RESULT.

To protect a method:

- 1. Select the method you want to protect by clicking the method window or choosing the method name in the Window menu.**
- 2. Choose Save Method As from the File menu.**

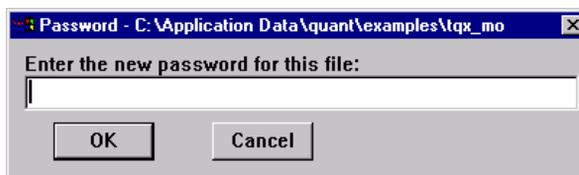
The Save Method As dialog box appears.

3. Turn on the Password check box in the Save Method As dialog box.



4. Choose OK.

A dialog box appears prompting your to enter a password.



5. Type a password for this method and choose OK.

Another dialog box appears prompting you to confirm your password.

6. Type exactly the same password a second time and choose OK.

Notice

Store your passwords in a safe place! Once you close a protected method, you won't be able to reopen or delete it without the correct password. ▲

Turning off password protection

You can turn off password protection by resaving the method with the "Password" *check box* off. You must know the correct password in order to turn off password protection.

To turn off password protection for a method:

1. Choose Open Method from the File menu.

The Open Method dialog box is displayed.

2. Select the file name of the method you want to change and choose OK.

A dialog box appears prompting you to enter the password for the selected method.

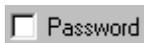
3. Enter the correct password and choose OK.

The method is opened in a new *method window*.

4. Choose Save Method As from the File menu.

The Save Method As dialog box appears.

5. Turn off the Password check box.



6. Choose OK.

Exporting data to a text file

Use the Export to Text File command in the File menu to copy the contents of a table that is displayed in the *active window* to a spreadsheet or text processing application. All of the data that can be displayed in the selected table will be exported. For example, if a *diagnostic task window* displays data for one *component* at a time, the diagnostic results for all components will be exported.

The data are displayed in an application window. If TQ Analyst locates Microsoft® Excel® on your computer, the data are displayed in an Excel application window. If the Excel application is not found, the data appear in a window for a text processing application, such as NotePad or Write. The results are formatted using the settings for the report parameters (see the Report tab) in the current method.

You can save the displayed information in a spreadsheet file (*.XLS extension) or text file (*.TXT extension) or copy portions of the displayed data or the entire contents of the file to the Clipboard. The contents of the Clipboard can then be pasted into a word processing or other application used for generating reports.

Exporting standards to a text file

Use the Standards to Text File command in the File menu to copy the concentration and spectral data for all of the *standards* in the current method to a spreadsheet or text file as comma-separated variables (.CSV file name extension).

When you choose Standards to Text File, the Save As dialog box is displayed showing the default file name for the CSV file (TQDATA.CSV). You can enter a unique name for the file (recommended) and specify a different location or leave the default file name and location. When you are finished, choose Save.

Note If you leave the default file name and *extension*, the software will overwrite your data the next time you save information using the Standards to Text File command. ▲

Use Explorer or My Computer to locate and open the *.CSV file. If TQ Analyst locates Microsoft® Excel® on your computer, the data are displayed in an Excel application window. If the Excel application is not found, the data appear in a window for a text processing application, such as NotePad or Write.

The data are saved in the following format:

Concentration data

	(Std1)	(Std2)	(Std3)	(Std4)	(Std5)	(Std6)
	A	B	C	D	E	F
0 (Usage)	0	0	1	1	1	1
1 (comp1)	82	65	62	59	44	33
2 (comp2)	10	19	20	21.5	28	32.5
3 (comp3)	101	120	145	171	194	225

Spectral data

4070.03 (Freq1)	-.046	-.025	0.068	0.083	0.001	0.024
4071.96 (Freq2)	0.037	0.001	0.002	0.009	0.004	0.02

Row 1 of the concentration data shows how the TQ Analyst Usage parameter is set for each standard, where 0 = calibration, 1 = validation, 2 = correction, and 3 = ignore. The concentration data for each component in each standard are provided next.

The spectral information for the standards appears after the concentration data. Row 1 of the spectral data gives the X-axis location (*frequency*) of the first measurement and the Y-axis (intensity) value at that location for each standard. Rows 2 through the end of the spectral data show the frequency and intensity values for any additional measurements.

Setting up the printer

Use the Printer Setup command in the File menu to specify a printer and set the print parameters before you print images or information on paper.

To set up the printer:

1. Choose Printer Setup from the File menu.

The Printer Setup dialog box appears. This is a standard Windows dialog box. For more information on using this type of dialog box, see your Windows documentation.

2. Select the printer you want to use from the list box.

You can also specify portrait or landscape page orientation.

Note The page orientation setting in Windows Control Panel or in the printer setup dialog box for other applications is not affected by the setting you make here. ▲

- 3. If you want to set the printer parameters, click the Options button in the Printer Setup dialog box. If you don't want to set the parameters, choose OK.**

If you choose Options, a dialog box appears showing the current settings of the printer parameters. This is a standard Windows dialog box and varies in appearance depending on your printer. See the manuals that came with your Windows software and printer for complete information on setting the printer parameters. After you set the printer parameters, choose OK to close the dialog box, and then choose OK to close the Print Setup dialog box.

Printing a method

Print a method when you want a paper copy of the *method parameter* settings, table entries and calibration data. You can also print the contents of a *spectral window* or the selected *task window*, such as the Region Selection or Corrections window.

To print a method:

- 1. Select the window you want to print.**

This can be a *method window*, a task window or a spectral window.

- 2. Make sure the printer is turned on and ready.**

- 3. Choose the Print command from the File menu.**

A Print dialog box appears allowing you to specify items such as the number of components to print. If you are printing a task window, you can also select which items in the window to print. Set the parameters as desired and then choose OK.

If a *method window* was selected, a summary of the method is printed. If a task window was selected, the items you selected in the Print dialog box will be printed.

Analyzing a sample from TQ Analyst

You can analyze an *unknown sample spectrum* using any TQ Analyst method without quitting the TQ Analyst software. The method must be *calibrated* before it can be used to analyze a sample spectrum.

The sample spectrum must be stored in a spectral data file before it can be analyzed. A large number of Thermo Scientific and other file formats are accepted.

The file name *extensions* for the common file types that can be selected for analysis are shown in the following table.

<i>File Type</i>	<i>Extension</i>
Spectra	.SPA
Group of spectra	.SPG
JCAMP-DX	.JDX
PCIR	.IRD, .IFG
*Nicolet SX/DX	.SPC, .NIC
Comma-separated text	.CSV
Perkin-Elmer	.SP
Mattson	.IGM, .ABS, .DRT, .SBM, .RAS
Spectacle	.IRS, .SDA, .UVD
GRAMS386	.SPC, .GLD

*Nicolet SX and DX files both use the Nicolet SX/DX file type.

Note If you want to analyze multiple samples from TQ Analyst and send the results to a spreadsheet or text file, use the Multiple Quantify command in the Diagnostics menu. See the next section for more information. ▲

To analyze a sample from TQ Analyst:

- 1. Select the method you want to run by clicking the method window or choosing the method name in the Window menu.**
- 2. Click the Quantify button on the TQ Analyst toolbar.**

The Open Spectrum dialog box is displayed.

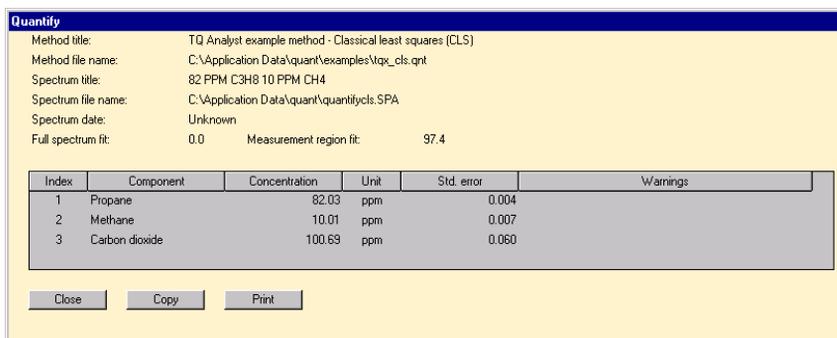
- 3. Select a spectral data file and choose OK.**

You can select spectra that were saved using any Thermo Scientific software or spectra that are in another format, or “file type,” such as GRAMS386 and CSV. Use the Files of Type list box to select a specific file type, or choose “All Files” to see all the files in the current directory.

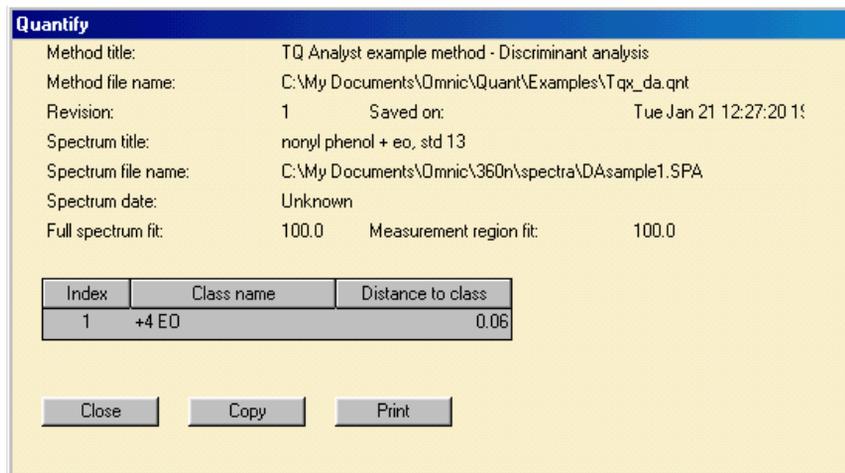
Note If a warning message about mismatched *parameters* appears when you select a *spectrum* to *quantify*, the parameter settings used to collect the sample spectrum are significantly different from the settings that are saved with the current method. For optimum performance, all *standards* and samples should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the spectrum again using the proper settings for the collection parameters, edit the settings in the current method or ignore the warning and continue analyzing samples. ▲

TQ Analyst begins analyzing the spectrum you selected using the current method. Depending on how the *method parameters* are set, you may be prompted to enter needed information, such as a pathlength value. Follow the instructions that appear on the screen.

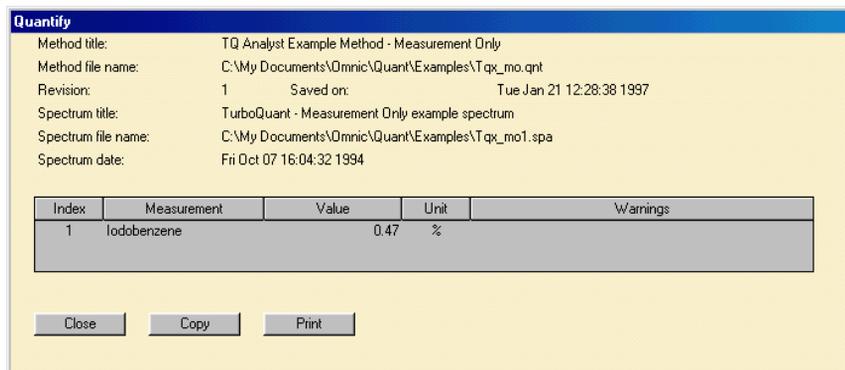
When the analysis is finished, a dialog box appears showing the results. If the current method is for *quantitative analysis* (see the Analysis Type parameter on the Description tab), the results will indicate the concentrations of the specified components in each sample.



If the method is designed to *classify* the unknown sample spectrum based on a set of known spectra, the names and *match values* of the reported spectra will be provided.



A *peak height* or peak area may be reported if the method is set up to make simple measurements of the spectra that are quantified.



Depending on how the parameters on the Report tab are set, the results may contain additional information, such as the titles and file names of the method and the spectra that are quantified.

If a problem occurred during the analysis, a warning may be printed with the *analysis results*. The parameters that produce the warnings are also included on the Report tab. Press the *Explain*

button when the Report tab is displayed for instructions on interpreting warnings.

Note You can print the contents of the Quantify dialog box by clicking the Print button in the dialog box. ▲

- 4. Click the arrows on the scroll bar or move the scroll box to bring each portion of the table of results into view.**
- 5. When you are finished viewing the analysis results, choose OK to close the dialog box.**

Analyzing multiple samples from TQ Analyst

If you want to analyze a group of *samples* from TQ Analyst, use the Multiple Quantify command from the Diagnostics menu. When you use Multiple Quantify, the *analysis results* are placed in a spreadsheet (Microsoft® Excel®) or text file.

You can run Multiple Quantify on any TQ Analyst method. The method must be *calibrated* before Multiple Quantify can be used.

You can select one or more spectral data files to be quantified or select an input file that lists the file names of the spectra to be quantified. A large number of Thermo Scientific and other file formats are accepted. The file name *extensions* for the common file types that can be analyzed using Multiple Quantify are shown in the following table.

<i>File Type</i>	<i>Extension</i>
Spectra	.SPA
Group of spectra	.SPG
JCAMP-DX	.JDX
PCIR	.IRD, .IFG
Nicolet SX/DX	.SPC, .NIC
Comma-separated text	.CSV
Perkin-Elmer	.SP
Mattson	.IGM, .ABS, .DRT, .SBM, .RAS
Spectacle	.IRS, .SDA, .UVD
GRAMS386	.SPC, .GLD

*Nicolet SX and DX files both use the Nicolet SX/DX file type.

To analyze multiple samples from TQ Analyst:

- 1. Select the method you want to run by clicking the method window or choosing the method name in the Window menu.**
- 2. Choose Multiple Quantify from the Diagnostics menu.**

The Open dialog box is displayed.

- 3. Select the spectral data files you want to quantify or select an input file.**

You can select spectra that were saved using any Thermo Scientific software or spectra that are in another format, or “file type,” such as GRAMS386 and CSV. Use the Files of Type list box to select a specific file type, or choose “All Files” to see all the files in the current directory. You may need to change directories or drives to locate the files you want to analyze.

- To select one or more spectral files to be quantified, set the Files of Type list box to “.SPA.” Then select the spectral data files you want to *quantify* and choose OK. To select more than one file, select the first file you want to include and then hold down the Control key while you click any additional file names.
- If you want to select a spectral group file, set the Files of Type list box to “.SPG.” Then select the spectral group file you want to quantify and choose OK.
- If you want to quantify a series of spectral files that are listed in a text file, change the Files of Type list box to “TXT.” Then select the text file that contains the list of file names.

Note The input file must be formatted properly for use with Multiply Quantify. For information on formatting the input file, refer to the Method Diagnostics chapter in this document. ▲

4. Click OK to begin the analysis.

Depending on how the *method parameters* are set, you may be prompted to enter needed information, such as a pathlength value. Follow the instructions that appear on the screen. (If you are quantifying spectral files that are listed in an input file and the file includes any additional information that is required for the analysis, these prompts will not appear.)

When the analysis is finished, the results are displayed in an application window. If TQ Analyst locates the Microsoft® Excel® spreadsheet application on your computer, the data are displayed in an Excel application window. If Excel is not found, the data appear in a window for a text processing application, such as NotePad or Write. The results are formatted using the settings for the report parameters (see the Report tab) in the current method.

If you close the result window, the data will be saved in the file QUANTIFY.TXT (or QUANTIFY.XLS).

Notice The contents of the file QUANTIFY.TXT (or QUANTIFY.XLS) will be overwritten the next time you use Multiple Quantify. If you want to keep your *analysis results*, use the Save As command to save them with a new file name. ▲

Running TQ Analyst methods from other applications

TQ Analyst is designed to work hand-in-hand with your Thermo Scientific spectral analysis software, such as OMNIC®, ECO®, Integra®, and RESULT™. When you are ready to use a TQ Analyst method for repeated *sample* analysis, review the procedures described here. If you need help, look in the documentation that came with your Thermo Scientific spectral analysis software for more information.

Running a method from OMNIC

OMNIC is Thermo Scientific general-purpose software package for collecting and analyzing spectral data. You can use the commands in the OMNIC Analyze menu to quickly open and run a TQ Analyst method. See the procedure below for specific instructions.

Note If you need help using an OMNIC command, see your OMNIC on-line help. ▲

To run a TQ Analyst method from OMNIC:

- 1. Start the OMNIC application.**
- 2. Choose Quant Setup from the OMNIC Analyze menu.**

The Open Method dialog box appears on the display.

3. Select a TQ Analyst method file and choose OK.

When you select a method, the name of the application that was used to create the method appears in the Origin box. The title of the selected method appears in the Method Title box. TQ Analyst method files have a “.QNT” extension.

4. If you need to collect the spectrum to be quantified, turn on the Collect and Bench check boxes in the Parameter group.

When these check boxes are on, OMNIC resets the parameters on the Collect and Bench tabs in the OMNIC Experiment Setup dialog box to match the settings in the selected TQ Analyst method. This ensures that the spectra of the unknown *samples* will be collected using the parameter settings that were used to collect the method’s calibration spectra.

Note If you turn on the Collect and Bench *check boxes* in the Parameter group, TQ Analyst will overwrite the Collect and Bench parameter settings in your OMNIC software. Leave the Collect and Bench *check boxes* off if you want to use the settings in your OMNIC software to collect the *spectrum* you want to *quantify*. ▲

5. Click OK to close the Open Method dialog box.

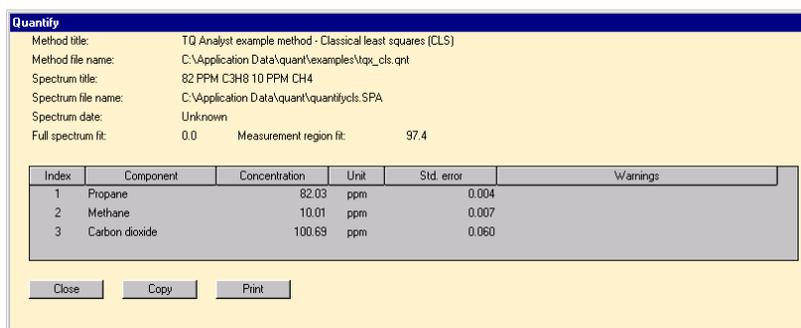
6. Use the commands in the OMNIC Collect menu to collect a sample spectrum or open a spectral data file that is stored on a disk.

7. Select the displayed spectrum.

8. Choose Quantify from the OMNIC Analyze menu.

The spectrum is then analyzed using the selected method. Depending on how the *method parameters* are set, you may be prompted to enter needed information, such as a pathlength value. Follow the instructions that appear on the screen.

When the analysis is finished, a dialog box appears showing the results. If the current method is for *quantitative analysis*, the results will indicate the concentrations of the specified components in each sample as shown in the example below.



The quantify dialog boxes that show up in OMNIC are the same as the TQ Analyst quantify dialogs. See the section titled “Analyzing a Sample From TQ Analyst” in this chapter for more examples of the quantify dialog boxes.

Note You can print the contents of the Quantify dialog box by clicking the Print button in the dialog box. ▲

- 9. Click the arrows on the scroll bar or move the scroll box to bring each portion of the table of results into view.**
- 10. When you are finished viewing the analysis results, choose OK to close the dialog box.**

See your OMNIC documentation if you need more information about running quantitative or other methods from OMNIC.

Running methods from other Thermo Scientific applications

We sell application-specific software, such as ECO, Integra and RESULT, that are fully compatible with TQ Analyst. The procedure for running a TQ Analyst method is different for each application. See the documentation that came with your application-specific spectral analysis software for details on analyzing *samples* using a TQ Analyst method.

Moving method files to another computer

TQ Analyst method files can be moved from one computer to another and then run without recalibration. This enables you to develop methods on one computer and run the methods on one or several other computers. Depending on your requirements for calibration transfer, the procedure may range from simply copying the proper files to rigorous testing and *validation*.

This section describes how to move a method by copying the appropriate method file and moving it to the new computer. In some cases, you will also need to copy the method's *standards library* to the new computer.

To move a method in order to edit or run the method in the new location, copy the files listed below to the new computer. The files can be placed in any subdirectory.

- The TQ Analyst method file (.QNT extension).
- The standards library that is associated with the method. The standards library is contained in two files which have the same base name as the method (methodfilename.LBD and methodfilename.LBT). The library files must be placed in the same directory as their associated method.

Note *Spectral measurement methods* do not have a standards library. To transfer these methods, you need to copy only the method (.QNT) file. ▲

To move a method in order to run (but not edit) the method in the new location, copy the files listed below to the new computer. The files can be placed in any subdirectory. In most cases, these minimum requirements will allow you to run the method on the new computer but not edit the method or update its calibration files.

- The TQ Analyst method file (.QNT extension).
- If you are moving a Search Standards or QC Compare search method, the library files that are associated with the method (.LBD and .LBT extensions) must also be copied onto the new computer. The library files are also required if you are moving a discriminant analysis method and you want to calculate fit values or use the *sample checking* features during sample analysis (see the Report tab). The library files must be placed in the same directory as their associated method.

Note If you move a Search Standards or QC Compare search method to another computer, you will be able to run and edit the method in the new location. There is no way to protect these methods from changes because the entire standards library is required in order to run the method. ▲

5 Managing TQ Analyst Windows

The TQ Analyst Window menu commands and *toolbar* can be used to manipulate *TQ Analyst windows*, such as method and *spectral windows*. This chapter describes how to use these tools to create, display, select, rearrange and close TQ Analyst windows.

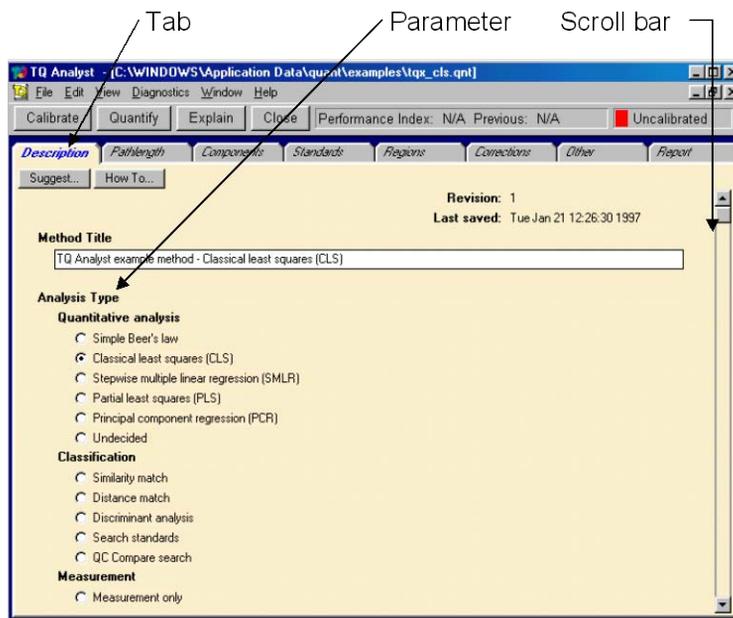
The main portion of the TQ Analyst window may be used to display *method windows*, *task windows*, and *spectral windows*. Many windows can be displayed on the screen at once. The exact number depends on the amount of memory in your computer.

You can maximize, minimize and resize a method window, task window, or spectral window just as you can the TQ Analyst window.

Method windows

A method window contains all of the *parameters* and functions needed to create a TQ Analyst method. The *method parameters* specify how the method will work.

To see descriptions of the parameters in the active method window, click the Explain button on the toolbar to open the Explain help window. Then click the tab that contains the parameter and click the parameter name.



Method Window

Method parameters are grouped by function. Each group of parameters is contained on a file card which has a *tab* that is always visible at the top of the method window. To display a group of parameters, click the corresponding tab. The file card containing the parameters is brought to the front.

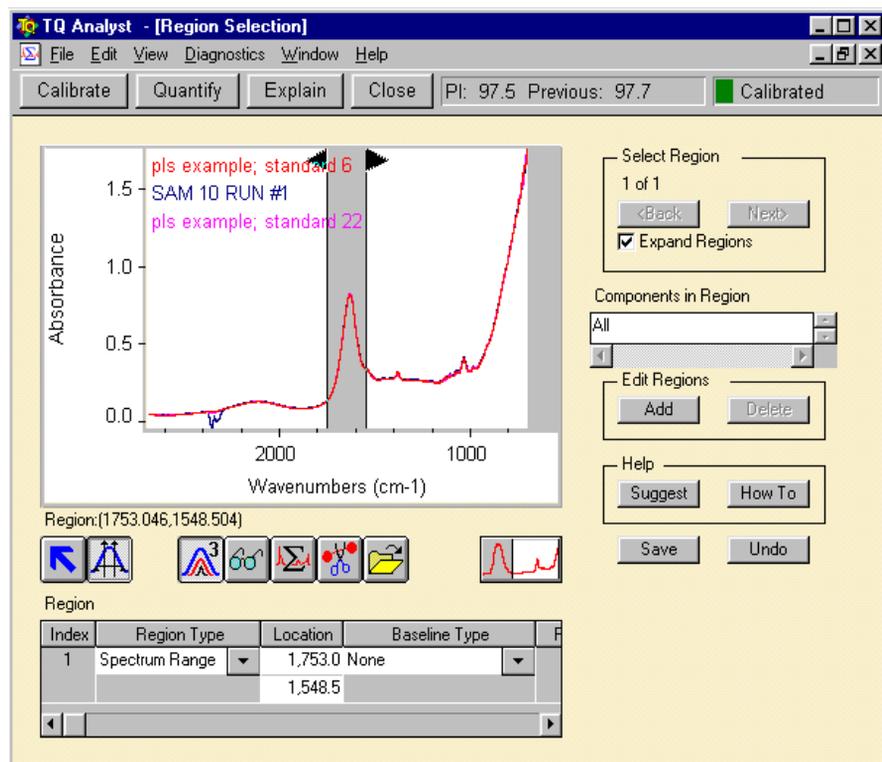
Use the *scroll bar* at the right edge of the method window to scroll additional parameters into view.

Task windows

Special *task windows* are available on several of the method tabs and all of the diagnostic displays. The task windows often contain tables and plots of spectral and method data to help you make a selection or complete a procedure. For example, the Region Selection task window shown in the illustration below allows you to choose the limits and *baseline* of the *analysis regions* for a method and to specify how the region and baseline will be measured.

To see descriptions of the features in a task window, click the Explain button on the toolbar to open the Explain help window and then click the feature.

For step-by-step instructions on completing the task (selecting regions, for example), click the How To button in the task window.



Task Window

You can save spectra that are displayed in a task window, such as the Region Selection or Corrections task window, by selecting the *spectrum* or spectra you want to save and then choosing Save Spectrum As from the File menu. See “Saving a Spectrum” in the chapter on File Management for more information.

If the task window contains a table of data, you can use the Export to Text File command in the File menu to copy the contents of the table to a spreadsheet or text processing application. All of the data that can be displayed in the selected table will be exported. For example, if a *diagnostic task window* displays data for one component at a time, the diagnostic results for all components will be exported. See “Exporting Data to a Text File” in the chapter on File Management for more information.

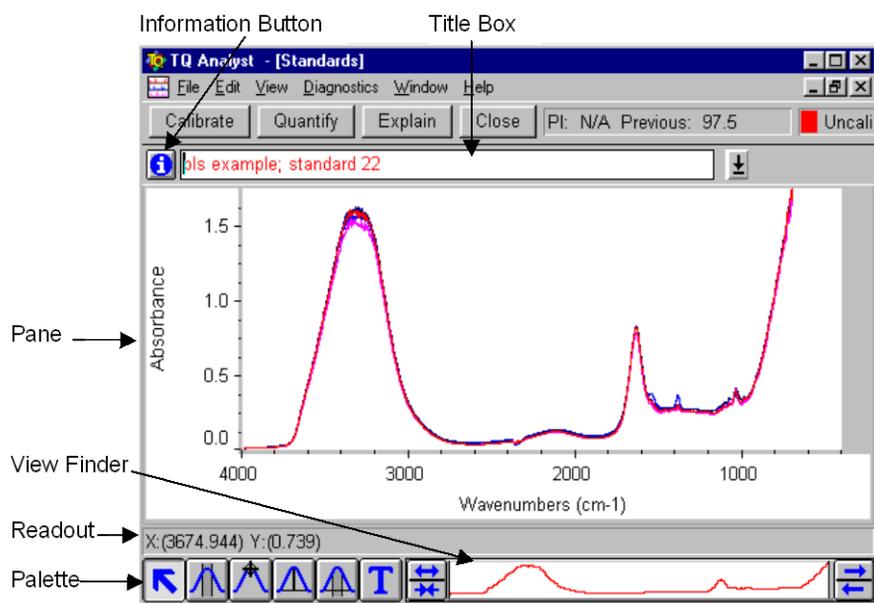
Spectral windows

TQ Analyst allows you to open and display spectral data files and work with displayed spectra. A *spectral window* is opened automatically when you open a *spectrum* or display the selected *standards* in your method. The illustration below shows the spectral window with several spectra displayed.

Use the commands in the View menu to rearrange the displayed spectra. The View menu becomes active whenever a spectral window is displayed.

For information on a command in the View menu, click the menu name and then use the up or down arrow key on the keyboard to select a command in the open menu.

Press the F1 key on the keyboard to display help information on the selected command. When you are finished reading the Help information, close the Help window by choosing Exit from the File menu.



Spectral Window

Spectral windows let you view several kinds of spectral data, such as spectra you have collected or processed or the results from a diagnostic analysis. The previous illustration shows a spectral window containing several spectra and identifies the main parts of the window. Each of these items is explained briefly in the sections that follow.

You can use the mouse to select a displayed spectrum and any of the commands in the View menu to rearrange the spectra in the window. When a spectrum is selected, it is displayed in red in the spectral window.

The View menu becomes active whenever a spectral window is displayed. For information on a command in the View menu, click the *menu name* and then use the up or down arrow key on the keyboard to select a command in the open menu. Press the F1 key on the keyboard to display help information on the selected command. When you are finished reading Help information, close the Help window by choosing Exit from the File menu.

You can customize the way TQ Analyst displays spectral data by setting the View and Window options. Use the Options command in the Edit menu to display the Options dialog box. Click the General tab and then click the General Options button. Click the View tab to see the View options or click the Window tab to see the Window options.

Title box



The title box near the top of a *spectral window* shows the title of the *spectrum* that was last selected or that is currently selected. If two spectra are selected, the title in the title box changes to "Two spectra selected." If more than two spectra are selected, "Multiple spectra selected" appears in the title box.

You can use the title box to perform the following operations.

- Display a list of *spectrum titles*.
- Select listed spectra.
- Deselect a listed spectrum.
- Select a different spectrum using the arrow keys.
- Change the title of the selected spectrum.

To remove the list of spectra, press Tab or Enter on the keyboard or simply click outside the title box. The currently selected spectra remain selected.

Collection and Processing Information



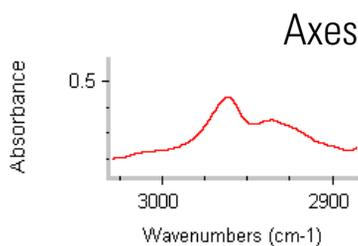
To see information about how the selected *spectrum* was collected and processed, click the Information button (labeled "i") to the left of the title box or double-click the spectrum's title in the title box. To see information about a spectrum that is not selected, first click the arrow to the right of the title box to display the list of *spectrum titles* and then double-click the title of the spectrum. The Collection And Processing Information window appears showing parameter settings and other information about the spectrum.

You can perform the following operations within the Collection And Processing Information window.

- Edit text in the Title, Comments, Custom Info 1 or Custom Info 2 boxes
- Copy information to the Clipboard

- Print information
- Enter custom information to be printed later
If you plan to print the spectrum, you can specify one or two pieces of information to be printed with it by typing the information in the Custom Info 1 and Custom Info 2 text boxes in the Collection And Processing Information window. Show Info Box must be turned on in the Print options (available through Options in the Edit menu) for this information to be printed.

When you are finished using the Collection And Processing Information window, close it by choosing Close. If you don't want to save the changes you made to the information, choose Cancel.



Along the bottom and left side of a *pane* (or panes) in a *spectral window* are the X-axis and Y-axis. The scale and display limits of the X-axis depend on the *spectral region* you are displaying. If you display a different region by using the view finder or selection tool, the X-axis is automatically adjusted for the new region.

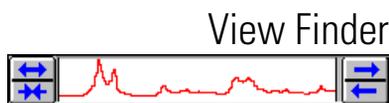
For FT-IR applications in the mid-infrared range, the most commonly used X-axis units are the *wavenumber* (cm^{-1}) and the *micrometer* (μm). Near-IR data are typically displayed in wavenumber or *wavelength*. You can specify the unit by using Display Setup in the View menu.

Note The scale of the X-axis is usually uniform across the *spectrum*. Sometimes, however, it is easier to see important *peaks* if a different X-axis scale is used for different regions. Use Display Setup in the View menu to split the X-axis into two or three sections, each with a different scale. ▲

The unit used for the Y-axis depends on the final format used when the spectrum was collected and whether you have converted the spectrum to another format. You can set the initial Y-axis unit for collected

spectra by setting Final Format in the collection parameters (click the Collection Parameters button on the Standards Tab).

The "Scale" commands and Display Limits in the View menu let you adjust the Y-axis scale.



The view finder lets you adjust the display of all the spectra in a *spectral window* or *task window* to show a larger or smaller *spectral region* or a different region of the same size.

The view finder contains a small image of the entire selected *spectrum*, regardless of which region of the spectrum is currently displayed. If more than one spectrum is selected in a spectral window, an image of the last spectrum selected appears in the view finder. If no spectrum is selected, the view finder is empty.

If more than one spectrum is displayed in a spectral window, the horizontal range of the view finder encompasses the ranges of all the spectra. The currently displayed spectral region is indicated by the region markers, the blue vertical lines within the view finder.

The next few sections provide information on using the view finder to adjust the display.

Expanding or contracting the display

To expand all the spectra horizontally about the center of the *pane*, click the top half of the view finder's Expand/Contract button. To contract the spectra horizontally about the center, click the bottom half of the button. You can press and hold down the mouse button during these operations to continuously expand or contract the display.

Displaying a different spectral region of the same size

There are three ways to use the view finder to display a different *spectral region* of the same size:

- To move your view of the *spectrum* to the right ("roll" to the right), click the top half of the Roll button.
- To roll to the left, click the bottom half of the button. You can press and hold down the mouse button during these operations to roll continuously to the right or left.
- Point anywhere between the region markers, press and hold down the mouse button, drag the markers to the desired location and then release the mouse button.
- Click to the left of the left region marker or to the right of the right region marker. Whenever possible, the new displayed region will be centered on the location you clicked. The markers will move so that the clicked location is halfway between the markers.

Changing the display limits by moving the region markers

Drag a marker in the view finder left or right to the desired location. The current X values of both markers appear in a box above the view finder while you drag the marker.

Displaying the entire spectrum

Double-click between the region markers in the view finder to display the entire spectrum.

Palette

The palette of a *spectral window* contains six tools that allow you to perform the operations described in the table below. The palette is located in the lower-left corner of every spectral window and the windows that appear during data collection. The names and appearance of the palette tools indicate their functions.

<i>To do this..</i>	<i>Use this tool..</i>
Select items on the screen.	 selection tool
Expand or contract a spectrum.	 selection tool
Move an overlaid spectrum up or down within its pane.	 selection tool
Move a stacked spectrum into another pane.	 selection tool
Drag a spectrum into another spectral window.	 selection tool
Select annotations to be deleted.	 selection tool
Select a region of a spectrum or measure the uncorrected area of a region.	 region tool
Display X and Y values of points.	 spectral cursor tool
Measure the height of a peak.	 peak height tool
Measure the area under a peak.	 peak area tool
Label spectral features.	 annotation tool
Add text to be displayed or printed.	 annotation tool
Move sampling information or annotations.	 annotation tool

Only one tool can be used at a time. To use a tool, select it by moving the mouse pointer over the tool and clicking. The selected tool is highlighted on the screen. A tool remains selected until you select another tool.

If you have selected any tool except the selection tool, when you move the pointer into a *pane*, a symbol representing the tool appears next to the pointer arrow to help you identify the tool being used.

When you use a tool, the *readout* above the palette may display information for the tool operation; for example, the X and Y values of the pointer location or the limits of the selected *spectral region*. To display the name of a tool on the palette, point to the tool and wait a moment.

Creating a new method window

A new *method window* is created when you perform the following operations:

- Open a method that is stored on a disk.
- Choose New Method from the TQ Analyst File menu.

To close a method window, click the Close button on the TQ Analyst *toolbar*.

If you make any changes to the parameters in a method window and then close the window, a message will appear asking whether to save the changes. Choose Yes to save the changes or choose No to close the window without saving. If you decide not to close the method window, choose Cancel.

Displaying task windows

To display a *task window* (if one exists) for a method tab, select the *tab* and then click the Edit button below the tab name. You can display the Region Selection task window, for example, by selecting the Regions tab and then clicking the Edit Regions button.

A *diagnostic task window* can be displayed by choosing the diagnostic command from the Diagnostics menu.

To close a task window, click the Close button on the TQ Analyst *toolbar*.

Displaying a spectral window

A *spectral window* is opened automatically when you open a *spectrum* or display the selected *standards* in your method. All of the standards you select for display will appear in the same spectral window.

To close the active spectral window, click the Close button on the TQ Analyst *toolbar*. All of the displayed spectra will be cleared from the spectral window when you close it.

Displaying collect windows

When you collect a *spectrum* in TQ Analyst, the collected data are displayed in the Collect Sample or Collect Background window. These windows operate the same in TQ Analyst as they do in Thermo Scientific general-purpose spectral analysis applications, such as OMNIC. See the documentation that came with your spectral analysis software for details about the collect windows.

About the active window

Only one window can be active at a time. To make a window active, click anywhere within it or choose the window name from the Window menu.

When a window is active, its title bar is displayed in the color you specify using the Windows Control Panel. (See your Windows documentation for information on using Control Panel.)

Note When the active window is maximized, it shares its title with the *TQ Analyst window*. This is how the TQ Analyst window is configured when you open the application. ▲

Selecting a window

The title of every open window is listed at the bottom of the Window menu. You can select one of these windows by choosing its title from the Window menu.

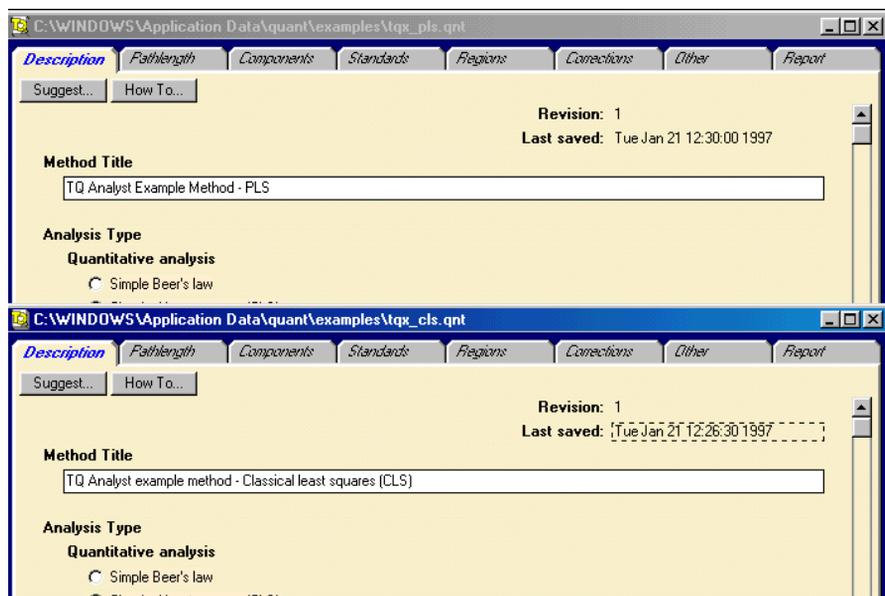
Resizing a window

You can resize the active window by dragging one of its borders or corners or by clicking the Maximize or Restore button.

Note When the active window is maximized, it shares a title bar with the *TQ Analyst window*. This is how the TQ Analyst window is configured when you open the application. ▲

Tiling the open windows

Use the Tile Windows command in the Window menu to resize and rearrange all the displayed windows so that they fit inside the *TQ Analyst window* without overlapping, like floor tiles. (Minimized windows and windows opened using other applications are not tiled.) The next illustration shows the TQ Analyst window with two *method windows* that are tiled.



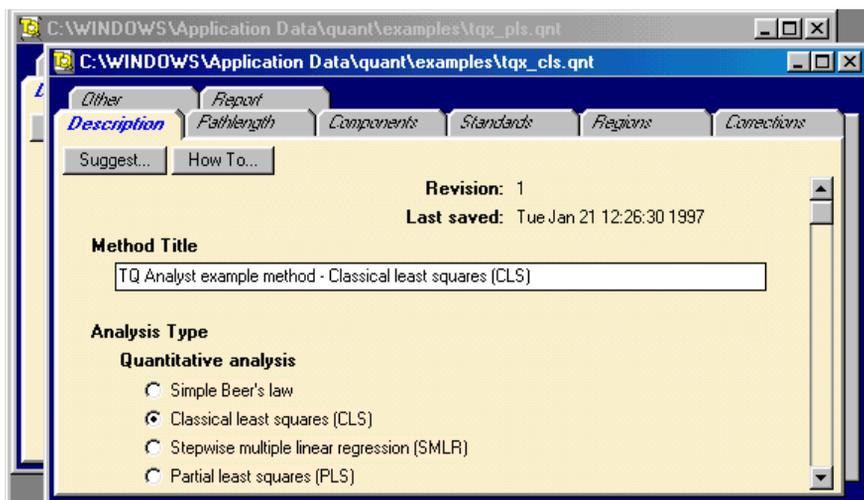
Tiled Method Windows

Tiling windows lets you easily move between windows when there are many windows on the screen.

Note If any windows have been minimized and appear as icons inside the TQ Analyst window, space will be left at the bottom of the TQ Analyst window to display these icons after the other windows are tiled. ▲

Cascading the open windows

Use the Cascade Windows command in the Window menu to resize and layer all the windows and arrange them within the TQ Analyst workspace so that their title bars are visible. The illustration below shows three *method windows* that are cascaded.



Cascaded Method Windows

Note If any windows have been minimized and appear as icons inside the TQ Analyst window, space will be left at the bottom of the TQ Analyst window to display these icons after the other windows are cascaded. ▲

Closing the active window

Use the Close button on the *toolbar* to close the current *method window*, *task window*, or *spectral window*. If you changed anything in the active window without saving your changes, a prompt will appear asking if you want to save the changes. Choose Yes to save the changes you made to the window or choose No to close the window without saving. If you decide to keep the window open, choose Cancel.

6 Working with Standards

You can use the tools on the Standards tab to open, collect and manipulate the spectra of the *standards* in a quantitative or *classification method*. Standards are known *samples* that are used to create the method *calibration model* or classification scheme.

This chapter describes how to use the tools in TQ Analyst to:

- Customize the format of the Standards table.
- Sort the entries in the Standards table.
- Open or collect a spectrum to use as a standard.
- Collect a background spectrum
- Replace the spectrum for a standard.
- Delete a standard.
- Display the spectrum of a standard and work with the displayed spectra.
- Displaying the processed version of a standard
- Choose the standards for a quantitative method.
- Evaluate the standards in a quantitative method.
- Identify and eliminate unnecessary standards in a quantitative method.
- Merge standards that are essentially the same.
- Restrict the Y-axis range.

If you are interested in using TQ Analyst to create methods that only measure spectral features in a series of spectra, you can skip this chapter. Standards are not used in measurement methods. See the

chapter on “Creating a Spectral Measurement Method” in this document for more information.

Standards tab

All of the software functions that deal with selecting, evaluating, measuring and displaying the *standards* that will be used in a TQ Analyst method are provided on the Standards tab. To display these features, click the *tab* labeled “Standards” in the *TQ Analyst window*.

Standards Tab

Index	Display	Spectrum Title	Usage	Glucose Wt %	BA Wt %	Pro Wt %	Glycerol Wt %
1		pls example; standard 1	Calibration	1.94	0.99	1.92	2.17
2		pls example; standard 2	Calibration	1.26	0.50	4.01	2.47
3		pls example; standard 3	Validation	2.59	0.57	1.49	3.04
4		pls example; standard 4	Calibration	0.36	2.01	2.69	1.41
5		pls example; standard 5	Validation	1.55	0.77	2.54	2.79
6		pls example; standard 6	Calibration	1.13	1.12	4.14	0.86

TQ Analyst Window

Standards table

The Standards table displays all of the *standards* in the current method and shows how they are used in the method. If the method has no standards, the Standards table will be blank.

Index	Display	Spectrum Title	Usage	Glucose Wt %	BA Wt %	Pro Wt %	Glycerol Wt %
2		pls example; standard 2	Calibration	1.26	0.50	4.01	2.47
3		pls example; standard 3	Validation	2.59	0.57	1.49	3.04
4		pls example; standard 4	Calibration	0.36	2.01	2.69	1.41
5		pls example; standard 5	Validation	1.55	0.77	2.54	2.79

The *spectrum title* should contain unique, identifying information that allows you to match the *spectrum* with the material that was measured.

The Usage column shows how each standard is used in the method. The options presented in the Usage drop-down list box depend on the current setting for Analysis Type (Description tab). For example, if Analysis Type is set to a quantitative technique, Usage can be set to Calibration, Validation, Correction, or Ignore. *Calibration standards* are used to create the method *calibration model* and *correction curve* if one is specified. The software uses *validation standards* to calculate the *performance index* after the method is *calibrated*. *Correction standards* are used along with the calibration standards to create a correction curve, if one is specified. Standards that have their Usage set to “Ignore” are excluded from the method.

If you are creating a Discriminant Analysis or QC Compare search method, the Usage can be set to Calibration, Validation, or Ignore. Similarity Match and Search Standards methods can have the Usage set to Calibration or Ignore.

If you are creating a quantitative method, the component columns show the concentration of each component in each standard. The Class column in a *classification method* shows the name of the group (class) that each standard is assigned to.

Customizing the format of the Standards table

You can specify whether the *standards* that are listed in the Standards table will be identified by the file names or the titles of the corresponding spectra or both.

To specify that the file names of the spectra will be listed in the Standards table, click the *check box* for Show Spectrum File Names on the Standards tab. If you want to see the titles of the spectra, click the *check box* for Show Spectrum Titles. When one or both of these check boxes are on, columns are added to the Standards table showing the file names and/or titles of the spectra for the standards.

Note File names will only be displayed for spectra that were opened using the Open Standard button on the Standards tab. Spectra that are collected in TQ Analyst are saved automatically in the *standards library* that is associated with the current method. As a result, these standards do not have individual file names. In addition, standards that are opened from a spectral group (.SPG) file will not save file names. ▲

Sorting the entries in the Standards table

Use the Sort Standards button to rearrange the entries in the Standards table. You can sort the entries based on *index number*, file name, *spectrum title*, class, usage, or the concentration of a measured *component*.

To sort the entries in the Standards table, click once on the column heading for the property you want the sort to be based on and then click the Sort Standards button at the top of the Standards tab. For example, to sort based on class, click the column heading labeled “Class” and then click Sort Standards.

If you are viewing a quantitative method, you can sort by index number, file name, spectrum title, component concentration, and usage (calibration, validation, correction, or ignore). *Classification methods* can be sorted by index number, file name, spectrum title, usage, and class.

When the entries are sorted by index number, the software arranges the *standards* in the order they were added to the method. Sorting by file name or title places the entries in alphabetical order. Sorting by usage places *calibration standards* first, followed by *validation standards* and then *correction standards*. Standards that are designated “Ignore” are placed last. Sorting the standards by component concentration places the standard with the lowest concentration of the selected component at the top of the list and the standard with the highest concentration at the bottom of the list. Sorting by *class* places the entries in alphabetical order by class name.

Opening a spectrum to use as a standard

You can open spectra that are stored on a disk and add them to the Standards table for the current method or replace an existing *standard*. This can be accomplished in a single step by using the Open Standard button on the Standards tab.

Note If you simply want to open a *spectrum* and display it in a *spectral window*, use the Open Spectrum command in the File menu rather than the Open Standard button on the Standards tab. ▲

If you use stored spectra as standards, make sure all of the spectra were collected using the same settings for the *data collection parameters*. You should also use these same parameter settings to collect the spectra you want to *quantify* with this method. Using consistent parameter settings helps minimize differences in the spectra which are due to variations in *sampling technique* rather than to differences in sample composition and will improve the accuracy of your analysis.

You can use Open Standard to open spectra that were saved in the standard Thermo Scientific format (*.SPA and *.SPG), or spectra that are in another format, or "file type," such as GRAMS386 or JCAMP-DX. The file name *extensions* for the commonly used file types are shown in the following table.

<i>File Type</i>	<i>Extension</i>
Spectra	.SPA
Group of spectra	.SPG
JCAMP-DX	.JDX
PCIR	.IRD, .IFG
Nicolet SX/DX	.SPC, .NIC
Comma-separated text	.CSV
Perkin-Elmer	.SP
Mattson	.IGM, .ABS, .DRT, .SBM, .RAS
Spectacle	.IRS, .SDA, .UVD
GRAMS386	.SPC, .GLD

*Nicolet SX and DX files both use the Nicolet SX/DX file type.

If you want to import spectra collected in another spectroscopic analysis program that we don't support, use that program to save the spectra in one of the formats listed above before attempting to import them into TQ Analyst.

The available file types are shown in a drop-down list box in the Open dialog box.

Note You can list all the files in the indicated directory by selecting All Files (*.*) from the Files of Type drop-down list box. ▲

To open a spectrum to use as a standard:

1. Select a row in the Standards table.

To add a standard, click anywhere in the open row at the bottom of the Standards table. If you want to replace a standard, click the row that contains the standard you want to replace.

2. Click the Open Standard button on the Standards tab.

The Open dialog box is displayed.

3. Select the spectral data file(s) you want to open from the list of available files.

If the spectrum you want to open is not listed, select a different drive or directory from the Drives or Directories list boxes or select another file format. When you select a file name, the spectrum's title appears in the Title box and a condensed version of the spectrum itself appears below the title.

TQ Analyst allows you to open spectra that were saved using TQ Analyst or spectra that are in another format, or "file type," such as GRAMS386 or JCAMP-DX. To list all the files in the indicated directory, select All Files (*.*) from the Files of Type drop-down list box.

4. Click OK.

The spectrum is added to the list of standards and saved in the method *standards library*. If this is the first standard in your method, the *data collection parameters* in the current method will be

set automatically to match the settings that were used to collect the spectrum you opened.

If you selected multiple spectra to open or opened a spectral group (.SPG) file, all of the selected spectra will be added to the end of the Standards table. New rows are added to the table to accommodate the number of standards in the group.

Note If a warning message about mismatched standards appears when you open additional standards, the parameter settings used to collect the new standard are significantly different from the settings that are saved with the current method. For optimum performance, all method standards should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the standard again using the proper settings for the collection parameters, edit the settings in the current method, or ignore the warning and continue adding standards. ▲

Collecting a spectrum to use as a standard

If data collection is enabled, you can collect the *spectrum* of a *standard* and add the spectrum to the Standards table directly from TQ Analyst. This section explains how to use the Collection Parameters and Collect Standards buttons on the Standards tab to set the collection parameters and collect the spectrum of a new standard. The collection parameters define the collection process and configure the spectral display.

Note Access to the data collection features of TQ Analyst can be controlled by some Thermo Scientific spectral analysis applications, such as RESULT. When data collection is disabled, the Collect Standard, Collection Parameters and Collect Background buttons are removed from the Standards tab. The Collect Standard feature of the Assess Feasibility wizard also becomes unavailable. If these features are not available in your TQ Analyst application, data collection is disabled and you must use your spectral analysis application to collect standards.

Skip to the sections on importing calibration spectra and concentration data later in this chapter. ▲

Optimizing the collection parameters

All of the spectra of the *standards* in your method should be collected using the same settings for the collection parameters. You should also use these same parameter settings to collect the spectra you want to *quantify* with this method. Using consistent parameter settings helps minimize differences in the spectra which are due to variations in *sampling technique* rather than to differences in sample composition. If you need to specify a parameter setting, it is important to do it *before* you collect the *spectrum* of the first standard in your method.

Note If you change the setting for a collection parameter after opening or collecting the spectra of one or more standards, TQ Analyst may warn you that the parameter setting should not be changed at this point. You will be given the option of overriding the warning or creating a new method with the new parameter settings. ▲

The standards must be collected in the same way you will collect the spectra of the unknown *samples*, using the same *data collection parameters* and the same spectrometer, if possible. The spectra of the standards should be of the highest achievable quality. Assuming that the sampling technique is reproducible, spectral quality is determined mainly by the *resolution* and *signal-to-noise ratio* of the spectral data.

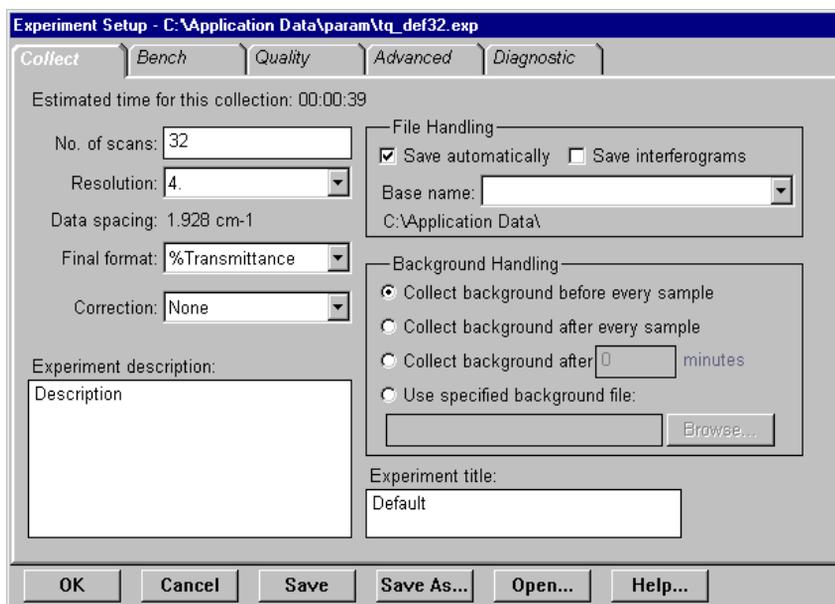
If you are analyzing gases, the spectra of the standards and samples should be collected at the same pressure and approximately the same temperature. This means if you have a concentrated gas standard that you need to dilute in order to lower its absorbance levels, you should back fill the cell to the targeted pressure with a gas that does not absorb in the *analysis range*, such as nitrogen or argon, or use a gas divider.

To display or change the collection parameters:

1. Click the Collection Parameters button on the Standards tab.

Note If this button does not appear in your TQ Analyst application, data collection is disabled and you must use your spectral analysis application to set the collection parameters. See the documentation that came with your Thermo Scientific spectral analysis software, such as RESULT, for more information. ▲

The Experiment Setup dialog box is displayed.



2. Set the data collection and processing parameters.

The Collect tab contains the parameters that control data collection and processing. This is the same software screen that allows you to set the collection and processing parameters in your Thermo Scientific spectral analysis software. See the

documentation that came with that software for detailed descriptions of the collection and processing parameters.

3. Set the Bench parameters.

The Bench tab contains the parameters that control the operation of your spectrometer or analyzer. (The term “bench” comes from the phrase “*optical bench*,” a generic name for FT-IR systems.) This is the same software screen that allows you to set the bench parameters in your Thermo Scientific spectral analysis software. See the documentation that came with that software for detailed descriptions of the bench parameters.

4. When you are finished, choose OK.

Any changes you make to these parameter settings will take effect immediately.

Collecting the calibration spectra

To collect the *spectrum* of a *standard*, first select a row in the Standards table. To add a standard, click anywhere in the open row at the bottom of the Standards table. If you want to replace a standard, click the row that contains the standard you want to replace. When you are finished making your selection, click the Collect Standard button on the Standards tab to start collecting data.

The process for collecting a spectrum in TQ Analyst is similar to the process for collecting a spectrum in your Thermo Scientific spectral analysis software. Refer to the documentation that came with that software for step-by-step instructions.

Note If the Collect Standard button does not appear in your TQ Analyst application, data collection is disabled and you must use your spectral analysis application to collect standards. See the documentation that came with your Thermo Scientific spectral analysis software, such as RESULT, for more information. ▲

Depending on how the *data collection parameters* are set, you may be prompted to collect a *background spectrum* or enter a *spectrum title* during data collection. If you don't enter a title during collection, the date and time of collection will be used. The new spectrum will be added to the open row at the bottom of the Standards table and saved in the method *standards library*.

Note When you collect a spectrum to use as a standard, TQ Analyst saves only a portion of the collection and processing information that is normally stored in a Thermo Scientific spectral data file. If you want to save all of the collection and processing information for your standards, save the standards as Thermo Scientific spectral data files by turning on the Save Automatically *check box* in Experiment Setup before collecting the standards. (Click the Collection Parameters button on the Standards tab to display the Experiment Setup dialog box. Save Automatically is located in the File Handling group on the Collect tab.) ▲

Importing the concentration values

If you set up the concentration data for your *standards* in a spreadsheet file, rather than entering the values directly in TQ Analyst, you must import the concentration data into TQ Analyst in order to complete your method. Use the copy and paste features of Excel and TQ Analyst to import the *concentration values*.

To import the concentration values:

1. Open TQ Analyst software and do the following:

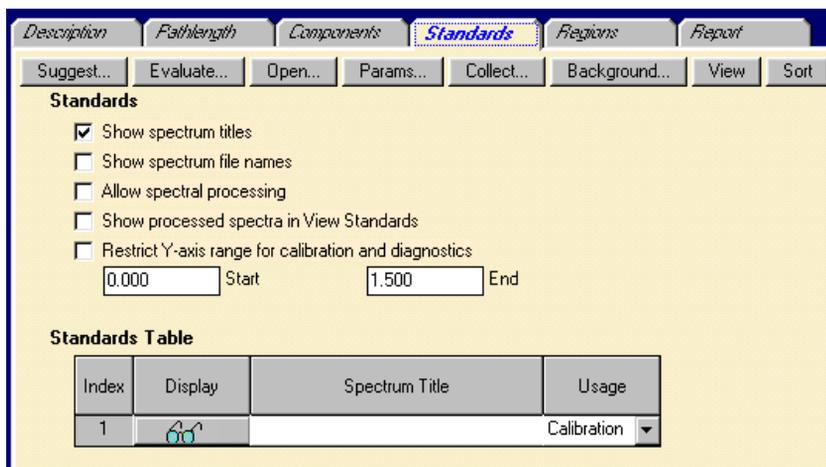
- Click the Components tab and enter the component names in the Components table, if you haven't done this already. If you want to copy the entire table of concentration values in one operation, make sure the components are listed in the Components table in the same sequence as they appear in the spreadsheet file.
- Click the Standards tab and make sure there are enough empty rows in the Standards table to add all of the data you want to copy. To add each blank row to the Standards table, click in the blank Spectrum Title cell at the bottom of the table, enter a placeholder name and then press the Enter key on your keyboard. If the Standards table already contains the file names and/or titles of the calibration spectra, make sure the standards are listed in the Standards table in the same sequence as they appear in the spreadsheet file.

2. Switch to the spreadsheet application and use the Copy command to copy only the concentration values onto the Clipboard.

Start with the first data cell in the upper left corner of the spreadsheet.

3. Select the TQ Analyst application, click the Standards tab and select the first cell that you want to paste the data into.

In most cases, this should be the first concentration column for the first component.



4. Choose Paste from the TQ Analyst Edit menu to paste the data into the Standards table.

Collecting a background spectrum

A *background spectrum* measures the response of the spectrometer without a *sample* in place. A background spectrum is used to eliminate signals that are due to the spectrometer and its environment from the *sample spectrum*.

The background *single-beam spectrum* shows how the energy of the *source* is distributed over the displayed frequency range. It includes the characteristics of the spectrometer, including the *detector*, *beamsplitter* and atmospheric conditions. Each sample single-beam spectrum is ratioed against the background single-beam spectrum so that the absorptions in the final spectrum are due solely to the sample.

The background spectrum you use to process your sample spectra should be collected under the same conditions as the sample spectra. If the conditions change over time, a new background may be needed.

Background spectra are typically collected in response to user prompts that appear during data collection. The software can be set up to prompt for background collection before or after every sample or after a specified period of time. You can also set up the software to use a specified background file. The options for specifying when background collection will occur are located with the *data collection parameters* in the Background Handling group. See “Optimizing the Collection Parameters” in this chapter for more information. Use the Collect Background button to collect a new background before the specified time has elapsed if your sampling or other conditions have changed.

Note Be sure to use the same settings for the data collection parameters when collecting a *background spectrum* as you plan to use for the *standards*. These same settings should also be used to collect the spectra of any samples you use this method to analyze. Using consistent parameter settings helps minimize differences in the spectra which are due to variations in *sampling technique* rather than to differences in sample composition. ▲

To collect a new background immediately, make sure there is not a sample in the spectrometer sample compartment, sampling module or accessory and then click the Collect Background button on the Standards tab. The Collect Background window appears and the spectrometer begins collecting data. During collection a live display of the data appears in the Collect Background window. When background collection is finished, the final background spectrum is displayed in the Collect Background window.

Note If the Collect Background button does not appear in your TQ Analyst application, data collection is disabled and you must use your spectral analysis application to collect background spectra. See the documentation that came with your Thermo Scientific spectral analysis software, such as RESULT, for more information. ▲

Click the Close button on the TQ Analyst *toolbar* to close the Collect Background window. The new background becomes the current background for the active method. Any spectra you collect of standards or samples will be ratioed against the current background spectrum.

Note If Use Specified Background File is currently on, you will not be able to collect a new background until you select a different background handling option (see “Optimizing the Collection Parameters” in this chapter for more information). ▲

Replacing a standard

If you suspect that the *spectrum* of a *standard* contains spectral errors, such as excessive instrument *noise* or strong absorptions in the *analysis region* or regions, you can easily replace the spectrum with a new one.

To replace the spectrum of a standard, first copy the component concentration values (if it is a quantitative method) to the open row at the end of the Standards table. Then click anywhere in the new row and either open a new spectrum or collect a new spectrum for that standard. If the new spectrum contains a title or has a file name and these features are selected for display in the Standards table, the title and/or file name of the new spectrum will replace the title and/or file name of the spectrum that was replaced.

Next, set the Usage and Class for the new standard, if those columns are present in the table. Finally, don't forget to delete the old standard. To delete a standard, click anywhere in the corresponding row in the Standards table and choose Delete Row from the Edit menu.

Deleting a standard

To delete a *standard*, click the appropriate row in the Standards table and choose Delete Row from the Edit menu.

If you want to delete multiple rows at the same time, click anywhere in the first row you want to delete. Then hold down the Shift key on the keyboard while you click any additional rows to be deleted. To delete the selected rows, choose Delete Row from the Edit menu.

Note The Sort button at the top of the Standards tab may help you place similar standards in sequential order in the Standards table so they can be easily deleted. ▲

Displaying the spectrum of a standard

The spectra of the *standards* that are listed in the Standards table can be displayed in a *spectral window*. Many spectra can be displayed in the spectral window at the same time. If processing operations are specified in the method, you can select whether you want to see the original data or the processed versions when spectra are displayed. See the section on “Displaying The Processed Version of a Standard” for more information.

Begin by selecting the standards you want to display. To select a standard for display, click its Display button in the Standards table.

The display buttons are labeled with an icon that looks like a pair of eyeglasses. When a Display button is pressed, a plus (+) sign appears on the button and the corresponding *spectrum* is selected for display.

Display buttons
↓

Standards Table							
Index	Display	Spectrum Title	Usage	Glucose Wt %	BA Wt %	Pro Wt %	Glycerol Wt %
1		pls example; standard 1	Calibration	1.94	0.99	1.92	2.17
2		pls example; standard 2	Calibration	1.26	0.50	4.01	2.47
3		pls example; standard 3	Validation	2.59	0.57	1.49	3.04
4		pls example; standard 4	Calibration	0.36	2.01	2.69	1.41
5		pls example; standard 5	Validation	1.55	0.77	2.54	2.79
6		pls example; standard 6	Calibration	1.13	1.12	4.14	0.86

To display the spectra of the selected standards, click the View Standards button on the Standards tab. When you click View Standards, a spectral window appears showing the spectra of the selected standards.

You can change the settings for the Display buttons while the spectral window is open. To update the contents of the spectral window, click the View Standards button again.

Selecting a displayed spectrum

You can select a displayed *spectrum* by clicking the spectrum in the *spectral window*. When a spectrum is selected, it is displayed in red in the spectral window.

Rearranging displayed spectra

The View menu becomes active whenever a *spectral window* is displayed. For information on a command in the View menu, click the *menu name* and then use the up or down arrow key on the keyboard to select a command in the open menu. Press the F1 key on the keyboard to display help information on the selected command.

When you are finished reading Help information, close the Help window by choosing Exit from the File menu.

Displaying the collection information for the standards

The software saves key information about the *standards* in a quantitative or *classification method*. The information is saved in the method *standards library*.

To display information about the standards in the current method, display the *spectrum* of any standard in a *spectral window*, click the spectrum to select it and then double-click the *spectrum title*. The Collection and Processing Information dialog box is displayed. The information in the Data Description group applies to all of the standards in the method.

Note The information in the Data Collection Information and Spectrometer Description groups does not apply to the selected standard or the current method and should be ignored. ▲

Displaying the processed version of a standard

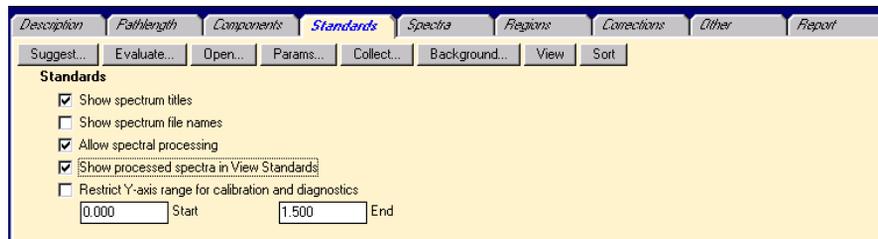
If you specify that spectral processing will be used in your method, TQ Analyst lets you choose which version of the data will be displayed in the *spectral window*—the original version or the processed version.

This applies to direct spectral processing, such as smoothing, *derivatives* and baseline correction, as well as any pathlength corrections that affect the spectral data, including the MSC (Multiplicative Signal Correction) and SNV (Standard Normal Variate) pathlength corrections. See the chapter titled “Processing Spectral Data” for information on setting up derivatives, smoothing or multipoint baseline correction for the method *standards*. The same corrections will be applied to any *samples* analyzed with the method. For information about the MSC and SNV pathlength options, see the chapter called “Principles of TQ Analyst” at the beginning of this document or the two chapters on creating a quantitative or *classification method*.

To configure the software to always show the processed spectra of any standards that are displayed, turn on the *check box* for “Show Processed Spectra in View Standards.” The check box appears on the Standards tab.

For instructions on displaying standards in a spectral window, click the

Explain button on the toolbar and then click the View Standards button on the Standards tab.



Note This *check box* is not available for QC Compare search and Search Standards methods. ▲

When this check box is on, the software checks whether any processing steps were applied to the standards before displaying them in a spectral window. If processing operations were specified, TQ Analyst displays the processed version of the standards rather than the original (unprocessed) version. This parameter is useful only when the check box for “Allow Spectral Processing” is on or when the MSC or SNV pathlength option is selected.

Note If you want to see the effects of a single processing step, such as smoothing, derivatives, or multipoint baseline correction, select the spectra you want to display by turning on their Display buttons in the Standards table (see the Standards tab) and then use the View Standards button on the *Spectra tab* (rather than the Standards tab) to display them. See the section “Viewing Selected Processing Operations” in the chapter “Processing Spectral Data” for more information. ▲

Using the Suggest Standards wizard to choose standards

TQ Analyst provides a handy tool to help you choose *standards* for quantitative methods. The Suggest Standards wizard provides the number and approximate concentrations of the standards that are necessary to create an accurate *calibration model* for each *component* over the specified *analysis range*. Both calibration and *validation standards* are recommended. If your method already contains standards, the wizard evaluates those standards and recommends concentration values for any additional standards that are needed.

Note You must enter *analysis limits* for each component before using Suggest Standards. See the Components tab for information on setting analysis limits. ▲

To start the wizard, click the Suggest Standards button on the Standards tab. The software displays a message asking whether you want recommendations for the minimum, typical, or optimum number of standards. After you respond to the message, the software fills in the Standards table with approximate concentration values for the recommended standards. If the Standards table already contains standards, the wizard prompts you to specify the number of standards you want to add. Then it recommends appropriate concentrations for the new standards. The recommended standards are titled "No spectrum assigned."

When the Suggest Standards wizard is finished, you must prepare the standards, collect their spectra, and specify accurate *concentration values* for each *component* and each *standard*.

Evaluating standards for quantitative methods

If your method already contains *standards*, TQ Analyst provides a tool to help you determine whether they were prepared correctly and whether additional standards are needed. To start the process, click the Evaluate Standards button on the Standards tab. TQ Analyst runs a series of tests to determine the following:

- Whether the concentrations of the standards are distributed throughout the specified *analysis range* for each *component*.
- If the concentrations of the various *components* in the standards vary independently.

Note You must enter concentration values for the current standards and *analysis limits* for each *component* before using Evaluate Standards. See the Components tab for information on setting analysis limits. If you want the software to recommend standards based on the spectral data for a group of known samples, use the Select Standards command in the Diagnostics menu rather than the Evaluate Standards wizard. See the chapter on "Method Diagnostics" for more information. ▲

When the wizard is finished, the software reports a “percent spanned” value, which indicates the percentage of the *analysis range* for each component that is described by the standards. Instructions on how to interpret the reported value are also provided.

If the reported value for percent spanned is less than 70, the software recommends adding standards to the method. A warning message is displayed if the number of independent *components* is less than the number of components in the method.

Note If you want TQ Analyst to recommend which standards to add, run the Suggest Standards wizard when the Evaluate Standards wizard is completed. See the previous section of this chapter for more information. ▲

If the percent spanned value for your method is less than 70 or if the number of independent components is less than the number of components, we recommend running the Pairwise Concentration diagnostic (see the Diagnostics menu). The diagnostic results can help you compare the concentration of each component in each standard to the concentrations of the other components in the method.

If the concentration of one or more components is dependent on the concentration of another component in all of the standards, the wizard will report that the standards were diluted serially. The wizard will also tell you if the concentrations of the components in all of the standards consistently sum to a constant value. Depending on the type of *samples* the method will analyze and the selected analysis type, these factors may or may not be important to the analysis. For brief descriptions of the algorithms available for *quantitative analysis* and the general requirements for each, click the *Explain button* on the *toolbar* to open the *Explain help window*, then click the Description tab and select each quantitative analysis type, or see the descriptions for the quantitative calibration techniques in the chapter on “Principles of TQ Analyst” in this document.

If the method contains a large number of standards (>100) and the standards span more than 95% of the *analysis range* for each *component*, the wizard gives you the opportunity to remove any standards that are not contributing significant information to the method *calibration model*. Removing unneeded standards helps simplify the method and minimize the amount of time it takes to *calibrate*. It also prevents the calibration model from overemphasizing *regions* that are represented by similar standards.

If a message appears asking whether you want to prune the standard set, enter the lowest Percent Spanned value that you feel is necessary for your analysis and choose Continue. The value you enter should not be less than 95%. The software uses either the *fractional factorial design model* or a random model (using the *maxi-min strategy*) to select appropriate calibration and validation standards until it reaches the value you specified. The Usage parameter for all of the remaining standards will be set to "Ignore." You may leave the extra standards in the method or delete them by clicking the appropriate row in the Standards table and choosing Delete Row from the Edit menu.

To display additional information about your standards, click the Detail button on the last screen in the Evaluate Standards sequence.

Eliminating unnecessary standards

If your method contains a large number of *standards* (>100) and the standards span more than 95% of the *analysis range* for each *component*, the Evaluate Standards wizard gives you the opportunity to remove any standards that are not contributing significant information to the method *calibration model*. Removing unneeded standards helps simplify the method and minimize the amount of time it takes to *calibrate*. It also prevents the calibration model from overemphasizing *regions* that are represented by similar standards.

To check if your method contains extra standards, run the Evaluate Standards wizard. To start the wizard, click the Evaluate Standards button on the Standards tab.

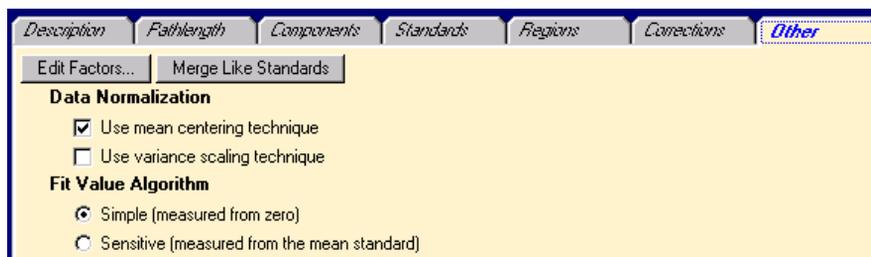
If the method contains more than 100 standards and the wizard reports a Percent Spanned value that is greater than 95%, you will be prompted to specify a minimum Percent Spanned value and to prune any unneeded standards. Enter the Percent Spanned value that you feel is necessary for your analysis and choose OK. The value you enter should not be less than 95%.

The software will randomly select appropriate calibration and *validation standards* until the Percent Spanned value reaches the value you specified. The Usage parameter for all of the remaining standards will be set to "Ignore." You may leave the extra standards in the method or delete them by clicking the appropriate row in the Standards table and choosing Delete Row from the Edit menu.

Merging standards

If your quantitative method includes two or more *standards* that have identical *concentration values* for all *components*, you can use the Merge Like Standards feature to create a composite standard that represents all of those standards. If you are developing a Discriminant Analysis or QC Compare search method, Merge Like Standards creates a composite standard for each *class* defined in your method. Each composite standard will represent all of the standards in the corresponding class.

Merge Like Standards is available for two of the *classification method* types (Discriminant Analysis and QC Compare search) and for all of the quantitative methods except Simple Beer's Law . The button is located on the Other tab.



This button is most useful when developing a Discriminant Analysis or QC Compare search method because it allows you to add a standard that is a composite of all the other standards in a given class. You may choose whether you want to use only the composite standard to represent the *class* or add the composite standard to the others by setting the Usage parameter in the Standards table. Adding the composite standard tends to weight the center of the class' spectral characteristics more heavily than spectral features that are unique to certain standards.

When you click Merge Like Standards for a quantitative method, TQ Analyst searches the *calibration set* for standards that have exactly the same *concentration values* for all *components* in the method. If any are found, the software creates an additional composite standard that can represent all of the standards in that "family." Using Merge Like Standards with a classification method (available for Discriminant Analysis and QC Compare search methods only) adds a composite standard to each class.

The spectral data for the original standards are averaged to create the *spectrum* for the composite standard. Composite standards are added to the end of the Standards table with titles "Composite standard A," "Composite standard B," etc.

The Usage for composite standards is automatically set to "Calibration," which means they will be included in the next calibration. If you want to exclude the original standards or any composite standard from calibration or any other operation, you must

change their usage from “calibration” to “ignore” (or remove the standard from your method).

Restricting the Y-axis range

If your method is set up to measure all of the data points in a *spectral region* or regions rather than *peak heights* or peak areas (i.e., if the Region Type parameter is set to Spectrum Range for all regions in the method), you can restrict the analysis to a specified Y-axis range. This allows you to eliminate data points in spectral regions that are or may be totally absorbing or in regions that contain excessive *noise*. Strong absorption *bands* tend to have more uncertainty and therefore should be excluded from the *analysis region* or regions of a quantitative or *classification method*.

To restrict the Y-axis range:

- 1. Turn on the check box for Restrict Analysis to Y-Axis Range.**
- 2. In the box labeled “Start,” enter the lowest intensity value that you want to include in the analysis.**
- 3. Select the box labeled “End” and enter the highest intensity value that will be included.**

Only the data points that fall between the specified Y-axis limits will be used during calibration. If you run any diagnostic routines on the method, the Y-axis range for the diagnostic routines will also be restricted to the limits you specified. See the “Method Diagnostics” chapter of this manual for detailed descriptions of the diagnostic tools available in TQ Analyst.

7 Processing Spectral Data

Although spectroscopy assumes a direct and linear relationship between absorbance, concentration, and *pathlength*, it is not always possible to collect spectra where this is completely true. Placing the data in another format or applying certain processing steps often allows the software to better model these relationships. For example, an analysis may work best on the first or *second derivative* spectrum rather than the original spectrum or perhaps with a *baseline* anomaly removed.

Processing steps such as smoothing, derivatives, and baseline correction are common practice in many *infrared* applications. If you plan to apply processing operations to your spectral data, keep the following points in mind:

- All of the spectral data must be in the same format. If you use the first or second derivatives of the *standards* to create the *calibration model*, you must also analyze the first or second derivative spectra of the unknown *samples*. TQ Analyst is set up to do this automatically. Any processing steps that are specified for the standards are automatically applied to any unknown samples you use the method to analyze. If you convert a *sample spectrum* to its first or second derivative outside of TQ Analyst and then try to analyze it with a method that specifies derivatives, the software will display an error message.
- Any processing operations that are applied to the standards must also be applied to the unknown samples (this also happens automatically in TQ Analyst). If you specify smoothing or baseline correction, it is important to first consider a representative pool of standards and known samples to be sure you will achieve the desired results. When applying baseline correction, for example,

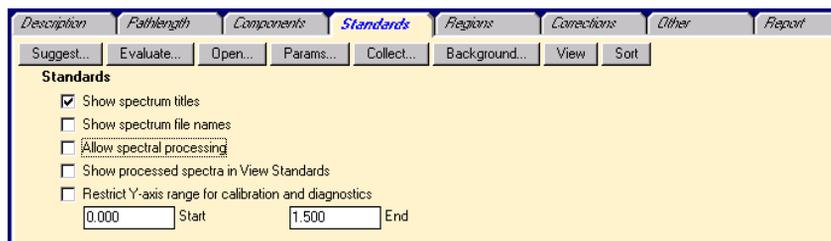
you must be sure the same or similar baseline anomaly appears in all of the standards as well as the unknown samples.

We recommend using the processing tools that are provided in TQ Analyst to apply smoothing, derivatives or baseline correction to your data. Processing your data in TQ Analyst will ensure that the standards and unknown samples are handled consistently, which will improve the accuracy of prediction and overall performance of your method. This chapter describes how to use certain tools on the Standards and Spectra tabs to:

- Show or hide the Spectra tab, which is used for Processing spectral data.
- Select the format of your spectral data (e.g., original, *first derivative*, second derivative).
- Set up automatic smoothing.
- Configure multipoint baseline correction.
- Specify whether the original or the processed version of your spectral data will be displayed in the *spectral window*.

If you want to work only with the original spectra with no additional spectral processing, make sure the following *check box* is off and skip to the next chapter.

Turn this check box off to work with your original spectra with no additional spectral processing.



This is the default configuration for TQ Analyst. If you are creating a Measurement Only method, the *check box* appears on the Measurements tab. For other analysis types, it appears on the Standards tab.

Note This *check box* is not available for QC Compare search and Search Standards methods. ▲

Showing or hiding the Spectra tab

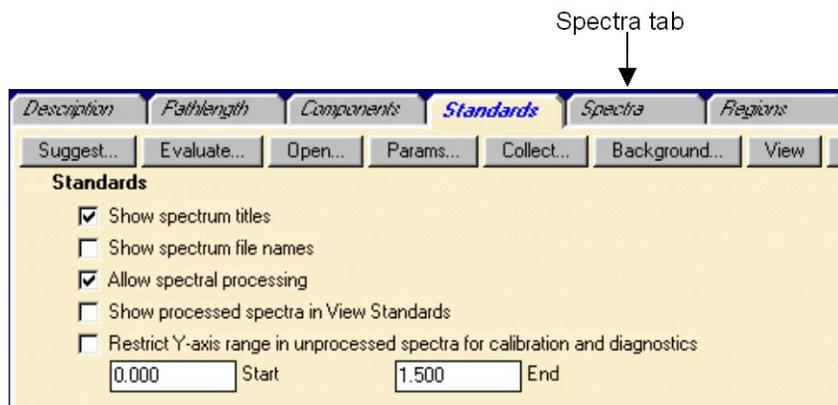
TQ Analyst is initially configured to use the original spectra of the *standards* to create the method *calibration model* and to analyze the original data for the unknown *samples*. In this configuration, the Spectra tab does not appear with the other tabs at the top of the *TQ Analyst window*.

If you decide you want to work with *derivative* spectra or apply smoothing or baseline correction to your spectral data, you must first turn on the *check box* called “Allow Spectral Processing.” If you are creating a Measurement Only method, this check box appears on the Measurements tab. For other analysis types, it appears on the Standards tab.

Allow spectral processing

Note This *check box* is not available for QC Compare search and Search Standards methods. ▲

When the check box is on, TQ Analyst adds a new tab, called the Spectra tab, to your method. The Spectra tab appears adjacent to the Measurements tab in a Measure Only method. For other analysis types, the new *tab* appears next to the Standards tab.



You can use the parameters on the Spectra to set up processing operations for your spectral data, such as smoothing the data, calculating *derivatives*, and correcting spectral *baselines*.

If you want to work only with the original spectra with no processing steps applied, make sure the *check box* for Allow Spectral Processing is off.

Turn this check box off to work with your original spectra with no additional spectral processing.

Allow spectral processing

This is the default configuration for TQ Analyst.

Displaying processed spectra

If you specify that spectral processing will be used in your method, TQ Analyst lets you choose which version of the data will be displayed in the *spectral window*—the original version or the processed version.

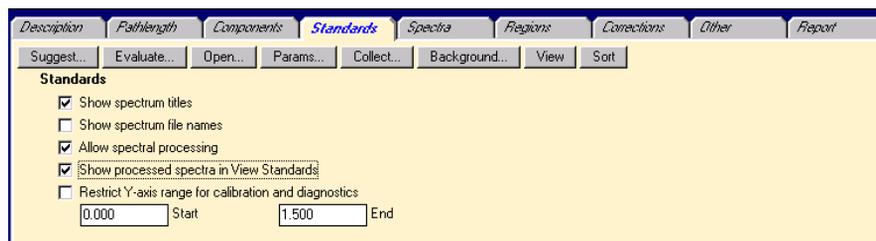
This applies to direct spectral processing, such as smoothing, derivatives and baseline correction, as well as any pathlength corrections that affect the spectral data, including the MSC (Multiplicative Signal Correction) and SNV (Standard Normal Variate) pathlength corrections. For information about the MSC and

SNV pathlength options, see the chapter called “Principles of TQ Analyst” at the beginning of this document.

To configure the software to always show the processed spectra of any *standards* that are displayed, turn on the check box for “Show Processed Spectra in View Standards.”

The *check box* appears on the Standards tab.

For instructions on displaying standards in a spectral window, click the Explain button on the toolbar and then click the View Standards button on the Standards tab.



When this check box is on, the software checks whether any processing steps were applied to the standards before displaying them in a spectral window. If processing operations were specified, TQ Analyst displays the processed version of the standards rather than the original version.

Note This *check box* is not available for QC Compare search and Search Standards methods. ▲

This parameter is useful only when the check box for “Allow Spectral Processing” is on or when the MSC or SNV pathlength option is selected.

Note If you want to see the effects of a single processing step, such as smoothing, derivatives, or baseline correction, select the spectra you want to display by turning on their Display buttons in the Standards table (see the Standards tab) and then use the View Standards button on the Spectra tab to display them. See the next section “Viewing Selected Processing Operations” for more information. ▲

Viewing selected processing operations

When setting up spectral processing, it is important to display a representative spectrum before and after the processing step in order to verify how the operation will work. Use the View Standards button on the Spectra tab to see the effects of a single processing step, such as smoothing, *derivatives*, or baseline correction.

To view a selected processing operation:

- 1. Click the Standards tab and select a few representative spectra to be displayed by turning on their Display buttons in the Standards table.**

The Display buttons are labeled with a pair of eyeglasses. When a Display button is on, a “+” sign appears on the button. The Display buttons for all of the other *standards* should be off.

- 2. Select the processing function you want to apply.**

For example, if you want to set up derivatives, smoothing or multipoint baseline correction, set the data format, smoothing or baseline correction options as desired. See the sections on “Specifying the Format of Your Spectral Data,” “Setting Up Automatic Smoothing” and “Setting Up Multipoint Baseline Correction” in this chapter for instructions.

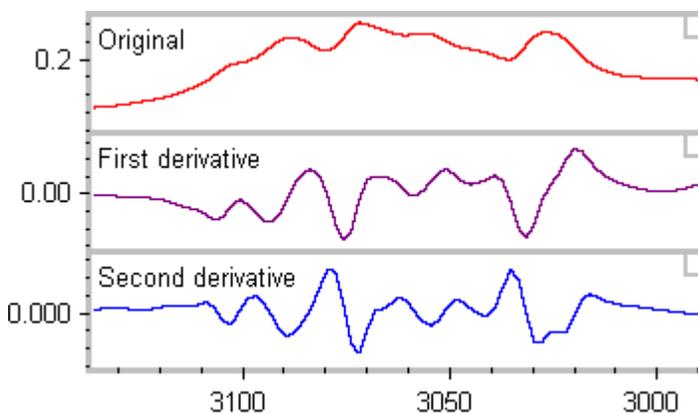
If you need to see the original spectrum in order to determine the number of points for smoothing or the locations of the *baseline points* to be corrected, click the View Standards button on the Spectra tab to display your representative spectra. When you are finished viewing the representative spectra, click the Close button on the TQ Analyst *toolbar* to close the *spectral window*.

3. **When you are finished setting up the processing operations, click the View Standards button on the Spectra tab to see the representative spectra with the specified changes.**

If you want to specify more than one processing step, we recommend setting them up in this order: Data Format, Smoothing, Multipoint Baseline Correction. That way, you can see the effect of each processing step before and after it is applied as well as the cumulative effect of all of the specified processing operations.

Specifying the format of your spectral data

TQ Analyst allows you to specify a format for the spectral data you want to analyze. You can elect to analyze the original spectrum, as it was opened or collected in TQ Analyst, or you can analyze the first or second *derivative* spectrum (see example below).



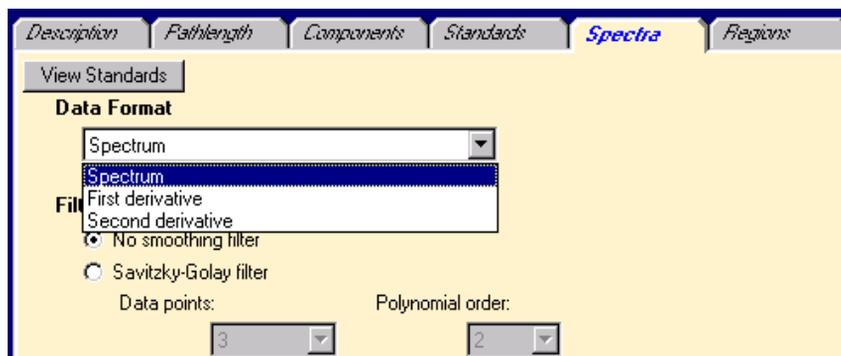
The first derivative is useful for revealing peaks that appear as shoulders in the original spectra. Use the second derivative to find the exact location (center) of shoulders in the original spectra.

The original spectra can be in any units; however, the derivative operation is usually applied to spectra that are in absorbance, Kubelka-Munk or photoacoustic units.

The parameter for selecting the data format is located on the Spectra tab. If the Spectra tab does not appear with the other tabs in the *TQ Analyst window*, the parameter that displays it is currently off. See the section on “Showing and Hiding the Spectra tab” in this chapter for more information.

The *default setting* for the Data Format parameter performs all processing and analysis steps on the original spectra.

To process and analyze your data in another format, click the arrow next to the list box to see the available options and then select a format.



The choices available for setting Data Format are described briefly below.

<i>Format</i>	<i>Description</i>
Spectrum	Original spectrum as it was opened or collected in TQ Analyst
First derivative	<i>First derivative</i> of the original spectrum
Second derivative	Second derivative of the original spectrum

What is the first derivative?

The first derivative shows the rate of change across the entire spectrum. This means that in the first derivative, shoulders become narrower and thus are easier to see. It is important to remember that the maximum and minimum points in the first derivative curve are the points of maximum rate of change and not the maximum and minimum points of the original peaks. The maximum and minimum points of the original peaks have a Y value of zero in the first derivative.

What is second derivative?

The second derivative shows the change in the rate of change across the spectrum. This curve is more complex than the first derivative, with significantly narrower bands. The second derivative is useful for finding exact peak locations since peaks in the second derivative appear at the same locations as peaks in the original spectrum. The second derivative has more baseline *noise* than the first derivative. For each derivative operation you perform, the noise level increases slightly, the signal strength decreases dramatically and the signal-to-noise ratio decreases.

The conversion will be applied to the spectra of the *standards* as well as to any *unknown sample spectrum* analyzed with the method. All processing steps that affect the spectral data will be done in the selected format, including MSC pathlength correction, SNV pathlength correction, smoothing, baseline correction, and any diagnostic analyses.

To see the effect of a first or second derivative setting, leave Data Format set to "Spectrum." Click the Standards tab and select a representative spectrum for display by clicking its Display button in the Standards table. See "Displaying the Spectrum of a Standard" in the chapter on "Working With Standards" for more information. Then click the Spectra tab and click the View Standards button. The original version of the selected spectrum will be displayed in a *spectral window*. Click the Close button on the TQ Analyst *toolbar* to close the spectral window. If you want to see the effects of *first derivative*, set Data

Format to “First Derivative” and then click View Standards again. The first derivative form of the representative spectrum will be displayed in a spectral window. When you are finished, click the Close button on the toolbar to close the spectral window.

If you want to analyze derivative spectra, we recommend using the Data Format feature of TQ Analyst to perform the derivative conversion. You can collect the spectra of the standards outside of TQ Analyst if you wish. However, don't import derivative spectra to set up a TQ Analyst method. The same goes for the unknown *samples*. For example, if you convert a sample spectrum to its first or second derivative outside of TQ Analyst and then try to analyze it with a method that specifies derivatives, the software will display an error message.

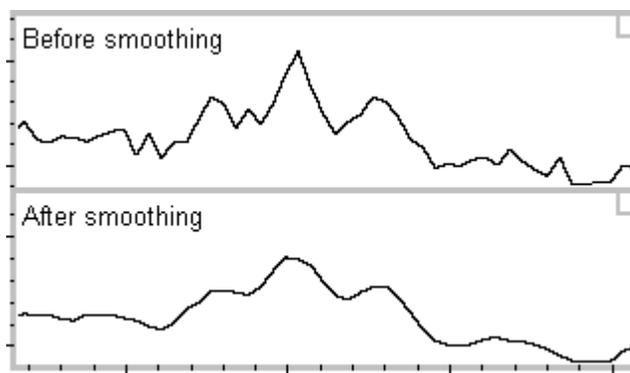
Note If you set up a method that uses first or second *derivative* spectra, don't select one of the derivative options for defining the *analysis regions*. You should also avoid using the “area” Region Type to measure *first derivative* spectra, since the area of a derivative peak often goes to zero. When using derivative spectra, we recommend setting the Region Type to “Spectrum Range” for all of the method analysis regions. See the sections titled “Selecting the Analysis Regions” in the individual chapters on creating a quantitative, classification or *spectral measurement method* in this document for instructions on selecting analysis regions. ▲

Setting up automatic smoothing

Smoothing allows you to improve the appearance of your spectra by smoothing *peaks* that are obscured by random *noise*. Reducing random noise can also decrease the error in a method *calibration model*. The smoothing algorithm is based on the assumption that spectral information due to sample absorptions varies slowly and is obscured by rapidly changing random noise.

The algorithm works by preferentially smoothing the high-frequency component of the spectral data. In this sense, “high frequency” refers to sequential data points that have dramatic differences in intensity.

In other words, the algorithm seeks out and smoothes peaks that resemble random noise (see the example below).



All of the software features for setting up smoothing are included on the Spectra tab. If the Spectra tab does not appear with the other tabs in the *TQ Analyst window*, the parameter that displays it is currently off. See the section on “Showing and Hiding the Spectra tab” in this chapter for more information.

To see descriptions of the smoothing parameters, click the Explain button on the toolbar to open the Explain help window and then click the parameter name on the Spectra tab.

Filter to Smooth Data

No smoothing filter

Savitzky-Golay filter

 Data points:

 Polynomial order:

Norris derivative filter

 Segment length:

 Gap between segments:

TQ Analyst provides two smoothing filters: the Savitzky-Golay filter and the Norris derivative filter. If you select the Savitzky-Golay smoothing filter, the settings for Data Points and Polynomial Order

determine the degree of smoothing. The Norris Derivative filter is defined by the settings for Segment Length and Gap Between Segments. You can smooth the original data or their *first* or *second derivatives* by setting the Data Format parameter.

Savitzky-Golay smoothing

To configure typical Savitzky-Golay smoothing, set the Data Format and smoothing parameters as described below. Savitzky-Golay smoothing can be applied to the original spectra or to the first or second derivative spectra.

Data Format:	“Spectrum” for simple smoothing “First Derivative” for smoothed <i>first derivative</i> “Second Derivative” for smoothed 2 nd derivative
Filter to Smooth Data:	Savitzky-Golay filter
Data Points:	Determines the degree of smoothing. Can be set to any odd integer from 3 to 25 (should be > setting for Polynomial Order). Set to 7 data points for average smoothing. Increase for more smoothing; decrease for less.
Polynomial Order	Defines shape of curve for smoothing operation. Can be set to any integer from 2 to 6. Should be less than the setting for Data Points. Set to 3 rd order (cubic) polynomial for average smoothing. Higher orders result in less smoothing; lower orders cause more smoothing.

Norris derivative smoothing

To configure typical Norris Derivative smoothing, set the Data Format and smoothing parameters as described below. The Norris-derivative smoothing filter is intended to be used with first or second derivative spectra. It is typically applied to Near-IR spectra.

Data Format:	“First Derivative” for smoothed <i>first derivative</i> “Second Derivative” for smoothed 2 nd derivative
Filter to Smooth Data:	Norris Derivative filter

- Segment Length:** Determines the degree of smoothing. Can be set to any odd integer from 1 to 25. Set to preferred number of points per segment (default is 5). Larger values increase smoothing (points in each segment are averaged); smaller values decrease smoothing.
- Gap Between Segments:** Defines the distance between two consecutive segments. Can be set to any integer from 0 to 12. Set to preferred gap between segments (default is 0). Increasing the gap lowers resolution. When working with derivatives, increased gap produces larger differences for broad peaks.

To see the effects of smoothing, leave Filter To Smooth Data set to “No smoothing filter.” Click the Standards tab and select a representative spectrum for display by clicking its Display button in the Standards table. See “Displaying the Spectrum of a Standard” in the chapter on “Working With Standards” for more information. Then click the Spectra tab and click the View Standards button. The original version of the selected spectrum will be displayed in a *spectral window*. Click the Close button on the TQ Analyst *toolbar* to close the spectral window. If you want to see the effects of Savitzky-Golay smoothing, select the Savitzky-Golay filter, set Data Points and Polynomial Order and then click View Standards again. The smoothed version of the representative spectrum will be displayed in a spectral window. Set and reset the smoothing parameters and display the results until you are able to determine the optimum settings. When you are finished, click the Close button on the toolbar to close the spectral window.

When smoothing is on, TQ Analyst applies the smoothing filter to the spectrum of every *standard* in the current method. The spectrum of any unknown *sample* you use the method to analyze will also be smoothed before the concentrations are measured (or the sample is classified).

Notice The Savitzky-Golay algorithm smooths all peaks. Smoothing degrades the effective spectral *resolution* of the data and can remove ("smooth out") small spectral features, including sample peaks (this is especially true for mid-IR spectra). If possible, increase the *signal-to-noise ratio* of the data by collecting more scans instead of smoothing. ▲

Setting up multipoint baseline correction

A *baseline* consists of those portions of a spectrum where there are no significant absorptions. Ideally the intensity in these portions of the spectrum is zero absorbance units (or 100% *transmittance*). In reality baselines may be tilted, shifted or curved.

Turn on multipoint baseline correction if you want to eliminate a significant baseline feature, such as a sloping, curving, shifted or otherwise undesirable baseline, that appears in the spectrum of every *standard* and affects the entire spectrum. Before deciding to use multipoint baseline correction, make sure the same baseline feature is present in the spectra of any *samples* the method will analyze.

TQ Analyst provides two algorithms for correcting spectral baselines using a multipoint correction: the piecewise linear correction and the spline correction.

All of the software features for setting up multipoint baseline corrections are included on the Spectra tab. If the Spectra tab does not appear with the other tabs in the *TQ Analyst window*, the parameter that displays it is currently off. See the section on "Showing and Hiding the Spectra Tab" in this chapter for more information.

If you are creating a quantitative, classification or *spectral measurement method* (excluding the SMLR method type), you may use the Regions tab to specify unique one- or two-point baselines for each region to be analyzed or measured rather than using the multipoint baseline correction provided on the Spectra tab. Using the Regions tab to define baselines is the preferred method for several reasons. First, you are

forced to consider the baseline for each *analysis region* independently so the correction is often more accurate than an overall correction. Second, the software can usually apply independent baselines more consistently than multipoint baselines, which will improve the accuracy of prediction and overall performance of your method. Third, you can use the tools and spectra displayed in the Region Selection task window to select the *baseline points* interactively for each region. This is easier and often more accurate than entering baseline points in a table. For information on specifying unique baselines for each region, see the sections titled “Selecting the Analysis Regions” in the individual chapters on creating a quantitative, classification or spectral measurement method.

Use the parameters in the Multipoint Baseline Correction group on the Spectra tab to turn on multipoint baseline correction and specify what kind of baseline correction will be used.

To see descriptions of these parameters, click the Explain button on the toolbar and then click the parameter name.

Multipoint Baseline Correction

No correction
 Piecewise linear correction
 Spline correction

Baseline Points Table

Index	Type	Start	End
1		0.000	0.000

The type and degree of baseline correction depends on the algorithm (linear or spline) and the number and locations of the *baseline points* used for the correction. The baseline points specify where the correction should occur. The algorithm determines how the software calculates the values that are subtracted from each data point in the spectrum to correct the baseline. You can apply a baseline correction to the original spectra or to their first or *second derivative* spectra by setting the Data Format parameter.

Piecewise linear correction

The algorithm for the piecewise linear correction interpolates a straight line between each baseline point you specify. It works best for correcting tilted baselines and other baselines that contain linear sections.

Spline correction

The spline correction algorithm interpolates a cubic polynomial curve based on at least four consecutive specified *baseline points*. Use it to correct baselines, or baseline sections, that are curved.

Use the Baseline Points table to select the baseline points that will be used for baseline correction. You can specify as many baseline points as you wish.

To see descriptions of the columns in this table, click the Explain button and then click each column heading.

Index	Type	Start	End
1		0.000	0.000

Each row in the table specifies a new baseline point. Each baseline point is defined by the starting and ending location of the region in which the point will be chosen and the method used to select the baseline point (Type).

TQ Analyst provides the following options for choosing baseline points:

- | | |
|------------------|--|
| Average in range | Takes the average of the points between the start and end of the specified range. The selected point may or may not fall on the spectrum. |
| Maximum in range | Uses the highest (<i>absorbance</i>) or lowest (% transmittance) Y value on the spectrum between the start and end of the specified range. |

Minimum in range	Uses the lowest (absorbance) or highest (% transmittance) Y value on the spectrum between the start and end of the specified range.
------------------	---

To select one of these options for the first *baseline point*, click the arrow in the first row of the Type column in the Baseline Points table to show the drop-down list box and then click the desired option. Then use the Start and End columns to define the X-axis location of the first and last data point in the first baseline region. The first baseline point will be chosen using the method and region you specified.

When you finish defining each baseline point, the software automatically adds another row to the Baseline Points table. Repeat the steps above for each additional correction point you want to specify.

To determine the best region for selecting each baseline point, use the View Standards button on the Spectra tab to display a representative spectrum of a method standard while making your selections. To select a spectrum for display, click the Standards tab and then click the Display button for a representative spectrum in the Standards table. See “Displaying the Spectrum of a Standard” in the chapter on Working With Standards” for more information. To see the uncorrected version of the selected spectrum, click the Spectra tab and then click the View Standards button. The spectrum will be displayed in a *spectral window*.

The regions you choose should be on (or near) the baseline of the representative spectrum. Choose all of the baseline points you want to correct and record them on a piece of paper. Then click the Close button on the TQ Analyst *toolbar* to close the spectral window. Enter Start and End values for each baseline point in the Baseline Points table and specify the Baseline Type by choosing an option in the Type column. The resulting “baseline” is subtracted from the actual spectrum so that the baseline of the corrected spectrum appears flat and near zero absorbance units (or 100% transmittance). To see the

baseline corrected spectrum, click the View Standards button again. The baseline corrected version of the representative spectrum will be displayed in a spectral window.

Set and reset the baseline points and display the results until you are able to determine the optimum settings that work for several representative spectra. When you are finished, click the Close button on the toolbar to close the spectral window.

By selecting points carefully, you can correct baselines without using a large number of points. In general, you should select points at the beginning and end of baseline regions that need to be corrected and "low points" that are above zero absorbance (or high points below 100% transmittance). When the selected points are brought to zero absorbance (or 100% transmittance), the points between the selected points will also be shifted so that their vertical position relative to the nearest selected points is maintained. Similarly, if you choose low points on both sides of a *peak*, the Y value of the peak will be decreased but the *peak height* measured from the low points will stay the same.

Notice Be careful not to introduce false peaks or other inaccuracies when you choose *baseline points*. ▲

When the linear or spline correction is selected and the baseline points are properly defined, TQ Analyst applies the correction to the spectrum of every standard in the current method. The spectrum of any unknown sample you use the method to analyze will also be baseline corrected before the concentrations are measured (or the sample is classified).

8 Creating a Quantitative Method

Read this chapter to learn the key steps in creating a successful quantitative method using TQ Analyst software. The following topics are covered:

- Defining the problem
- Choosing a sampling technique
- Creating a new method file
- Giving the method a title
- Selecting a calibration technique
- Choosing a pathlength option
- Defining the method components
- Running the *feasibility test*
- Collecting the standards
- Selecting the analysis regions
- Customizing the method
- Saving the method
- Calibrating the method
- Validating the method
- Correcting component linearity
- Where to go from here.

Most of the operations described in this chapter are carried out in a *method window*. The method window is broken into a series of tabs. The tabs that appear when one quantitative method type is selected

may be different from the tabs that appear for another quantitative method. There are more tabs for the Partial Least Squares analysis type, for example, than for Simple Beer's Law.

The tabs are arranged in an order that is convenient for creating a quantitative method. If you select the tabs in sequence from left to right, starting with the Description tab, they will lead you step by step through the method development process.

If you need information on updating the calibration data, running method diagnostics, or setting up sample reports, individual chapters on those topics are provided later in this document.

If you want to create a *classification method* or one that only measures and reports spectral information, skip this chapter. Instructions for creating classification and *spectral measurement methods* are provided in the next two chapters of this document.

Wizards for quantitative methods

TQ Analyst offers a number of wizards to help you complete each quantitative method development task. From choosing the proper settings for *method parameters* to setting up your experimental design, the wizards can help make even your first attempt at developing a method a success.

The following table gives brief descriptions of the wizards available for creating quantitative methods.

<i>Location</i>	<i>Wizard</i>	<i>Function</i>
Description tab	Suggest Analysis Type	Recommends a setting for the Analysis Type parameter.
Pathlength tab	Suggest Pathlength Type	Recommends a setting for the Pathlength Type parameter.
Components tab	Assess Feasibility	Determines whether there is sufficient variability in the sample data that correlates with differences in sample composition. (Tells you whether your method is feasible.)
Standards tab	Suggest Standards	Fills in the Standards table with the names and concentrations of the <i>standards</i> you should prepare and collect.
	Evaluate Standards	Determines whether the standards that are listed in the Standards table were prepared correctly and whether additional standards are needed.
Regions tab	Suggest Regions	Chooses appropriate <i>spectral regions</i> for the analysis.

Continued on next page

<i>Location</i>	<i>Wizard</i>	<i>Function</i>
Other tab	Suggest Factors	Chooses <i>factors</i> or <i>principal components</i> (PCs) in a <i>partial least squares</i> or <i>principal component regression</i> method. Use the Edit Factors button to view or edit the chosen factors (PCs).
Corrections tab	Suggest Corrections	Determines whether a <i>correction curve</i> will improve the accuracy of a quantitative method and suggests settings for the correction parameters for each component.

To start a wizard, click the appropriate button on the specified tab. For example, to start the Analysis Type wizard, click the Suggest Analysis Type button on the Description tab.

The wizards are intended to be used in the order shown in the table above. However, some of the wizards' recommendations will be limited if they can't access certain information. You may use the Pathlength Type wizard, for example, on a completely new method (all tables are blank) but the wizard can only help you distinguish the simple pathlength options, such as Constant, Known and Predict. The method must be fairly complete (components, standards and regions defined) before the Pathlength wizard can recommend a pathlength correction, such as Internal Reference or Multiplicative Signal Correction. The same is true for the Analysis Type wizard. If you select the "Undecided" option for Analysis Type and Pathlength Type, define your components and standards and then use the Regions wizard to recommend spectral regions for your method, the Regions wizard will recommend Analysis Type and Pathlength Type options as part of the region selection process.

The Suggest Factors wizard is available only for PLS and PCR methods. It suggests appropriate *factors* automatically during the calibration process. The Suggest Corrections wizard is not automatic (nor should it be). If you want to see what, if any, corrections are recommended, *calibrate* your method and then click the Suggest Corrections button on the Corrections tab.

To learn the important steps and considerations for good quantitative method design and understand how to get the most out of the wizards, we recommend reviewing all of the material in this chapter in the order in which they are presented.

You may use the wizard's recommendations, alter them, delete them or overwrite them completely. We suggest using the wizards' recommended settings as a starting point. Then add your knowledge of spectroscopy and the chemical system you are measuring to determine the optimum settings.

Defining the problem

Most researchers know which compound they need to measure before the *sample* arrives in their lab. You may be asked, for example, to set up a method to measure the amount of olefin in an oil sample or to identify the type of antioxidant in a polymer material.

Knowing *what* to measure is not usually a problem; knowing *how* to measure it often is. The key is finding information in the *sample spectrum* that correlates with the *components* you need to measure.

The first step in creating a quantitative method is to clearly define the system you are measuring by answering the following questions:

- How many components are in the sample?
- Which components do I need to measure?

- What range of concentrations of each component do I expect to find in the unknown samples?
- What are the possible interferences?
- How does the *sample matrix* affect each measured component?

The answers will help you choose an appropriate *sampling technique* and define the experimental conditions that will be used for the analysis.

Choosing a sampling technique

Once you've defined the problem, the next step is to choose a *sampling technique* and define the experimental conditions for the analysis. In many cases, the physical characteristics of the *sample* dictate the sampling technique. For example, if you are analyzing powder samples in bottles that have narrow openings, you might use a fiber optic probe. Other samples, such as a polyol lubricant, might require a temperature-controlled, fixed-pathlength transmission cell.

Choosing the optimum sampling technique for the material you want to measure is a critical step in achieving accurate quantitative results. It requires knowledge of *infrared* spectroscopy and the effects of interferences, which can adversely affect your results.

The instrumentation industry offers a wide range of tools and accessories for material sampling and analysis. Each has benefits and drawbacks which may or may not be important to your analysis. Select the technique that consistently produces a quality spectrum with the least amount of effort and expense.

You generally have some latitude in setting the experimental conditions. Two characteristics of spectral data that are especially important to quantitative measurements are spectral *resolution* and *signal-to-noise ratio*.

The signal-to-noise ratio of a spectrum defines how well the sample signal can be distinguished from signals that are due to random *noise*. In spectroscopy, signal-to-noise ratio is affected by the number of scans that are added together to produce the spectrum as well as the current settings for the *source* aperture and the electronic gain. See the documents that came with your spectrometer for instructions on optimizing these and other settings that define data collection.

Choosing the proper spectral resolution depends on the selected sampling technique and the physical state of the samples. If you are analyzing solid or liquid samples in the mid-infrared range, we recommend starting with a resolution setting of 4 or 2 cm^{-1} . Gas phase measurements may require 1 or even 0.5 cm^{-1} resolution to resolve the *peaks*, especially if the gas molecules are small (6 atoms or less). If the peaks in the spectra you collect at these recommended resolutions are not fully separated, switch to a lower resolution setting. Remember, the *smaller* the resolution value, the *higher* (better) is the resolution.

Near-infrared peaks are overtones and combination *bands* which are broader than mid-infrared peaks and often overlap. We recommend starting with a resolution setting of 16 or 8 cm^{-1} for near-infrared data collection. If correlation values for minor constituents are low, increasing resolution (choosing a smaller resolution value) may improve separation.

Keep in mind however, that the signal-to-noise ratio of the spectrum decreases as the resolution increases so you may want to also increase the number of scans. If you're still not getting the resolution and peak shape you need after increasing the resolution, try another sampling technique.

When creating quantitative methods, we recommend collecting or converting the spectra to absorbance or $\log(1/R)$ units. Since these units vary linearly with concentration in most cases, they are ideal for *quantitative analysis*. If you want to work with derivative spectra, use

the Data Format parameter on the Spectra tab to make the derivative conversion. See the chapter on “Processing Spectral Data” in this document for information on setting Data Format.

Creating a new method file

The first step in creating a method using TQ Analyst is to create a new method file. A method file contains all of the parameters that define how your method will operate. Your method must contain valid settings for all of the *method parameters* before it can be *calibrated* or run.

To create a new method file:

- 1. Choose New Method from the File menu.**

The method parameters are displayed in a new *method window*. The Description tab, which contains the first group of method parameters, is already open.

- 2. Choose Save Method As from the File menu.**

The Save Method As dialog box is displayed.

- 3. Type a file name for the new method in the File Name box and select the directory and disk where you want the method saved.**

- 4. Choose OK.**

When you create a new method file, the *method parameters* are automatically set to their default values. You can change any of the parameter settings or use the *default settings*.

Note Save your method frequently while creating the method or editing the method parameters. ▲

Giving the method a title

Use the Method Title box on the Description tab to enter a title for your new method.

Method Title

TQ Analyst example method - Classical least squares (CLS)

You will use the *method titles* along with their file names to select a method to open. The title can also be displayed or printed with the *analysis results*.

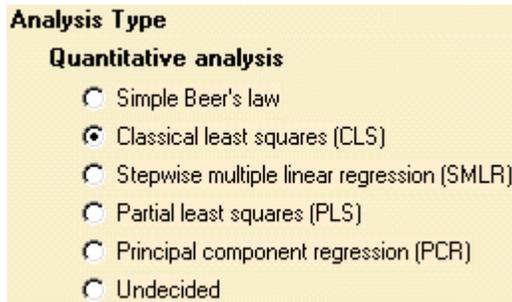
You may enter a longer description of the method in the Method Description box. You may also enter your name in the Developer's Name box so people know who created the method.

The Method Description and Developer's Name parameters are also on the Description tab. Use the *scroll bar* to bring these parameters into view.

Selecting a calibration technique

Another important but difficult step in developing a quantitative method is choosing the algorithm used to create the method *calibration model*. The Analysis Type parameter on the Description tab provides a range of calibration techniques for *quantitative analysis* (see the list below).

To see detailed descriptions of the calibration options for quantitative methods, click the Explain button on the toolbar to open the Explain help window and then click each option in the software.



We recommend leaving the Analysis Type set to “Undecided” until you’ve collected some spectral data. At this point, you don’t have enough information about the problem to make a good decision and you don’t need to decide yet.

However, if you are experienced in chemistry and statistical analysis and understand the chemical system you are studying and its spectral response, you may feel comfortable selecting a calibration option now. Use the Explain Help feature of your TQ Analyst software to see descriptions of the calibration options available in TQ Analyst or see the chapter called “Principles of TQ Analyst” in this document.

It is best to select the simplest technique that is capable of modeling the *absorbance* versus concentration response of the *samples* you want to analyze. You can always switch to another technique later if the results from the first attempt are unsatisfactory or try several techniques and choose the one that calculates component concentrations with the most accuracy.

Each time you change the analysis type, the main *TQ Analyst window* is reconfigured so that only the method tabs and parameters which are needed to develop the type of method you selected are displayed on the screen. If you switch the analysis type between two options in the same group, for example if you change from Simple Beer’s Law to CLS, the information you entered and the settings you chose for the previous analysis type will be saved.

Choosing a pathlength option

The parameters on the Pathlength tab define how the method will determine sample *pathlength* or thickness. Sample pathlength is important to quantitative measurements using spectral data because the intensities of the *peaks* in a *sample spectrum* are determined by the thickness as well as the composition of the *sample*. For more information on the effects of sample pathlength on quantitative measurements, see the chapter called “Principles of TQ Analyst” at the beginning of this document.

There are several ways to account for differences in sample pathlength for quantitative methods, even if the exact pathlengths are unknown. To choose a pathlength treatment, select an option for the Pathlength Type parameter on the Pathlength tab.

To see detailed descriptions of the pathlength options for quantitative methods, click the Explain button on the toolbar to open the Explain help window and then click each option in the software.

Pathlength Type

- Undecided
- Constant
- Known
- Predict
- Internal reference ($A=k*b*c$)
- Peak ratio or Normalize ($A/b=k*c$)
- Multiplicative signal correction (MSC)
- Standard normal variate (SNV)

In most cases, your *sampling technique* will dictate the correct pathlength option (frequently Constant or Known). If the pathlengths of your samples aren't constant or known, we recommend setting the pathlength option to “Undecided.” Using this option will allow the TQ Analyst Pathlength wizard to recommend a pathlength setting for your method. The Pathlength wizard performs a statistical analysis of the spectral data from the *standards* to find the spectral peak or *region* that correlates best with variations in sample pathlength.

Keep in mind, however, that the method must be fairly complete before the Pathlength wizard can recommend a pathlength setting

other than Constant or Known. For example, you must define the *components*, collect and identify the standards and select the *spectral regions* to be used for the analysis before the wizard can recommend a pathlength correction, such as Internal Reference or Multiplicative Signal Correction. If you select the “Undecided” option for Pathlength Type, define your components and standards and then use the Regions wizard to recommend spectral regions for your method, the Regions wizard will recommend a pathlength option as part of the region selection process.

You may also try several pathlength types and then choose the one that produces the best results. Here are some brief guidelines:

<u><i>If sample pathlengths are...</i></u>	<u><i>Use this pathlength type...</i></u>
Always the same	Constant
Variable and known (or easily measured)	Known
Variable and unknown (or not easily measured)	Predict Internal Reference Peak Ratio MSC SNV

Some of the pathlength options require additional information, such as a default pathlength value or a *pathlength peak*. If a pathlength option requires additional information, parameters for entering the information will appear on the Pathlength tab when that pathlength option is selected. If you choose the Predict pathlength option, additional parameters to define the predicted pathlength component are displayed on the Components tab.

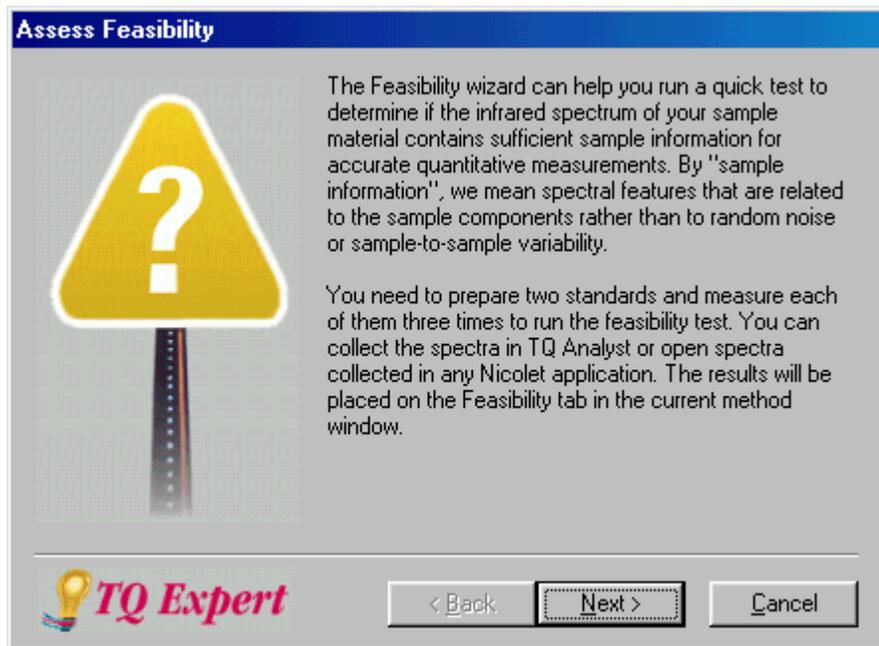
Note The software offers more pathlength options for the multivariate quantitative techniques (CLS, SMLR, PLS and PCR) than for the Simple Beer's Law technique. More pathlength options are available for multivariate analyses because a multivariate *calibration model* is better able to identify subtle changes in the spectral data that are due to differences in sample pathlength. If sample pathlength is unknown and difficult for you to measure, a multivariate analysis may be required to accurately analyze your samples. ▲

Running the feasibility test

When developing a quantitative method, you can run a quick test to determine if there is sufficient sample information in the *infrared* spectra for accurate quantitative measurements. By “sample information,” we mean spectral features that are related to the sample *components* rather than to random *noise* or sample-to-sample variability.

The *feasibility test* can be run as soon as you've finished setting the parameters on the Components tab. To start the feasibility test, click the Assess Feasibility button on the Components tab.

The first screen for the Assess Feasibility wizard appears on your displayed.



The Assess Feasibility wizard guides you through a series of steps to collect or open the test spectra, define the *spectral region* you want to assess and run the test. You need to prepare only two *standards* and to measure them each three times to test for spectral variance. The two standards must contain different concentrations of each component you want to measure. The exact concentrations of the components in the standards are not important; but make sure the concentrations fall within the component *analysis limits* you specified. See “Specifying Analysis Limits” in the previous section for more information.

You can collect the spectra in TQ Analyst (the wizard steps you through data collection) or open spectra that were collected in another Thermo Scientific spectral analysis application, such as OMNIC, Integra or RESULT. The wizard lets you specify how the data will be acquired.

Note If the Collect Standard button does not appear in the Feasibility wizard, data collection is disabled and you must use your spectral analysis application to collect the standards for the feasibility test. When you are finished collecting the standards, use the Feasibility wizard to open the feasibility standards and run the feasibility test. See the documentation that came with your Thermo Scientific spectral analysis software, such as RESULT, for information on collecting standards. ▲

The feasibility test is a standard analysis of variance (*ANOVA*) statistical analysis that calculates both variation due to sample composition and variation due to *sampling technique*. When the test is completed, a Feasibility tab is added to the *method window*. The test results are displayed on the Feasibility tab along with a recommendation of whether you should continue developing your method.

If the test results indicate that your method is not feasible, adjust the measurement parameters or try another sampling technique and then rerun the feasibility test.

If these adjustments don't change the feasibility test results, there is either too little variation between standards or too much variation between measurements of one or both standards. You must identify and eliminate the source of the problem before proceeding with method development. A small variation between standards usually occurs when the absorptions that are due to the species being measured are too weak. A low signal-to-noise ratio or inconsistent sampling technique can produce a large variation between measurements of the same standard.

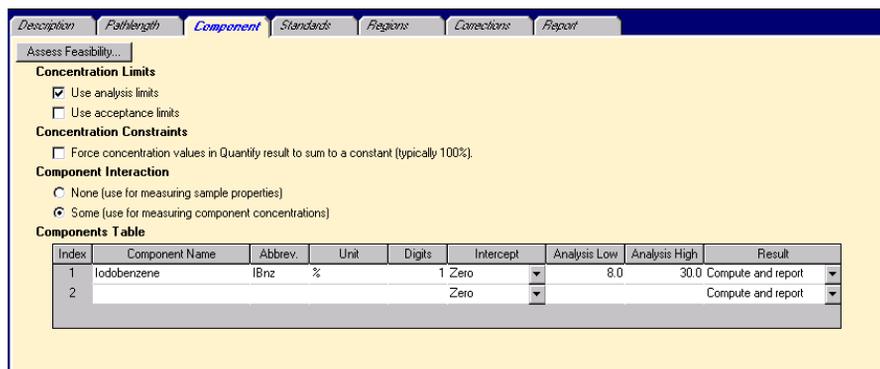
In some cases, the problem simply cannot be solved by *spectral measurement*. The Assess Feasibility wizard is designed to identify problems with the analysis early in development so you can avoid unnecessary work.

Defining the method components

The next step in developing a new method is to specify what you want to measure. Use the Components tab to enter information about the *components* in a quantitative method, such as the component names and the unit that will be used to report their concentrations.

This section covers the basic options that are important to defining components for any quantitative method. Depending on the current setting for Analysis Type (see the Description tab), other component settings may appear on the Components tab. For example, you may be able to set an option that forces the *concentration values* to sum to a constant or one that forces a component *calibration curve* through the origin. Use the Explain feature of TQ Analyst to see detailed descriptions of these and other options displayed on the screen.

To see detailed descriptions of the items on this tab, click the Explain button on the toolbar to open the Explain help window and then click the item in the software.



Assess Feasibility...

Concentration Limits

- Use analysis limits
- Use acceptance limits

Concentration Constraints

- Force concentration values in Quantify result to sum to a constant (typically 100%).

Component Interaction

- None (use for measuring sample properties)
- Some (use for measuring component concentrations)

Components Table

Index	Component Name	Abbrev.	Unit	Digits	Intercept	Analysis Low	Analysis High	Result
1	Iodobenzene	IBnz	%	1	Zero	8.0	30.0	Compute and report
2					Zero			Compute and report

Components Tab

Entering the component names

A unique name must be specified for each *component* the method will measure. The component names are entered in the Components table on the Components tab. When you use the method to analyze a *sample spectrum*, each component name will be displayed or printed next to the corresponding *concentration value*.

To enter the component names:

- 1. Select the Components tab.**
- 2. Click in the first row of the Component Name column, type the name of the first component and press Enter.**

When you press Enter, the software adds a blank row to the end of the table.

- 3. Continue entering names until all of the components have been specified.**
- 4. Use the Abbrev column to enter an abbreviated name for each component.**
- 5. Use the Unit column to enter the unit that will be used to measure the concentration of each component.**

For example, if a component is measured in milligrams per liter, enter mg/liter for the unit.

6. Use the **Digits** column to specify the number of digits after the decimal symbol that will be used to enter and report the concentration values for each component.

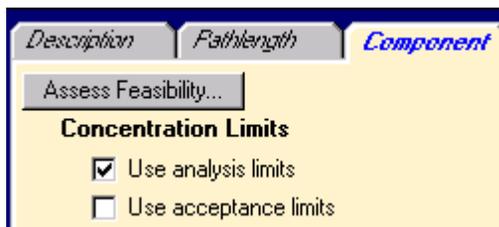
Note The digits parameter defines the number of digits that are displayed in TQ Analyst or printed with the *analysis results*, not the number of significant digits that are used in the calculations. ▲

Specifying analysis limits

You should also define the limits of the concentration range you want to measure for each *component*. These limits are needed to use the method performance and *sample checking* features of TQ Analyst as well as the Analysis Type wizard. We'll talk more about these options later.

To specify analysis limits for the components in your method:

1. Turn on the check box for **Use Analysis Limits** at the top of the **Components** tab.



When the check box is on, TQ Analyst adds two columns to the Components table.

2. In the column labeled “Analysis Low,” enter the lower limit of the analysis range for each component.

3. Use the column labeled “Analysis High” to specify the upper limit of each analysis range.

For optimum results, make the upper and lower limits of the analysis range about 5 percent higher and lower than the highest and lowest concentration values you expect to find in the unknown samples.

Note You must enter analysis limits for each *component* in order to use all of the wizards available in TQ Analyst software. ▲

Note If you enter analysis limits for the components in your method, you can use the *analysis limits sample checking* feature to monitor the results from each sample. When analysis limits sample checking is active, a warning can be displayed or printed with the *analysis results* when a component *concentration value* falls outside the specified limits. See the chapter on “Preparing A Method For Sample Analysis” in this document for more information on sample checking. ▲

Note Analysis limits are used to scale the method *performance index*. If you change the analysis limits for one or more *components* after the method has been *calibrated* once, the new performance index may be different from the performance index for the previous calibration. See the section titled “Calibrating the Method” in this chapter for more information on the performance index. ▲

Collecting the standards

When you are finished defining the method *components*, running the *feasibility test* and selecting a pathlength option, you are ready to collect the *standards* that will be used to develop and validate the *calibration model*. This section defines the various kinds of standards used in a TQ Analyst method and explains how to choose standards and collect their *infrared* spectra.

What are standards?

For *quantitative analysis*, *standards* are *samples* which have known concentrations of each *component* the method will be used to measure. TQ Analyst uses three types of standards for quantitative methods: *calibration standards*, *validation standards*, and *correction standards*.

For now, we are only interested in standards used for calibration and *validation*. See the chapter on “Correcting Component Linearity” in this document for information on using correction standards in a TQ Analyst method.

Calibration standards are used during the method calibration step to build a *calibration model*, which relates spectral features to *component* concentration. In order to do this, you must specify the concentration of each component in each calibration standard. We may also refer to the calibration standards as the “*calibration set*.”

Validation standards are used only to evaluate the performance of a *calibrated* method. Since validation standards are not used to calculate the calibration model, they are an unbiased test of performance. Each time you calibrate a method, TQ Analyst uses the new calibration model to predict the component concentrations of the validation standards. Because the composition of the validation standards is known (you must supply this information just as you do for calibration standards), TQ Analyst can calculate the difference between the calculated concentrations and the actual concentrations.

Validation standards – a measure of performance

Although validation standards are not required to calibrate or run a TQ Analyst method, they are key to the TQ Analyst philosophy. The software uses the validation standards to calculate a method *performance index* after each calibration. You can use the performance index to track the performance of your method as you develop or modify it. We strongly recommend that you include a few validation standards in every method you develop. If you don't, you will miss out on this powerful feature of your TQ Analyst software.

Choosing standards for quantitative methods

Choosing the proper *standards* is probably the most critical factor in achieving accurate quantitative results. For example, if you don't include enough standards or don't distribute their concentrations or other properties throughout the specified *analysis range*, the *calibration model* may not accurately describe the behavior of the *components* you want to measure. In addition, some method types won't work if specific conditions aren't met. A CLS method, for example, won't give accurate results if the concentrations of the components in the standards don't vary independently.

When using TQ Analyst software, there are two ways to select standards for a quantitative method. You can do it yourself, based on your experience and the guidelines provided here and elsewhere in this document. Or, you can use the software to help you determine the proper standards for your analysis.

There are three techniques for selecting standards: the Suggest Standards wizard, the Evaluate Standards wizard and the Select Standards diagnostic. The Suggest Standards wizard recommends the number and approximate concentrations of the standards for the current method. This is useful only if you are making new standards from scratch. If you already have standards or spectra from materials that could serve as standards, you can import the spectra and then use either the Evaluate Standards wizard or the Select Standards diagnostic

to determine which ones to include in the method. All three of these techniques follow the recommended guidelines for selecting standards for quantitative methods but let the computer do most of the work.

The three techniques for selecting standards are explained in the sections that follow.

Using the Suggest Standards wizard to select standards

The Suggest Standards wizard provides the number and approximate concentrations of the *standards* that are necessary to create an accurate *calibration model* for each *component* over the specified *analysis range*. Both calibration and *validation standards* are recommended.

Note The Suggest Standards wizard is useful only if you are making new standards. If you already have standards or spectra from materials that could serve as standards, you can import the spectra and then use the Evaluate Standards wizard to determine which ones to include in the method. See the next section for more information. ▲

You can use the Suggest Standards wizard when the Standards table is blank. However, you must enter *analysis limits* for each component before using this wizard. See the section called “Defining the Method Components” for instructions. If the Standards table contains spectra or concentration values, the Suggest Standards wizard will evaluate the current standards and then recommend any additional standards it feels are necessary.

The wizard uses the information in the Components table to construct a multidimensional sample space. Then it uses statistical guidelines to randomly select candidates from the sample space model. This process is repeated until the software generates a complete set of calibration and validation standards.

To start the wizard, click the Suggest Standards button on the Standards tab. The software displays a message asking whether you

want recommendations for the minimum, typical, or optimum number of standards. After you respond to the message, the software fills in the Standards table with approximate concentration values for the recommended standards. The recommended standards are titled "No spectrum assigned."

When the wizard is finished, you must prepare the standards, collect their spectra, and specify accurate concentration values for each component in each standard. All of these operations are described in later in this chapter.

Using the Evaluate Standards wizard to select standards

The Evaluate Standards wizard reviews the *standards* that are currently in the Standards table and lets you know if additional standards are recommended. It can also recommend appropriate concentrations for the new standards. Both calibration and *validation standards* are recommended.

Note The Evaluate Standards wizard is useful only if the Standards table already contains standards or spectra from materials that could serve as standards. See the section titled "Importing the Calibration Spectra" later in this chapter for instructions on importing standards. If you are making new standards, you can use the Suggest Standards wizard to recommend the number and approximate concentrations of the standards to include in the method. See the previous section for more information. ▲

The Evaluate Standards wizard works only when the Standards table contains spectra or *concentration values* for at least one standard. You must also enter analysis limits for each component before using this wizard. See the section called "Defining the Method Components" for instructions.

Note If you want the software to recommend standards based on the spectral data for a group of known samples, use the Select Standards command in the Diagnostics menu rather than the Evaluate Standards wizard. See the chapter on “Method Diagnostics” for more information. ▲

The wizard uses the information in the Components table to construct a multidimensional sample space. Following statistical guidelines, the wizard matches the spectral and/or concentration data in the Standards table with candidates from the sample space model.

To start the wizard, click the Evaluate Standards button on the Standards tab. When the wizard is finished, the software reports a “percent spanned” value, which indicates the percentage of the analysis range for each *component* that is described by the standards. Instructions on how to interpret the reported value are also provided. See “Evaluating Standards” in the chapter called “Working With Standards” for more information.

Note If you want TQ Analyst to recommend which standards to add, run the Suggest Standards wizard when the Evaluate Standards wizard is completed. See the previous section of this chapter for more information. ▲

When the wizard is finished, you must analyze the standards and then specify accurate concentration values for each component in each standard. Both of these operations are described in the sections that follow.

Selecting standards manually

You may prefer to select your own *standards*, based on your experience in chemistry and statistical analysis. If you want to select the standards for a quantitative method, consider the following guidelines before you begin.

- **Use sufficient quantity.**

To determine the number of standards required, consider the number of components and the range of concentrations you expect to find in the unknown *samples*. The more components there are, the more standards are required. Similarly, the wider the concentration range, the more standards are required. In general, the more standards used to create the calibration model the better it will represent “real world” samples.

Be sure to include at least two additional standards that can be used to validate the model. The concentration of each component in the *validation standards* should fall within the specified concentration range for each component in the method. See the section titled “What are Standards?” to learn how TQ Analyst uses validation standards to measure method performance.

You should also consider the calibration technique that will be used and any requirements it has for choosing standards. Refer to the section called “Principles of TQ Analyst” at the beginning of this document for more information.

- **Use representative mixtures.**

The composition of the standards should be similar to the composition of the samples you want to measure. This insures that spectral *baselines* are handled properly and that all substances in the sample are properly modeled.

For example, if your method must measure olefins in the presence of aromatics, your standards should be mixtures of olefins and aromatics. If you used the spectra of pure olefin for the standards, the *calibrated* method would not know how to deal with the additional presence of the aromatics.

- **Use representative concentrations.**

If you know that the concentration of a particular component will vary in “real world” samples, do not prepare standards in which the concentration of that component is constant. The component concentrations for the calibration standards should reflect the expected concentration range for each component that will be measured.

Generally, you should bracket the expected concentrations with your standards. For example, if you are measuring a single component, make one standard with a component concentration that is slightly lower than the lowest concentration you expect and make another standard with a component concentration which is slightly higher than the highest concentration you expect. The remaining concentrations should fall somewhere in between. *Never use a method to calculate concentrations beyond the concentration range of the standards.*

It is extremely important that you verify the reported concentrations of the standards. Your quantitative results can be, at best, only as accurate as the standards used to create the calibration model.

- **Vary concentrations independently.**

The concentrations of the components in the standards should vary independently. Avoid using serial dilutions of one standard to make the other standards.

For example, suppose you have a two component mixture for one standard and then you dilute it to make a second standard. The concentrations of the components in the second standard will be different from those in the first standard. However, the variation in each component concentration will be the same; that is, the component concentrations may not vary independently. As a

result, the *calibrated* method will not be able to distinguish one component from the other even though you have two standards.

- **Avoid using zero concentrations.**

When preparing solid or liquid mixture standards that may have molecular interactions, make sure each component in the standards has some reasonable concentration. In other words, don't allow the concentration of any component to be so low that it is essentially zero. This ensures that the calibration model accounts for molecular interactions, which frequently occur between components in liquid and solid *sample mixtures*.

Use the Standards table on the Standards tab to design your experiment. Following the guidelines provided above, enter proposed concentration values for all of the standards you plan to make.

The column headings on the right side of the Standards table should show the name and *measurement unit* for each component the method is set up to analyze. Use these fields to fill in the concentration of each component in each standard. Make sure the concentration values you enter are correct and match the specified unit. If the component columns do not appear in the Standards table, switch to the Components tab and fill out the Components table. See the section called “Defining the Method Components” earlier in this chapter for instructions.

When you are finished, let the TQ Analyst Standards wizard evaluate your experiment design. To start the wizard, click the Evaluate Standards button on the Standards tab. The wizard reviews the concentration values are currently in the Standards table and lets you know if additional standards are recommended. It can also recommend appropriate concentrations for the new standards. See “Evaluating Standards” in the chapter called “Working With Standards” for more information.

Some people prefer to design the experiment in a spreadsheet file, such as Microsoft® Excel®, and then paste the concentration data into the Standards table. See “Importing the Calibration Data” in this chapter for information on setting up the spreadsheet file and copying the data to the Standards table in TQ Analyst.

When you are finished designing the experiment, you must prepare the standards, collect their spectra, and specify accurate concentration values for each component in each standard. All of these operations are described in the sections that follow.

Preparing and analyzing the standards

When you are finished designing your experiment and testing the design, it is time to prepare and analyze the real *standards* and update the concentration data.

If you are making the standards now, carefully prepare the component mixtures following the guidelines set up in your experiment. As you prepare the component mixtures, there will inevitably be differences between the proposed concentrations and the actual values. For example, if you are preparing mixtures of caffeine and starch, standard #1 may call for 10.85 mg caffeine and 4.73 mg of starch. As you weigh the materials, carefully enter the actual concentration values and units into the Standards table or spreadsheet file, if one was used. Make sure the concentration values you enter are correct and match the specified unit. Some labs require that standards are analyzed by another (non-infrared) method to verify or determine their actual concentration values before using them in a quantitative method.

If you imported the calibration spectra, use a non-infrared method to determine the actual concentration values of the standards and then enter the actual concentration values in the Standards table or spreadsheet file, if that is preferred. Make sure the concentration values you enter are correct and match the specified unit.

Collecting the spectra of the standards

Once the *standards* are prepared and the correct concentration data recorded, the spectra must be carefully collected and stored on a disk. You can use TQ Analyst or another Thermo Scientific spectral analysis package, such as OMNIC, Integra, or RESULT, to collect the spectra of the standards.

Before collecting the spectral data, you must make sure the *data collection parameters* are set to optimum values. See the next section for details.

Note If you imported the spectra of the standards, skip the sections on optimizing the collection parameters and collecting the spectra of the standards. ▲

Optimizing the collection parameters

The *standards* must be collected in the same way you will collect the spectra of the unknown *samples*, using the same data collection parameters, if that is possible. The spectra of the standards should be of the highest achievable quality. Assuming that the *sampling technique* is reproducible, spectral quality is determined mainly by the *resolution* and *signal-to-noise ratio* of the spectral data. The parameters that define resolution and signal-to-noise ratio are part of the data collection parameter set.

If you are using TQ Analyst to collect the calibration spectra, use the Collection Parameters button on the Standards tab to display the data collection parameters. See the chapter called “Working With Standards” in this document for more information.

Note If the Collection Parameters button does not appear in your TQ Analyst application, data collection is disabled in TQ Analyst. You must use your spectral analysis application, such as RESULT, to set the data collection parameters. See the documentation that came with your spectral analysis software for more information. ▲

If you are using another Thermo Scientific spectral analysis package to collect the calibration spectra, see the documentation that came with that software for instructions on setting the data collection parameters.

Once the parameters have been set, we recommend using the chosen settings to collect all of the calibration and *validation standards* as well every unknown sample you use the method to analyze.

Note If you change the setting for a collection parameter after opening or collecting the spectra of one or more standards, TQ Analyst may warn you that the parameter setting should not be changed at this point. You will be given the option of overriding the warning or creating a new method with the new parameter settings. ▲

If you are analyzing gases, the spectra of the standards and samples should be collected at the same pressure and approximately the same temperature. This means if you have a concentrated gas standard that you need to dilute in order to lower its absorbance levels, you should back fill the cell to the targeted pressure with a gas that does not absorb in the *analysis range*, such as nitrogen or argon, or use a gas divider.

Collecting the spectral data

When you are finished setting (and testing) the *data collection parameters*, you are ready to collect the spectrum of each *standard*. You can use TQ Analyst, another Thermo Scientific spectral analysis package, such as OMNIC, Integra, or RESULT, or another application to collect the calibration spectra.

To collect the spectrum of a standard in TQ Analyst, click the Collect Standard button on the Standards tab.

Note If the Collect Standard button does not appear in your TQ Analyst application, data collection is disabled in TQ Analyst. You must use your spectral analysis application, such as RESULT, to collect standards. See the documentation that came with your spectral analysis software for more information. ▲

The process for collecting a spectrum in TQ Analyst is exactly like the process for collecting a spectrum in your Thermo Scientific spectral analysis software. Refer to the documentation that came with that software for step-by-step instructions.

Depending on how the data collection parameters are set, you may be prompted to collect a *background spectrum* or enter a *spectrum title* during data collection. If you don't enter a title during collection, the date and time of collection will be used. The new spectrum will be added to the open row at the bottom of the Standards table and saved in the method *standards library*.

Note When you collect a spectrum to use as a standard, TQ Analyst saves only a portion of the collection and processing information that is normally stored in a Thermo Scientific spectral data file. If you want to save all of the collection and processing information for your standards, save the standards as Thermo Scientific spectral data files by turning on the Save Automatically *checkbox* in Experiment Setup before collecting the standards. (Click the Collection Parameters button on the Standards tab to display the Experiment Setup dialog box. Save Automatically is located in the File Handling group on the Collect tab.) ▲

As you collect the spectra, be sure to note any special sampling conditions that may affect the spectral data, such as the temperature and pressure of a gas cell, the cell *pathlength* or the packing pressure of a diffuse reflectance accessory. If you used TQ Analyst software to collect the standards, this information can be stored with the *data collection parameters* in the Experiment Description entry box. To see

the Experiment Description entry box, click the Collection Parameters button on the Standards tab. When you are finished entering the experiment description, click Save and then click OK to close the dialog box.

If you are collecting the spectra outside of TQ Analyst, be sure to carefully name and save each spectrum in a file when you are finished collecting it. If you are using a software package from another manufacturer to collect the calibration spectra, be sure to save the spectra in a format that is compatible with TQ Analyst. See the section titled “Opening a spectrum to use as a standard” in the chapter called “Working With Standards” for a list of compatible file types.

Importing the calibration spectra

If you used another spectral analysis package to collect the calibration spectra rather than collecting them directly in TQ Analyst, you must import the spectra into TQ Analyst in order to complete your method.

Use the Open Standard button on the Standards tab to import the spectra of the *standards*. Begin by clicking anywhere in the first blank row of the Standards table. Then click the Open Standard button and select the first file you want to import.

TQ Analyst allows you to open spectra that were saved using TQ Analyst or spectra that are in another format, or “file type,” such as GRAMS386 or JCAMP-DX. To list all the files in the indicated directory, select All Files (*.*) from the Files of Type drop-down list box.

If you want to open spectra you collected in an application from another manufacturer, make sure you save the spectra in a compatible format before attempting to import them into TQ Analyst. See the section titled “Opening a spectrum to use as a standard” in the chapter called “Working With Standards” for a list of compatible file types.

The spectrum is added to the list of standards and saved in the method *standards library*. If this is the first standard in your method, the data collection parameters in the current method will be set automatically to match the settings that were used to collect the spectrum you opened.

You can add each spectrum individually or select a number of spectra and add them all at once. If the spectra are stored in a spectral group file (.SPG extension), set the File Type list box to “.SPG” and then select the group file you want to add.

If you selected multiple spectra to open or opened a spectral group (.SPG) file, all of the selected spectra will be added to the end of the Standards table. New rows are added to the table to accommodate the number of standards in the group.

Note If a warning message about mismatched standards appears when you try to add a standard, the parameter settings used to collect the new standard are significantly different from the settings that are saved with the current method. For optimum performance, all method standards should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the standard again using the proper settings for the collection parameters, edit the settings in the current method, or ignore the warning and continue adding standards. ▲

Importing the concentration values

If you set up the concentration data in a spreadsheet file, rather than entering the values directly in TQ Analyst, you must import the concentration data into TQ Analyst in order to complete your method. Follow the instructions provided below to set up the spreadsheet file. Then use the copy and paste features of Excel and TQ Analyst to import the *concentration values*.

Setting up a spreadsheet file

Some people prefer to enter the concentration data for the *standards* in a spreadsheet file, such as Microsoft® Excel®, and then paste the data into the Standards table. To do this, follow the template below to set up the spreadsheet file.

Standard	<i>Concentration Values</i>		
	Component 1	Component 2	Component 3
1	0.462	1.037	0.869
2	0.628	1.954	1.027
3	0.937	2.895	1.359

Note If you've already entered the component names in the Components table in TQ Analyst software, make sure the components are listed in the spreadsheet file in the same sequence. For example, if Glucose appears in row 1 of the Components table, make sure Glucose appears in column 1 of the spreadsheet file. If you need help, see the section on "Entering the Component Names" earlier in this chapter. ▲

Importing the spreadsheet data

When you are finished entering or updating the *concentration values* in the spreadsheet file, such as Microsoft® Excel®, you are ready to import the data into the Standards table in TQ Analyst.

To import the concentration values for the standards:

1. **Open TQ Analyst software and do the following:**

- Click the Components tab and enter the component names in the Components table, if you haven't done this already. If you want to copy the entire table of concentration values in one operation, make sure the components are listed in the Components table in the same sequence as they appear in the spreadsheet file.

- Click the Standards tab and make sure there are enough empty rows in the Standards table to add all of the data you want to copy. To add each blank row to the Standards table, click in the blank Spectrum Title cell at the bottom of the table, enter a placeholder name and then press the Enter key on your keyboard. If the Standards table already contains the file names and/or titles of the calibration spectra, make sure the standards are listed in the Standards table in the same sequence as they appear in the spreadsheet file.

2. Switch to the spreadsheet application and use the Copy command to copy only the concentration values onto the Clipboard.

Start with the first data cell in the upper left corner of the spreadsheet.

3. Select the TQ Analyst application, click the Standards tab and select the first cell that you want to paste the data into.

In most cases, this should be the first concentration column for the first component.

Standards Table

Index	Display	Spectrum Title	Usage	C1 %	C2 %	C3 %
1			Calibration ▾	0.00	0.00	0.00

4. Choose Paste from the TQ Analyst Edit menu to paste the data into the Standards table.

Once the data are in TQ Analyst, you can use a wizard to review the concentration values. To start the wizard, click the Evaluate Standards button on the Standards tab.

Specifying Usage

The column labeled “Usage” in the Standards table allows you to specify how each *standard* will be used in the method. To set the usage for a standard, simply choose an option in the Usage list box.

For quantitative methods, you can set the usage to “calibration,” “validation,” “correction,” or “ignore.”

Standards designated as “Calibration” will be used to create the *method model* during calibration. In TQ Analyst, *calibration standards* are also used to calculate a *correction curve*, if one is specified.

Validation standards will be used to evaluate the performance of a method once the method is *calibrated*. The results from the validation standards are also used to calculate the *performance index*.

Correction standards are used along with the calibration standards to calculate a *correction curve*, if one is specified. Correction standards are not used in calibration. See the section called “Correcting Component Linearity” in this chapter for more information.

Use the “ignore” setting to temporarily exclude a standard from the method.

Selecting the analysis regions

The final step in building a method is to identify the spectral information in the *standards* which correlates best with the *components* you want to measure. Similar to the process for selecting the standards, choosing the component peaks or regions is another difficult task in quantitative method design. Specialized training in chemistry and spectral interpretation is sometimes required, especially when choosing regions for a CLS or PLS analysis.

If you are creating an SMLR method, you may want to divide one or more of the selected regions by another peak or region by specifying a denominator peak. If you specify denominator peaks, the software uses the second region for each component as the denominator peak.

The following sections describe how to use the wizard to select the *analysis regions* for your method and explain how to specify denominator peaks for an SMLR method.

Using the wizard to select the analysis regions

Use the Regions tab to select the *analysis regions* and specify how they will be measured. If you need help choosing the proper analysis regions, take advantage of the software's built-in expertise by using the Suggest Regions wizard. The wizard performs a statistical analysis of the *calibration standards* and then recommends one or more spectral *peaks* or regions for each *component* the method is set up to measure. It also indicates how the peaks or regions should be measured and what *baseline* should be used.

If you are creating a quantitative method, the wizard starts automatically when you select the Regions tab. When you are finished interacting with the wizard, click the Edit Regions button on the Regions tab. The suggested peaks or regions are displayed in the Region Selection task window. The peaks or regions the wizard recommends will depend on the spectral information for the standards and the current settings for the Analysis Type and Pathlength Type parameters.

Note If the Analysis Type or Pathlength Type parameter is set to “Undecided,” TQ Analyst will choose appropriate settings for one or both parameters while recommending *analysis regions*. When selecting a calibration technique (Analysis Type) and pathlength treatment (Pathlength Type), the wizard chooses the simplest option that adequately models the chemical system to be measured. If the Pathlength Type is set to Internal Reference or Peak Ratio, which require a *pathlength peak*, but no pathlength peak is currently specified, the wizard will also recommend a pathlength peak. ▲

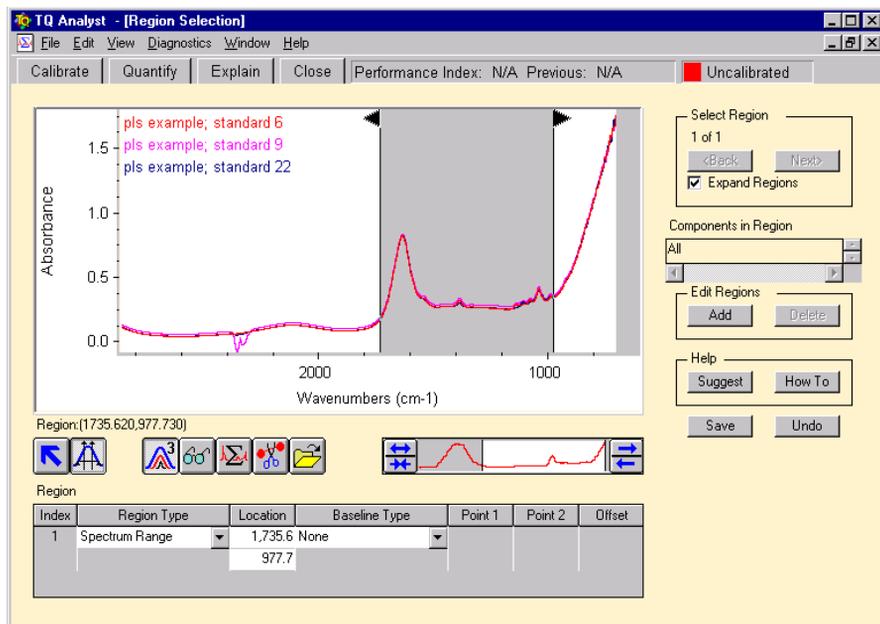
All of the wizard’s recommendations are based solely on the concentration and spectral information for the calibration standards. If the standards truly represent the unknown *samples* you wish to measure, the recommendations are statistically valid.

You are free to change the recommended Analyst Type or Pathlength Type setting or the recommended regions at any time. When setting these and other parameters in a quantitative method, we recommend using the simplest option that adequately models the chemical system to be measured. You can always switch to another technique later if the results are unsatisfactory or try several techniques and choose the one that calculates component concentrations with the most accuracy.

Note If you switch the analysis type between two options in the same group, for example if you change from Simple Beer’s Law to CLS, the information you entered and the settings you chose for the previous analysis type will be saved. ▲

To see a description of an item in this task window, click the Explain button on the toolbar to open the Explain help window and then click the item.

For step-by-step instruction on editing regions, click the How To button in the task window.



Region Selection Task Window

The spectra of the standards that contain low, medium and high concentrations of the components that absorb in region 1 are displayed in the graphical window. Additional information about the current region is provided in the Regions table, located at the bottom of the task window.

Since region selection is usually an iterative process, consider the recommended regions only as a starting point. You may need to adjust the regions or other settings in the Region Selection task window, such as the Region Type and Baseline Type, to achieve the best results. See the Explain and How To help for the Region Selection task window for instructions on optimizing regions using the tools in the Region Selection task window.

Any changes you make to the Region Selection task window will be saved automatically when you close the window. To close the Region Selection task window, click the Close button on the TQ Analyst *toolbar*.

To display the Region Selection window after one or more regions have been selected, click the Edit Regions button on the Regions tab.

Setting up a denominator peak or region for an SMLR method

You can design a method that calculates the ratio of your *component* peaks by setting Calculate Ratios on the Other tab. This can be useful for minimizing *pathlength* differences in the *standards* and *samples*.

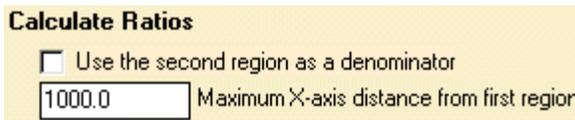
If you specify ratios, the software uses the second *region* for each component as the denominator peak. All of the other regions will be divided by the result for the second region.

Note If you want to limit the ratio to a single component peak, set the number of regions used for each component to 2. See the Component Regions table on the Other tab for details on specifying regions for SMLR methods. ▲

To define a denominator peak or region for the components in an SMLR method:

- 1. Click the Other tab.**
- 2. In the Calculate Ratios group, turn on the check box for Use Second Region As Denominator.**

To see detailed descriptions of the items in this group, click the Explain button on the toolbar to and then click the item in the software.



Calculate Ratios

Use the second region as a denominator

1000.0 Maximum X-axis distance from first region

3. Indicate whether you want to limit the region the software will use to locate a denominator peak by setting the check box for Limit Region For Denominator Peak.

This parameter is designed for advanced users who are familiar with SMLR analyses. Make sure you look at some typical spectra of samples and standards before deciding to limit the region for the denominator peak. People typically limit the region only when they want the software to locate a denominator peak that is in the same area as the first component peak. This helps ensure that the two peaks have similar shapes, which makes the ratio more accurate.

4. If you turned on the Limit Region For Denominator Peak check box, specify the region limits by entering a value for Maximum Distance From First Region.

Limit the region for locating the denominator peak by entering its maximum distance in data points from the peak maximum in the first component region.

Customizing the method

If you have been following the guidelines provided in this chapter, your method contains all of the information needed for calibration, including:

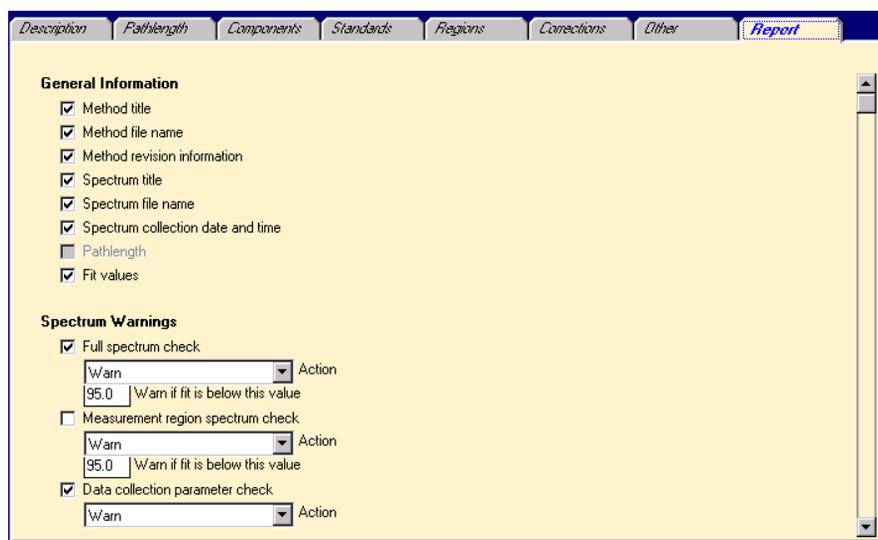
- Descriptions of the *components* being measured.
- Representative *standards* measured using reproducible *sampling technique*.
- *Spectral regions* which correlate to known component information.

Depending on the calibration technique (Analysis Type) you selected, there are other features and settings in the software that may be used to

customize your method. For example, if you want to calculate *derivative* spectra or apply smoothing or baseline correction, turn on the *check box* for Allow Spectral Processing on the Standards tab. (See the chapter called “Processing Spectral Data” in this document for more information.)

Use the parameters on the Report tab to configure the sample reports. See the section on “Setting Up Sample Reports” in the chapter called “Preparing a Method for Sample Analysis” for details.

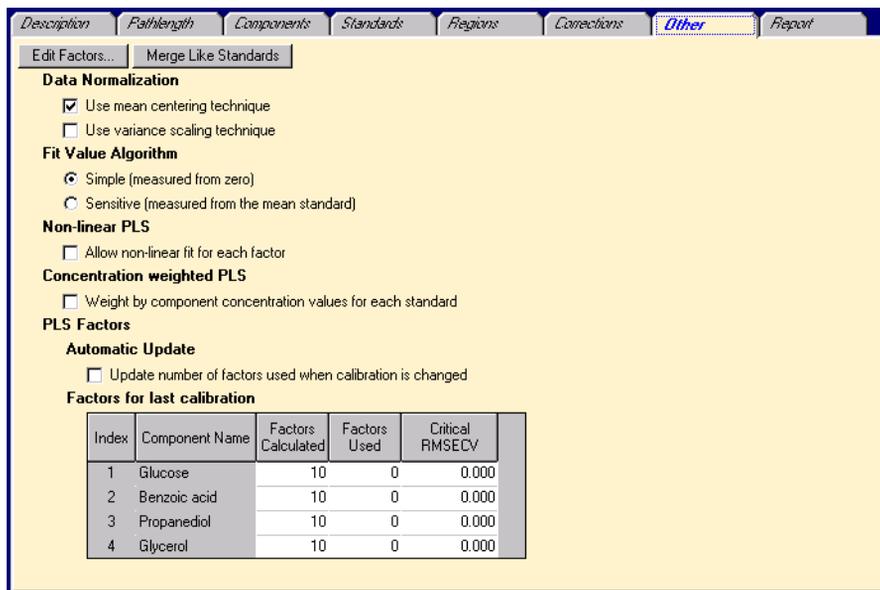
To see detailed descriptions of the features on the Report tab, click the Explain button on the toolbar to open the Explain help window and then click the feature.



Report Tab for PLS Method

The parameters on the Other tab allow you to customize certain features of a quantitative method, such as the number of *factors* used for a PLS method or the number of regions used for each component in an SMLR method.

To see detailed descriptions of the features on the Other tab, click the Explain button on the toolbar to open the Explain help window and then click the feature.



Other Tab for PLS Method

Feel free to consider or try all of the features included in your method. If you don't have time to review them or don't need special enhancements, leave the *default settings* provided in the software. TQ Analyst is designed to provide a working method with a minimum of effort.

The method should produce acceptable results for most *sample* types when the *method parameters* are set to their default values. To see the default settings for a quantitative method, choose New Method from the File Menu and set the Analysis Type to one of the quantitative options.

If you prefer using other method specifications or have specifications you need to follow, you may change the settings for any method parameter at any time.

Saving the method

We recommend that you save your work regularly when creating or editing methods. Be sure to save the method again before you *calibrate* it.

To save your method:

- 1. Select the method you want to save by clicking the method window or by choosing the method name in the Window menu.**

- 2. Choose Save Method from the File menu.**

If the method does not have a file name, the Save Method As dialog box appears.

- 3. If the Save Method As dialog box appears, type a file name and select the directory and disk where you want the method saved.**

- 4. Choose OK.**

Calibrating the method

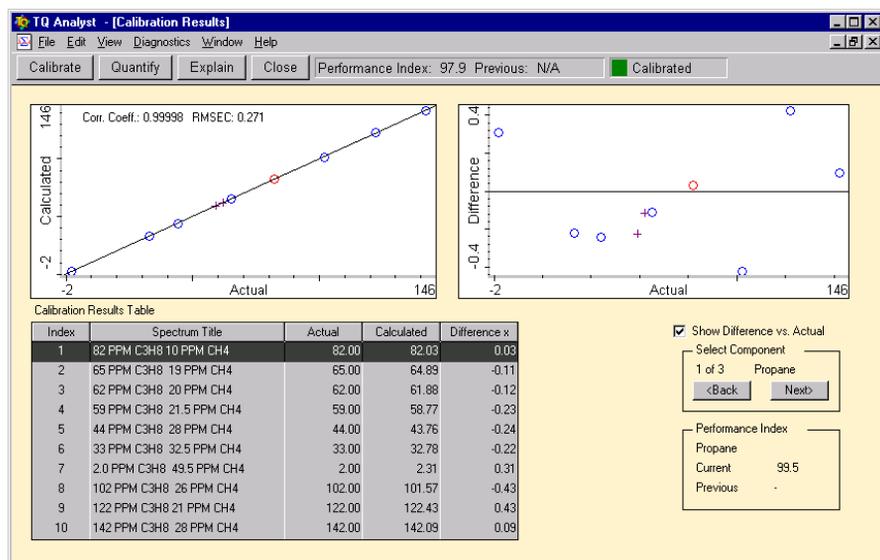
When you are finished selecting a calibration technique, choosing a pathlength option, defining the *components*, running the *feasibility test*, collecting the *standards*, selecting the *analysis regions* and saving, your method is ready for calibration. For quantitative methods, calibration produces the mathematical model that will be used to calculate component concentrations in the unknown *samples*.

To *calibrate* a quantitative method, click the Calibrate button on the TQ Analyst *toolbar*. Calibration may take 1 second or several hours, depending on the number of *calibration standards* used and the selected calibration technique. When calibration is completed, the calibration

readout on the TQ Analyst toolbar changes from red to green and the “Uncalibrated” message changes to “Calibrated.”

When you calibrate a quantitative method, the software calculates a *calibration model* and then uses the model to *quantify* the method's calibration standards. If *validation* and *correction standards* are specified in the method, the validation and correction standards are also quantified. The calibration data are displayed in the Calibration Results task window.

To see descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.



Calibration Results Task Window

The information provided in the Calibration Results task window can help you identify standards that may be *outliers*.

The table in the lower half of the task window shows the calculated and actual concentration values and the % difference value for each standard that contains the selected component.

Calibration Results Table				
Index	Spectrum Title	Actual	Calculated	Difference x
1	82 PPM C3H8 10 PPM CH4	82.00	82.03	0.03
2	65 PPM C3H8 19 PPM CH4	65.00	64.89	-0.11
3	62 PPM C3H8 20 PPM CH4	62.00	61.88	-0.12
4	59 PPM C3H8 21.5 PPM CH4	59.00	58.77	-0.23
5	44 PPM C3H8 28 PPM CH4	44.00	43.76	-0.24
6	33 PPM C3H8 32.5 PPM CH4	33.00	32.78	-0.22
7	2.0 PPM C3H8 49.5 PPM CH4	2.00	2.31	0.31
8	102 PPM C3H8 26 PPM CH4	102.00	101.57	-0.43
9	122 PPM C3H8 21 PPM CH4	122.00	122.43	0.43
10	142 PPM C3H8 28 PPM CH4	142.00	142.09	0.09

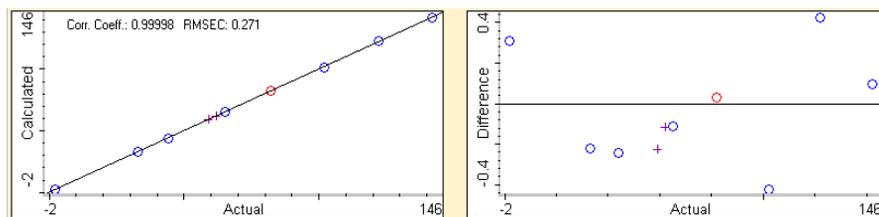
Calibration Table

Compare the % difference values for the *validation standards* to those of the *calibration standards*. If the validation standards consistently show larger difference values than the calibration standards, the calibration model may be *overfit*. This can result when too many *factors* are used in a PLS method or the polynomial order of a component *correction curve* is too high. If linearity corrections are specified in your method, make sure the polynomial order for each correction curve is the lowest order that provides the desired correction.

To see the error associated with each factor used in the current PLS method, click the Edit Factors button on the Other tab or run the PRESS diagnostic from the Diagnostic menu. You may edit the number of factors used in a PLS method from the Edit Factors window or the Other tab.

Note TQ Analyst performs a *cross validation* step as part of the calibration process for PLS methods. During cross validation, the software removes individual or multiple standards and then analyzes them as validation standards. If duplicate standards are present in the method, the software will remove and analyze all of them during the same cross validation iteration. This process removes some bias when calculating the recommended number of *factors* for a PLS analysis. We define “duplicate standard” as any standard that has exactly the same concentration values as another standard for all components in the method. ▲

Two plots are shown at the top of the task window.



Calibration Plots

The Calculated versus Actual plot compares the calculated concentration value to the actual concentration value for each standard that contains the selected component. The calculated values are the concentration values that were calculated using the calibrated method. The actual values are the known concentration values that were entered in the Standards table. The % Difference plot shows the differences between the calculated and the actual concentration values relative to the actual values.

All of the standards that contain the selected component and have their usage set to "calibration," "correction," and "validation" are represented in the plots. *Calibration standards* are represented with

circles, validation standards are represented with plus signs (+), and correction standards are represented with triangles. You use the Usage parameter on the Standards tab to set the usage of each standard.

If a method calculates concentration values perfectly (i.e., the calculated value for every standard matches its actual value exactly), the data points in the Calculated vs. Actual plot will form a line exactly 45 degrees from both axes and the data points in the % Difference plot will form a horizontal line at exactly zero % difference. A typical % Difference plot will show data points distributed randomly above and below the zero line within a narrow concentration range.

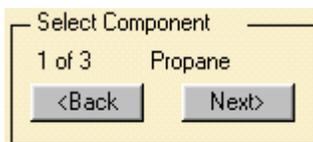
Outliers show up in the calibration plots as data points that are far away from the others. The greater the distance between a data point and these ideal lines, the greater is the difference between the calculated value and the actual value for the corresponding standard.

If you suspect that a standard is an outlier, check that the concentration and pathlength values were entered correctly in the Standards table (see Standards tab). You should also display the spectrum of the *outlier* standard. If the quality of the spectrum is poor, replace the spectrum with a new one. If the data look okay, consider the chemical makeup of the standard and how the standard is used in the method. For example, a validation standard is a better indicator of the accuracy of a *calibration model* than a calibration or *correction standard* because calibration and correction standards are used to generate the model while validation standards are not. It is up to you to decide whether or not an outlier standard should be included in the calibration or *validation set*.

If a pathlength column is displayed in the table, the concentration values shown in the plots have been adjusted for differences in *pathlength*. The pathlength adjusted concentration values are the calculated and actual concentration values displayed in the table multiplied by the appropriate pathlength value.

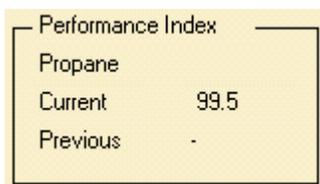
Each data point in the plots is linked to a standard in the table. To identify the standard that is associated with a data point in a plot, click the data point. The corresponding standard will be highlighted in the table. To find the data point that corresponds with a standard in the table, click anywhere in the row. The associated data point will be highlighted in the plots.

To select the next or previous component in the method, click the Next or Back button in the Select Component group. The name (or abbreviated name) and *index number* for the selected component are displayed above the buttons.



When you select another component, the concentration values for the new component are displayed in the Standards table and plots.

If the method includes at least two validation standards, TQ Analyst calculates a *performance index* for each component in the method as well as an overall performance index for all the components combined. The performance indices indicate how accurately a *calibrated* method can quantify the validation standards. The component performance values are displayed in the Calibration Results window (see below). The overall performance index appears on the TQ Analyst toolbar.



Performance Index	
Propane	
Current	99.5
Previous	-

Depending on the selected Analysis Type, additional performance information may appear on the Calculated versus Actual plot in the upper left corner of the Calibration Results window.

For Simple Beer's Law methods, the information string will look like the following:

$$Y = 0.0319x + 0.000 \quad \text{Corr. Coeff.: } 0.99990$$

where m equals the *slope* of the Calculated versus Actual plot and b is the Y-axis *intercept*. The correlation coefficient for the plotted values is also provided. The closer this value is to "one" the more linear is the relationship between the calculated and actual values for the selected component.

If you are calibrating a CLS, PLS or PCR method, the following information may also be provided:

$$\text{Corr. Coeff.: } 0.96762 \quad \text{Bias: } 0.231 \quad \text{RMSEC: } 0.165$$

The correlation coefficient provides the same information for these methods as it does for Simple Beer's Law. The RMSEC (Root Mean Square Error of Calibration) refers to the uncertainty of calibration for the selected component (square error values, calculate the average and then take the square root). Bias refers to a systematic difference between the expected values and the true values. Bias is reported only if the difference is significant (above zero).

When you are finished viewing the calibration results, click the Close button on the TQ Analyst toolbar to close the Calibration Results window.

Specifying the intercept for Beer's Law calibration

If you are creating a Simple Beer's Law method, you can specify whether you want to force each component *calibration curve* through the origin (0,0) or allow the curve to *intercept* the Y-axis at any point.

The ideal concentration versus absorbance curve is a straight line that runs through the origin (0,0). However, certain experimental conditions can increase the absorption readings for the *standards*. This effect is more pronounced in standards that contain low concentrations of the compound that is being measured than in the higher concentration standards. As a result, the real calibration curve may not be straight and it may not pass through the origin. The Intercept parameter allows you to choose whether you want to use the unaltered calibration data to calculate the calibration curve for your method or force the curve through the origin.

Use the Intercept list box in the Components table to specify the *intercept* for each *component* in your method.

To see descriptions of the columns in this table, click the Explain button on the toolbar and then click the column heading.

Index	Component Name	Abbrev.	Unit	Digits	Intercept	Analysis Low	Analysis High	Result
1	Iodobenzene	IBnz	%	1	Zero	8.0	30.0	Compute and report
2					Zero			Compute and report
					Nonzero			
					Zero			

Components Table

If the Intercept parameter is set to NonZero, TQ Analyst uses the measured absorbance values for all of the standards to calculate the best linear fit calibration curve.

When Intercept is set to Zero, the software forces the component calibration curve through the origin even if the data points from the standards that contain low concentrations of the component are no longer on the curve.

Note Forcing the curve to pass through the origin is not the same as adding the zero point to the calibration curve. Adding the zero point artificially weights the calibration curve in favor of passing through zero but does not force it to do so. If you want to add the zero point to a component calibration, add a *calibration standard* that contains zero concentration of the component to your method. ▲

Validating the method

The final step in developing a quantitative method is to validate the method. *Validation* simply means to verify that the method can analyze real world *samples* with sufficient accuracy.

There are several ways to validate a method using TQ Analyst software. For example, if method includes *validation standards*, you can use the *performance index* and the validation data displayed in the Calibration Results task window to evaluate performance. If you did not include validation standards in the method, you can prepare a few additional standards, analyze them with the completed method and use the results to validate the method. You can also use the Cross Validation diagnostic to validate a method. All of these options are discussed in the sections that follow.

Performance index

If the method includes at least one *validation standard*, TQ Analyst calculates a performance index during calibration. The performance index is a number that indicates how accurately a *calibrated* method can *quantify* the validation standards. TQ Analyst provides two algorithms for calculating the performance index for quantitative methods: *% Difference (default)* and *RMSE*.

Note To select which algorithm the software will use to calculate the performance index, choose Options from the Edit menu and then click the Diagnostics tab. See the glossary entries for *% Difference* and *RMSE* for details on these performance index algorithms. ▲

The software calculates a performance index for each *component* in the method as well as an overall performance index for all the components combined. The component performance values are displayed with the calibration results (see the previous section). The overall performance index appears on the TQ Analyst *toolbar*.

If you calibrate the method more than once, the overall performance value from the previous calibration is retained. This allows you to determine whether the changes you make to a method actually improve its performance. It's up to you to decide what is an acceptable performance value.

Validation data

The calibration data from the *validation standards* are also important indicators of performance. If the calculated values for the *components* in the validation standards are close enough to the actual values, your method is complete and ready for repeated *sample* analysis.

If you did not include validation standards, you can still validate the method by preparing a few additional standards and using the completed method to analyze them.

Note We do not recommend using *calibration standards* to validate a method. Since calibration standards are included in the *calibration model*, their results would be significantly biased. ▲

You can run the validation standards individually or use the External Validation diagnostic routine to run them automatically. External Validation can be used to analyze a large group of validation standards that are listed in a spreadsheet or text file. The validation data are placed in a spreadsheet (Microsoft® Excel®) or text file. See the chapter on “Method Diagnostics” in this document for more information on External Validation.

If the method calculates the component concentrations in the validation standards with sufficient accuracy, your method is complete and ready for repeated sample analysis.

Cross Validation diagnostic

If you are calibrating a quantitative method that does not include *validation standards*, you can use the Cross Validation diagnostic routine to evaluate the method's performance. The Cross Validation diagnostic quantifies each *calibration standard* in the method as if it were a validation standard. This is accomplished by sequentially removing the specified number of standards from the *calibration set*, calibrating the method and using the new *calibration model* to *quantify* the standards that were removed from the calibration set.

The process is repeated until all of the standards in the calibration set have been quantified as validation standards. You specify the number of standards to remove for each iteration in a dialog box that is displayed when you start the Cross Validation diagnostic. If the method calculates the *component* concentrations in the validation standards with sufficient accuracy, your method is complete and ready for repeated *sample* analysis.

Sources of error

If the % difference value for one or more *components* in a *validation standard* is too high, you must identify what caused the error and try to correct it. Some typical sources of error are listed below:

- The concentration of a component in a *standard* is incorrect.
- The concentration of a component in a standard was entered wrong.
- The standards don't accurately describe the component's absorbance versus concentration behavior (i.e., there are too few standards or the concentrations of the standards are not spaced correctly).

- Spectral errors caused by sampling, instrument *noise*, or strong absorptions are present in the calibration spectra.
- The *analysis region* or regions don't correlate well with changes in component concentrations.
- Other components in the standards are producing interfering *peaks* in the *analysis region* for this component.
- An inappropriate pathlength treatment was chosen. See “Choosing a Pathlength Option” in this chapter for more information.

Continue checking for errors and recalibrating the method until the *validation* results for all components are acceptable. If you are sure the method is free of errors but the calculated concentrations for one or more components still differ significantly from the actual concentrations and the errors tend to change regularly as a function of concentration, you may need to calculate a *correction curve* for that component. See “Correcting Component Linearity” for more information.

Correcting component linearity

If the *absorbance* vs. concentration plot for a *component* in a quantitative method is slightly curved instead of linear, you can use the Corrections tab to set up a *correction curve* for the component. You may also generate a bias correction.

To see descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.

Corrections Standards

Correct with Calibration and Correction standards
 Correct with Correction standards only

Corrections Table

Index	Component Name	Calculate	Force Through Zero	Constant	First Order	Second Order	Third Order
1	Propane			-0.639244	1.155565	-0.006913	0.000121
2	Methane			0.000000	1.162597	-0.033372	0.002251
3	Carbon dioxide			0.000000	1.006159	0.000000	0.000000

Corrections Tab

Note The Corrections tab is not available for Simple Beer's Law methods. ▲

There are two types of corrections you may specify: linearity corrections and bias corrections. You may specify a different correction for each component in the method.

If you're not sure whether corrections are needed, press the Suggest Corrections button on the Corrections tab. The Corrections wizard uses the performance index to determine if corrections will improve the method's performance for each component. If a linearity correction is chosen, the wizard increases the order of the *correction curve* until the predicted results from the validation spectra no longer improve or an inflection occurs in the correction curve. The spectral information used to determine the correction for the selected component, the suggested curve order, and the coefficients and *performance index* for the selected curve order are displayed in the Corrections task window.

Note If you think a bias correction may be needed due to a slight change in experimental conditions, collect a few standards under the new conditions and set their Usage (Standards table) to "Correction." Then run click the Corrections tab, set Correction Standards to "Correct with correction standards only" and then click Suggest to run the Corrections wizard. A bias correction will be recommended only if the wizard finds a significant bias between the original calibration curve and the correction curve. ▲

A different *correction curve* may be recommended for each component. Use the Back and Next buttons in the Corrections task window to display the recommended correction for each component in the method.

If you want to use the recommended corrections exactly as you see them, click the Close button on the TQ Analyst *toolbar*. The recommended corrections are saved automatically when you close the Corrections task window. If the check box for Calculate Coefficients

After Each Calibration in the Corrections task window is on, the wizard will reevaluate the corrections each time you calibrate the method and, if necessary, update the *correction coefficients*.

Note The settings for the other correction parameters (Curve Order and Force Through Zero) will not be updated even if the Calculate Coefficients After Each Calibration check box is on. ▲

When you use the method to *quantify* a corrected component, a correction factor is extracted from the curve and applied to the component results before they are reported. The final result is the *corrected concentration*.

Editing correction parameters

You may edit the recommended correction for any component by changing the settings in the Corrections task window. To display the Corrections task window, click the Edit Correction button on the Corrections tab.

For brief instructions on how to interact with the Corrections task window, click the How To button in the window. To see a detailed description of a feature in the Corrections task window, open the Explain window and then select the feature. The information in the Explain window will change each time you select another feature in the Corrections task window.

Use the Correction list box in the Edit Correction group to choose the type (linearity or bias) and degree of correction. If you don't want to apply a correction to a component calibration, set the Correction list box to "No Correction." You can also force the curve through the zero point by turning on the Force Through Zero check box.

Note If you are measuring component concentrations that are close to zero, you may improve the accuracy of your method by forcing the component *correction curve* through the origin. We do not recommend forcing the curve through the origin if you are measuring component concentrations which are well above zero. ▲

If you change either of these settings, the new correction curve is calculated and immediately displayed. The curve coefficients and the method *performance index* are also updated.

If you want to enter values for the *correction coefficients*, you must first turn off the Calculate Coefficients After Each Calibration *check box* at the bottom of the Corrections window. When the check box is off, the fields in the Coefficients table become active. Then type the value for each correction coefficient in the appropriate field.

If you edit the correction parameters for one or more components, leave the Calculate Coefficients After Each Calibration check box off. This prevents the Corrections wizard from updating the correction coefficients and overwriting the values you specified each time the method is *calibrated*.

Notice When the Calculate Coefficients After Each Calibration *check box* is off, the *correction coefficients* will not be updated, even if you change the method so that the correction is no longer valid. You must be sure to check any specified corrections each time you calibrate the method. ▲

If you want to edit the linearity correction for another component, use the Back or Next button in the Corrections task window to select the component, then repeat the procedure described above to edit the correction parameters.

When you are finished editing the correction parameters for all components, click the Close button on the TQ Analyst *toolbar* to close

Where to go from here

the Corrections task window. The corrections you specified will be saved automatically.

Method development is an iterative process. Take time to try the options available for developing a quantitative method to see if there is room for further improvement. We also recommend reviewing the information on quantitative methods in the chapter called “Principles of TQ Analyst” at the beginning of this document.

Each time you make a significant change to the method, *calibrate* the method and look at the performance indices for the *component* you are trying to improve. If the new *performance index* is higher than the previous one, keep going. If the performance index goes down, undo the changes you made to the method since the last calibration.

TQ Analyst also provides a collection of useful diagnostic routines for *quantitative analysis*. See the chapter called “Method Diagnostics” in this document for more information. Detailed explanations of the diagnostic displays, including tips on how to interpret the diagnostic results, are also available on-line by pressing the *Explain button* when the diagnostic window is open.

If you need information on updating the calibration data or setting up sample reports, individual chapters on those topics are provided later in this document.

9 Creating a Classification Method

Read this chapter to learn how to build a successful classification (qualitative) method using TQ Analyst software. The following topics are covered:

- Defining the problem
- Choosing a sampling technique
- Creating a new method file
- Giving the method a title
- Selecting a classification technique
- Choosing a pathlength option
- Defining the method classes
- Collecting the standards
- Selecting the analysis regions
- Customizing the method
- Saving the method
- Calibrating the method
- Validating the method
- Where to go from here.

Most of the operations described in this chapter are carried out in a *method window*. The method window is broken into a series of tabs. The tabs that appear when one *classification method* type is selected may be different from the tabs that appear for another classification method. There are more tabs for Discriminant Analysis methods, for example, than for the Search Standards analysis type.

The tabs are arranged in an order that is convenient for creating a classification method. If you select the tabs in sequence from left to right, starting with the Description tab, they will lead you step by step through the method development process.

If you need information on updating the calibration data, running method diagnostics, or setting up sample reports, individual chapters on those topics are provided later in this document.

If you want to create a quantitative method or one that only measures and reports spectral information, skip this chapter. Instructions for creating quantitative and *spectral measurement methods* are provided in other chapters of this document.

Wizards for classification methods

TQ Analyst offers a number of wizards to help you complete each *classification method* development task. From choosing the proper settings for *method parameters* to setting up your experimental design, the wizards can help make even your first attempt at developing a method a success.

The following table gives brief descriptions of the wizards available for creating classification methods.

<i>Location</i>	<i>Wizard</i>	<i>Function</i>
Description tab	Suggest Analysis Type	Recommends a setting for the Analysis Type parameter.
Regions tab	Suggest Regions	Chooses appropriate <i>spectral regions</i> for the analysis.

To start a wizard, click the appropriate button on the specified tab. For example, to start the Analysis Type wizard, click the Suggest Analysis Type button on the Description tab.

The wizards are intended to be used in the order shown in the table above. You may use the wizard's recommendations, alter them, or overwrite them completely. We suggest using the wizards' recommended settings as a starting point. Then add your knowledge of spectroscopy and the chemical system you are measuring to determine the optimum settings.

To learn the important steps and considerations for good classification method design and understand how to get the most out of the wizards, we recommend reviewing all of the material in this chapter in the order in which they are presented.

Defining the problem

TQ Analyst offers a variety of techniques for comparing and identifying unknown *samples* based on the spectra of known materials. These “classification” techniques are all qualitative measurements because they can tell you what's in a sample (or which *class* it belongs to), but not how much of a *component* the sample contains. In other words, classification methods can't produce concentration values like quantitative methods. However, you can set up a classification method that classifies samples based on target concentrations of a specific component or combination of components. In this case, each class defines one of the target concentrations. Qualitative techniques can also indicate how closely a sample matches a group of known materials so you can distinguish “good” samples from “bad” ones.

There are a number of factors to consider when setting up a *classification method* using absorption spectroscopy. The most important considerations are the number of different materials (or different quantities of the same material) the algorithm must distinguish, the number of *standards* available to define each known

material and the quality of the spectral information available for the standards and unknown samples. Each of these factors plays an important role in helping you determine the proper experimental design.

The first step in creating a classification method is to define the analytical problem by answering the following questions:

- How much information do you have about the unknown samples?
- What do you want to compare them to?
- What information is needed from the comparison?

For example, are you simply trying to verify the purity of a known sample or determine whether it is compound a, b, or c or are you trying to identify a material that is completely unknown?

The answers will help you choose an appropriate *sampling technique* and define the experimental conditions that will be used for the analysis.

Choosing a sampling technique

Once you've defined the problem, the next step is to choose a *sampling technique* and define the experimental conditions for the analysis. In many cases, the physical characteristics of the *sample* dictate the sampling technique. For example, if you are analyzing different grades of cellulose, you might use an integrating sphere to collect the sample data. Other samples, such as an edible oil, might require a quartz cuvette for transmission analysis.

Choosing the optimum sampling technique for the material you want to measure is a critical step in achieving accurate classification results. It requires knowledge of spectroscopy and the effects of interferences, which can adversely affect your results.

The instrumentation industry offers a wide range of tools and accessories for material sampling and analysis. Each has benefits and drawbacks which may or may not be important to your analysis. Select the technique that consistently produces a quality spectrum with the least amount of effort and expense.

You generally have some latitude in setting the experimental conditions. Two characteristics of spectral data that are especially important to qualitative measurements are spectral *resolution* and *signal-to-noise ratio*.

Increasing spectral resolution may improve a classification method's ability to distinguish classes. Remember, the *smaller* the resolution value, the *higher* (better) is the resolution. Keep in mind however, that the signal-to-noise ratio of the spectrum decreases as the resolution increases so you may want to also increase the number of scans (or the collection time). If you're still not getting the resolution and peak shape you need after increasing the resolution, try another sampling technique.

When creating classification methods, we recommend collecting or converting the spectra to absorbance or log (1/R) units. Since these units tend to vary linearly with concentration and many qualitative methods need to distinguish classes based on *component* concentrations, they are preferred for classification analyses. If you want to work with *derivative* spectra, use the Data Format parameter on the Spectra tab to make the derivative conversion. See the chapter on "Processing Spectral Data" in this document for information on setting Data Format.

Creating a new method file

The first step in creating a method using TQ Analyst is to create a new method file. A method file contains all of the parameters that define how your method will operate. Your method must contain valid settings for all of the *method parameters* before it can be *calibrated* or run.

To create a new method file:

1. Choose New Method from the File menu.

The method parameters are displayed in a new *method window*. The Description tab, which contains the first group of method parameters, is already open.

2. Choose Save Method As from the File menu.

The Save Method As dialog box is displayed.

3. Type a file name for the new method in the File Name box and select the directory and disk where you want the method saved.

4. Choose OK.

When you create a new method file, the method parameters are automatically set to their default values. You can change any of the parameter settings or use the default settings.

Note Save your method frequently while creating the method or editing the method parameters. ▲

Giving the method a title

Use the Method Title box on the Description tab to enter a title for your new method.

Method Title

TQ Analyst example method - Discriminant analysis

You will use the *method titles* along with their file names to select a method to open. The title can also be displayed or printed with the *analysis results*.

You may enter a longer description of the method in the Method Description box. You may also enter your name in the Developer's Name box so people know who created the method.

The Method Description and Developer's Name parameters are also on the Description tab. Use the *scroll bar* to bring these parameters into view.

Selecting a classification technique

Another important step in developing a *classification method* is choosing the technique that will be used to compare and *classify* the unknown *samples*. The Analysis Type parameter on the Description tab provides a range of techniques for classifying samples (see the list below).

To see detailed descriptions of the options for classification methods, click the Explain button on the toolbar to open the Explain help window and then click each option in the software.

Analysis Type

Quantitative analysis

- Simple Beer's law
- Classical least squares (CLS)
- Stepwise multiple linear regression (SMLR)
- Partial least squares (PLS)
- Principal component regression (PCR)
- Undecided

Classification

- Similarity match
- Distance match
- Discriminant analysis
- Search standards
- QC Compare search

All of the classification methods compare the spectrum of an unknown sample with the spectra of one or more groups (classes) of known materials (*standards*). Similarity Match and Distance Match methods may be used to compare the spectrum of an unknown material to a single group (class) of similar standards. Discriminant Analysis, Distance Match and QC Compare search methods are typically used to compare an unknown to several classes of standards in order to find the best matched class. A Search Standards method compares the unknown to many different materials, where each material is represented by only one standard, and finds the closest match.

A Search Standards method uses only one standard to define each *class* (TQ Analyst consider each standard as a separate class). Similarity Match methods need at least one standard in each class but allow multiple standards per class. The other method types use statistical algorithms to determine similarity and thus require at least two standards to define each class.

The various classification methods also differ in the kind of information they provide about an unknown sample. For example, a Similarity Match method compares the unknown sample to a single class of standards and reports a *match value*, which is a measure of similarity. A Search Standards method reports the *index number* and title of each selected class (standard) as well as a match value between 0 and 100.

The result of a discriminant analysis is the name of the *class* that is most similar to the *unknown sample spectrum* and a measurement of the distance between the unknown sample and each reported class. The closer each *distance value* is to zero, the better is the match.

The result of a QC Compare search is the single best *match* from each reported class. The classes are listed in order of importance; the class that contains the best matched spectrum is listed first. The *index number* of each standard is given as well as a *match value* between 0 and

100. The match value tells you how well the standard matches the unknown. A match value of 100 indicates a perfect match.

The result of a Distance Match method is the percentage of frequencies that exceed a specified distance threshold. The answers will range from 100 to 0, where 0 (percent frequencies exceeding the threshold) is a perfect match. If one class is defined in the method, the software reports the *distance value* for that class. If multiple classes are used, the software reports the class name and distance value for the specified number of classes. The best matched class (the one with the smallest distance value) is listed first.

The basic concepts behind the classification techniques are summarized in the table below.

<i>If you . . .</i>	<i>Then use . . .</i>
are interested in monitoring the purity of a material being produced by some process	Similarity Match Distance Match
want to screen incoming materials to determine if they are compound a, b, or c	Discriminant Analysis Distance Match QC Compare search
are trying to identify a material that you know little or nothing about	Search Standards

Unlike the quantitative group, there is no “Undecided” option for *classification methods*. You must select a classification option in the first stage of development. If you need help choosing a classification option, TQ Analyst provides a wizard to help you decide. To start the wizard, click the Suggest Analysis Type button on the Description tab and follow the path for qualitative methods. The wizard poses a series of questions and then selects a classification option based on your answers.

You are free to change the recommended classification option (Analysis Type) setting at any time. If you still can't decide, we recommend starting with the Discriminant Analysis option because it provides the most flexibility and produces a more thorough classification report. You can always switch to another technique later if the results from the first attempt are unsatisfactory or try several techniques and choose the one that classifies your samples with the most accuracy.

Each time you change the analysis type, the main *TQ Analyst window* is reconfigured so that only the method tabs and parameters which are needed to develop the type of method you selected are displayed on the screen. If you switch the analysis type between two options in the same group, for example if you change from Similarity Match to Discriminant Analysis, the information you entered and the settings you chose for the previous analysis type will be saved.

Use the Explain Help feature of your TQ Analyst software to see detailed descriptions of the classification options available in TQ Analyst or see the chapter called "Principles of TQ Analyst" in this document.

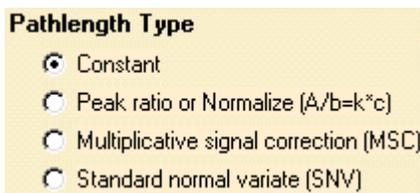
Choosing a pathlength option

Sample *pathlength* (thickness) is important to classification analyses because it affects the concentrations of the sample *components*. This is especially true for Distance Match and Discriminant Analysis methods when the method must discriminate between various materials that contain different amounts of a key compound.

If the pathlengths of the *standards* and the unknown *samples* are different and component concentrations are important to your classification analysis, the method should include a pathlength correction to account for the differences. A pathlength correction may also allow you to tighten class distribution for spectra that have significant anomalies, due to scattering or some other problem.

The parameters on the Pathlength tab define how the method will handle sample *pathlength*. TQ Analyst offers the following pathlength options for Similarity Match, Distance Match and Discriminant Analysis methods.

To see detailed descriptions of these pathlength options, click the Explain button on the toolbar and then click each option in the software.



Note No pathlength options are available for the Search Standards and QC Compare search classification techniques. If you are creating a Search Standards or QC Compare search method, skip to the section titled “Collecting the Standards.” ▲

Some of the pathlength options require additional information, such as a *pathlength peak* or *region*. If a pathlength option requires additional information, parameters for entering the information will appear on the Pathlength tab when that pathlength option is selected.

In most cases, your *sampling technique* will dictate the correct pathlength option (usually Constant, which means it has no effect on your spectral data). You may also try several pathlength types and then choose the one that produces the best results. Here are some brief guidelines:

<u><i>If sample pathlengths are...</i></u>	<u><i>Use this pathlength type...</i></u>
Always the same	Constant
Variable and unknown but Pathlength peak exists	Peak Ratio
Variable and unknown and no Pathlength peak exists	MSC SNV

The Peak Ratio, MSC and SNV pathlength options all correct the spectral data rather than the calculated concentration values. Pathlength corrections that operate on the calculated concentration values would be useless for a classification method since no concentration values are available.

If you are creating a classification method that contains standards collected at different pathlengths and you can find a peak that varies only with pathlength, use the Peak Ratio pathlength option. The Peak Ratio pathlength correction is especially useful for Distance Match and Discriminant Analysis methods when the method must discriminate between various materials that contain different amounts of a key component. Peak Ratio scales the spectrum based on the *pathlength peak*, leaving the concentration differences intact.

If the pathlengths of the standards and the unknown samples are different but you can't find a peak that varies only with pathlength, use the MSC or SNV pathlength correction.

If the pathlengths of the standards and the unknown samples are all the same, use the Constant pathlength option.

For descriptions of the Constant, Peak Ratio, MSC and SNV pathlength options, see the sections with those names in the chapter called "Principles of TQ Analyst" at the beginning of this document.

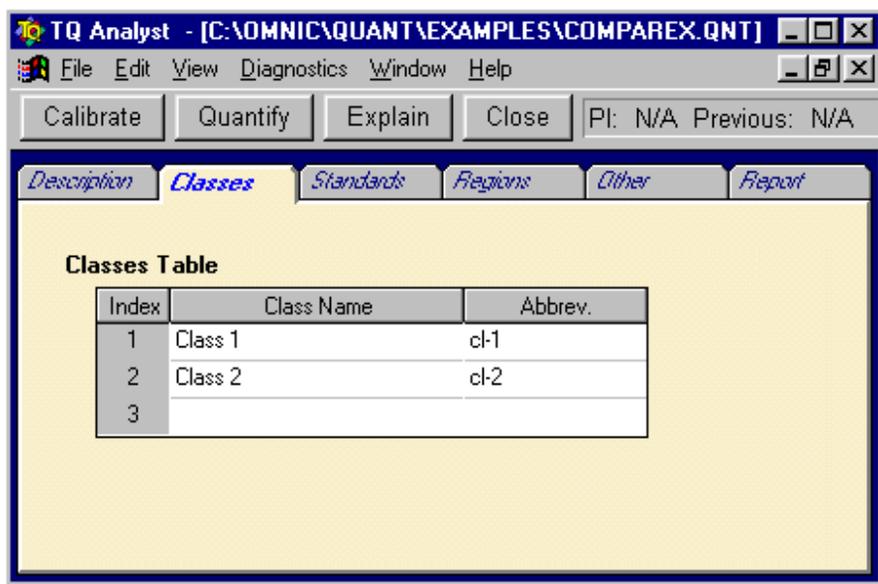
Defining the method classes

If you are creating a Discriminant Analysis or QC Compare search method, the next step is to define the groups (classes) the unknown *samples* will be compared to.

Note If you are creating a Similarity Match or Search Standards method, you don't need to specify classes. Skip to the next section, titled "Collecting the Standards." ▲

Use the Classes tab to enter information about the classes of spectra in a Discriminant Analysis or QC Compare search method (see the picture below).

To see descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.



Classes Tab

A unique name must be specified for each class. The *class* names are entered in the Classes table. When you use the method to analyze a *sample spectrum*, the class names will be displayed or printed along with the *analysis results*.

To enter the class names:

- 1. Select the Classes tab.**

2. **Click in the first row of the Class Name column and type the name of the first class.**

3. **Press Enter.**

TQ Analyst adds a blank row to the end of the table.

4. **Continue entering class names until all of the classes have been specified.**

5. **Use the Abbrev column to enter abbreviated names for each class.**

The abbreviated name will be used whenever the full class name doesn't fit in the available space. If no abbreviated names are entered, the default abbreviations are used.

Collecting the standards

When you are finished selecting a classification technique, defining the method classes and selecting a pathlength option, you are ready to collect the *standards* that will be used to develop and validate the method. This section defines the various kinds of standards used in *classification methods* and explains how to choose standards and collect their *infrared* spectra.

What are standards?

For classification analyses, *standards* are simply *samples* that have the characteristic you want to track. For example, if you want to set up a method that classifies materials based on composition or purity, you don't need to know everything that is present in the standards. However, you must know which *class* each standard belongs to.

TQ Analyst uses two kinds of standards for *classification methods*: *calibration standards* and *validation standards*.

Calibration standards are used during the method calibration step to build a distribution model, if one is needed. In order to do this, you must specify the class for each calibration standard in the method. If your classification method does not use a distribution model (models are not used for Search Standards and Similarity Match methods), the calibration standards are simply used for comparison. We may also refer to the calibration standards as the “*calibration set*.”

Validation standards are used to evaluate the performance of a *calibrated* Discriminant Analysis or QC Compare search method (Similarity Match and Search Standards methods do not use validation standards). You must specify the class for each validation standard included in your method. Validation standards are an unbiased test of performance because they are not used to calculate the distribution model. Each time you calibrate a Discriminant Analysis or QC Compare search method, the software uses the new distribution model to *classify* the validation standards.

Validation standards – a measure of performance

Although validation standards are not required to calibrate or run a Discriminant Analysis or QC Compare search method, they are key to the TQ Analyst philosophy. The software uses the validation standards to calculate a method *performance index* after each calibration. You can use the performance index to track the performance of your method as you develop or modify it. We strongly recommend that you include a few validation standards in every Discriminant Analysis or QC Compare search method you develop. If you don't, you will miss out on this powerful feature of your TQ Analyst software.

Choosing standards for classification methods

Choosing the proper *standards* is probably the most critical factor in achieving accurate classification results. For example, if you don't include enough standards or if they don't describe all of the variation in a class, the distribution model may not accurately describe the behavior of the *samples* you want to *classify*.

Consider the following guidelines before you begin selecting standards for a *classification method*.

- **Use sufficient quantity.**
To determine the number of standards required for each class, consider the classification technique. The Search Standards technique allows only one standard per class. All of the others require multiple standards to define each class.
- If the method requires multiple standards, we generally recommend using at least three standards per class. The key point is to make sure you include enough standards to adequately represent the variation you expect to see in the samples the method will *classify*. In general, the more standards used to define a *class* the better it will represent “real world” samples. However, be careful not to overrepresent certain variations (by using too many standards that have the characteristic) so the distribution is well balanced.

If you are creating a Discriminant Analysis or QC Compare search method, be sure to include at least two additional standards that can be used to validate the distribution model (at least one *validation standard* per class is ideal). The validation standards should represent a typical spectrum in the corresponding class. See the section titled “What are Standards?” to learn how TQ Analyst uses validation standards to measure method performance.

Optimizing the collection parameters

- **Use representative mixtures.**

The composition of the standards should be similar to the composition of the samples you want to measure. This insures that baseline features are handled properly and that all substances in the sample are properly modeled. This is especially true for Search Standards methods, which use only one standard to define each class. If you are creating a method that allows multiple standards per class, make sure you include examples of any variation you expect to see in the unknown samples. The *calibrated* method can then model this variation and *classify* the unknown samples with greater accuracy.

The *standards* must be collected in the same way you will collect the spectra of the unknown *samples*, using the same *data collection parameters* and the same spectrometer, if possible. The spectra of the standards should be of the highest achievable quality. Assuming that the *sampling technique* is reproducible, spectral quality is determined mainly by the *resolution* and *signal-to-noise ratio* of the spectral data. The parameters that define resolution and signal-to-noise ratio are part of the data collection parameter set.

Use the Collection Parameters button on the Standards tab to display the data collection parameters. See the chapter called “Working With Standards” in this document for more information.

Note If the Collection Parameters button does not appear in your TQ Analyst application, data collection is disabled in TQ Analyst. You must use your spectral analysis application, such as RESULT, to set the data collection parameters. See the documentation that came with your spectral analysis software for more information. ▲

Once the parameters have been set, we recommend using the chosen settings to collect all of the calibration and *validation standards* as well every unknown sample you use the method to analyze.

Note If you change the setting for a collection parameter after opening or collecting the spectra of one or more standards, TQ Analyst may warn you that the parameter setting should not be changed at this point. You will be given the option of overriding the warning or creating a new method with the new parameter settings. ▲

Collecting the spectra of the standards

Once the *standards* are prepared and the optimal parameter settings defined, the calibration spectra must be carefully collected and stored on a disk.

You can use TQ Analyst or another Thermo Scientific spectral analysis package, such as OMNIC or RESULT, to collect the calibration spectra. To collect the spectrum of a standard in TQ Analyst, click the Collect Standard button on the Standards tab.

Note If the Collect Standard button does not appear in your TQ Analyst application, data collection is disabled in TQ Analyst. You must use your spectral analysis application, such as RESULT, to collect standards. See the documentation that came with your spectral analysis software for more information. ▲

The process for collecting a spectrum in TQ Analyst is exactly like the process for collecting a spectrum in your Thermo Scientific spectral analysis software. Refer to the documentation that came with that software for step-by-step instructions.

Depending on how the data collection parameters are set, you may be prompted to collect a background spectrum or enter a *spectrum title* during data collection. If you don't enter a title during collection, the date and time of collection will be used. The new spectrum will be added to the open row at the bottom of the Standards table and saved in the method *standards library*.

Note When you collect a spectrum to use as a standard, TQ Analyst saves only a portion of the collection and processing information that is normally stored in a Thermo Scientific spectral data file. If you want to save all of the collection and processing information for your standards, save the standards as Thermo Scientific spectral data files by turning on the Save Automatically *check box* in Experiment Setup before collecting the standards. (Click the Collection Parameters button on the Standards tab to display the Experiment Setup dialog box. Save Automatically is located in the File Handling group on the Collect tab.) ▲

As you collect the spectra, be sure to note any special sampling conditions that may affect the spectral data, such as the temperature and pressure of a gas cell, the cell *pathlength* or the packing pressure of a diffuse reflectance accessory.

If you are collecting the spectra outside of TQ Analyst, be sure to name and save each spectrum in a file when you are finished collecting it. Then use the Open Standard button on the Standards tab to add each spectrum to the Standards table. See the chapter called “Working With Standards” in this document for more information.

Note If a warning message about mismatched standards appears when you open additional standards, the parameter settings used to collect the new standard are significantly different from the settings that are saved with the current method. For optimum performance, all method standards should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the standard again using the proper settings for the collection parameters, edit the settings in the current method, or ignore the warning and continue adding standards. ▲

Specifying class and usage

After adding each spectrum to the Standards table, you must indicate how it will be used in the method. For example, if you are creating a Discriminant Analysis, Distance Match, or QC Compare search method, you must specify the *class* each *standard* belongs to and how each standard will be used in the method. Similarity Match and Search Standards methods only require information about usage.

Selecting the class

If you are building a Discriminant Analysis, Distance Match, or QC Compare search method, a column labeled “Class” appears in the Standards table. Use the Class column to specify the class each standard belongs to.

To select the class for a standard, choose an option in the Class list box. The items in the list box are the classes that are specified on the Classes tab. If no class names were entered on the Classes tab, the Class list box in the Standards table will be blank.

Note You must enter *class* names and specify the class each standard belongs to before calibrating the method. ▲

Specifying Usage

The column labeled “Usage” in the Standards table allows you to specify how each *standard* will be used in the method. To set the usage for a standard, simply choose an option in the Usage list box.

For Discriminant Analysis and Distance Match methods, you can set the usage to “calibration,” “validation,” or “ignore.” Standards that are specified as “calibration” will be used to create the distribution model during calibration. *Validation standards* will be used to evaluate the performance of a method once the method is calibrated. The results from the validation standards are also used to calculate the *performance index*. Use the “ignore” setting to temporarily exclude a standard from the method.

The standards in a Similarity Match, QC Compare search, or Search Standards method can be set to “calibration” or “ignore.” Use the “calibration” setting for standards that you want to include in the comparison. If you want to temporarily exclude a standard from the method, set its usage to “ignore.”

Selecting the analysis regions

The final step in building a method is to identify the usable spectral information in the *standards*. We recommend using as much of the spectral information as possible for *classification methods*. However, the following features should be excluded:

- *Peaks* or *regions* that are totally absorbing (for example, absorbance values that are higher than 1.5 absorbance units or $1.5 \log 1/R$ units).
- Regions that contain excessive random *noise*.
- Peaks or regions that you know are unrelated to the measured components, such as those from carbon dioxide or water.

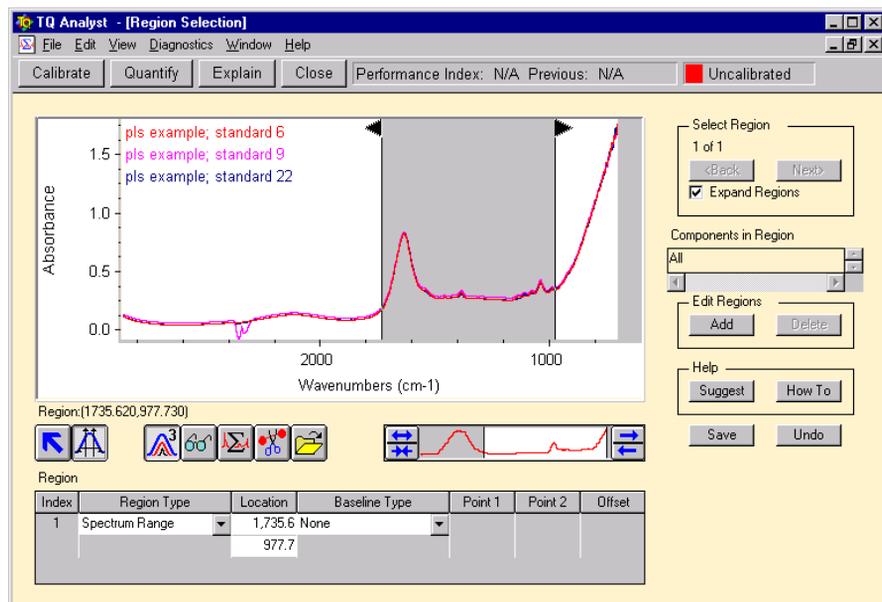
If you want the software to select the regions, use the Regions wizard on the Regions tab. The wizard starts automatically when you select the Regions tab.

Note If the Pathlength Type is set to Peak Ratio, make sure you specify a *pathlength peak* before running the Regions wizard. To specify a pathlength peak, click the Pathlength tab and enter appropriate values in the Pathlength Region table. If you start the Regions wizard without specifying a pathlength peak, the wizard will reset the Pathlength Type to Constant. ▲

For most classification applications, all of the useful information in the spectrum is used in the analysis. The wizard provides a few tips on the types of peaks or regions you should exclude from the analysis.

When you are finished interacting with the wizard, click the Edit Regions button on the Regions tab. The Region Selection task window appears on the display.

To see a description of an item in this task window, click the Explain button on the toolbar to open the Explain help window and then click the item. For step-by-step instruction on editing regions, click the How To button in the task window.



Region Selection Task Window

The spectra of the first three standards that are listed in the Standards table are displayed in the graphical window and one *analysis region* is recommended. The recommended region will contain all of the data points between the X-axis limits of the displayed spectra. Additional information about the selected region is provided in the Regions table, located at the bottom of the Region Selection task window.

Since region selection is usually an iterative process, the recommended region should be considered only as a starting point. You may need to adjust the region or other settings in the Region Selection task window to achieve the best results. Any changes you make to the Region Selection task window will be saved automatically when you close the

window. To close the task window, click the Close button on the TQ Analyst *toolbar*.

To display the Region Selection window after one or more regions have been selected, click the Edit Regions button on the Regions tab.

Customizing the method

If you have been following the guidelines provided in this chapter, your method contains all of the information needed for calibration, including:

- Descriptions of the classes.
- Representative *standards* measured using reproducible *sampling technique*.
- *Spectral regions* which correlate to known component information.

Depending on the classification technique (Analysis Type) you selected, there are other features and settings in the software that may be used to customize your method. For example, if you want to calculate *derivative* spectra or apply smoothing or baseline correction, turn on the *check box* for Allow Spectral Processing on the Standards tab. (See the chapter called “Processing Spectral Data” in this document for more information.)

Use the parameters on the Report tab to configure the sample reports. See the section on “Setting Up Sample Reports” in the chapter called “Preparing a Method for Sample Analysis” for details.

To see detailed descriptions of the features on the Report tab, click the Explain button on the toolbar to open the Explain help window and then click the feature.

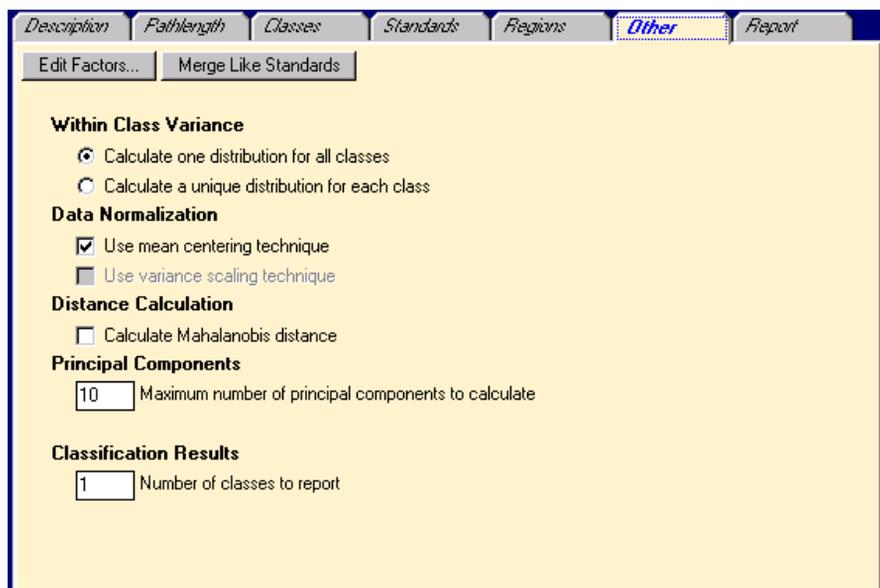
The screenshot shows the 'Report' tab of a software interface. The tab is highlighted in blue and contains the following sections:

- General Information:** A list of checkboxes for including various report elements: Method title, Method file name, Method revision information, Spectrum title, Spectrum file name, Spectrum collection date and time, Pathlength (unchecked), and Fit values.
- Spectrum Warnings:** Two checked checkboxes. The first is 'Full spectrum check' with a dropdown menu set to 'Warn' and a text input field containing '95.0', with the instruction 'Warn if fit is below this value'. The second is 'Data collection parameter check' with a dropdown menu set to 'Warn' and the instruction 'Warn if fit is below this value'.
- Classification Warning:** An unchecked checkbox for 'Distance value check' with a dropdown menu set to 'Warn' and a text input field containing '3.0', with the instruction 'Warn if distance to nearest class is above this value'.
- Results:** A checked checkbox for 'Distance value' with a 'Digits' label and a text input field containing '2'. An unchecked checkbox for 'Append results to spectrum comments' is also present.

Report Tab for Discriminant Analysis Method

The parameters on the Other tab allow you to customize certain features of a *classification method*, such as the number of *principal components* used for a Discriminant Analysis method or the *search algorithm* for a Search Standards method.

To see detailed descriptions of the features on the Other tab, click the Explain button on the toolbar to open the Explain help window and then click the feature.



Other Tab for Discriminant Analysis Method

Feel free to consider or try all of the features included in your method. If you don't have time to review them or don't need special enhancements, leave the *default settings* provided in the software. TQ Analyst is designed to provide a working method with a minimum of effort.

The method should produce acceptable results for most sample types when the *method parameters* are set to their default values. To see the default settings for a *classification method*, choose New Method from the File Menu and set the Analysis Type to one of the classification options.

If you prefer using other method specifications or have specifications you need to follow, you may change the settings for any method parameter at any time.

Saving the method

We recommend that you save your work regularly when creating or editing methods. Be sure to save the method again before you *calibrate* it.

To save your method:

- 1. Select the method you want to save by clicking the method window or by choosing the method name in the Window menu.**

- 2. Choose Save Method from the File menu.**

If the method does not have a file name, the Save Method As dialog box appears.

- 3. If the Save Method As dialog box appears, type a file name and select the directory and disk where you want the method saved.**

- 4. Choose OK.**

Calibrating the method

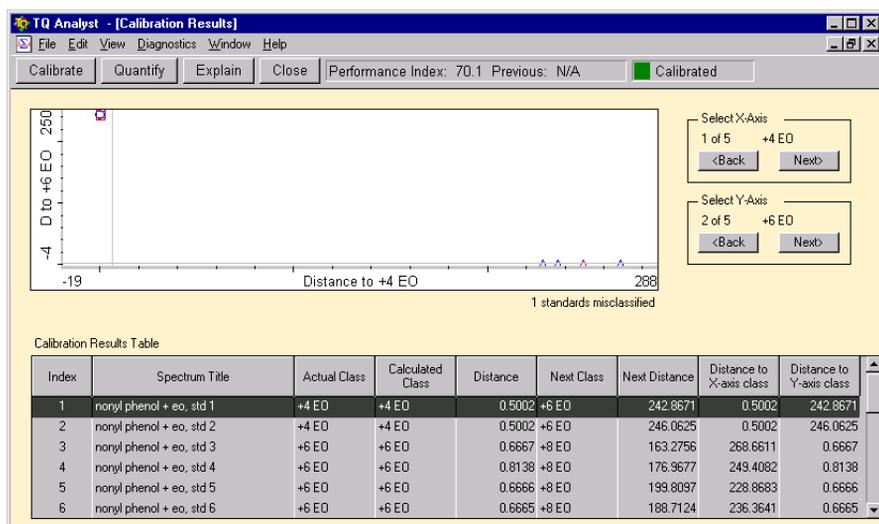
When you are finished selecting a classification technique, choosing a pathlength option, defining the classes, collecting the *standards*, selecting the *analysis regions* and saving, your method is ready for calibration. For discriminant analysis methods, calibration produces the distribution model that will be used to *classify* the unknown *samples*. No distribution model is created for similarity match, search standards and QC Compare search methods. However, the software uses the “calibration” step to test the method for consistency and proper experimental design.

To *calibrate* a *classification method*, click the Calibrate button on the TQ Analyst *toolbar*. Calibration takes only a second. When calibration is completed, the calibration *readout* on the TQ Analyst toolbar

changes from red to green and the “Uncalibrated” message changes to “calibrated.”

When you calibrate a discriminant analysis method, the software calculates a distribution model and then uses the model to *classify* the method's *calibration standards*. If *validation standards* are specified in the method, the validation standards are also classified. The calibration data are displayed in the Calibration Results task window.

To see descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.



The information provided in the Calibration Results task window can help you identify standards that were assigned to the wrong *class* and possibly understand why they were misclassified.

The table in the lower half of the task window shows the actual and the calculated class as well as the *distance value* for each standard in the method. The name and distance value for the next closest class are also provided.

Index	Spectrum Title	Actual Class	Calculated Class	Distance	Next Class	Next Distance	Distance to X-axis class	Distance to Y-axis class
1	nonyl phenol + eo, std 1	+4 EO	+4 EO	0.5002	+6 EO	242.8671	0.5002	242.8671
2	nonyl phenol + eo, std 2	+4 EO	+4 EO	0.5002	+6 EO	246.0625	0.5002	246.0625
3	nonyl phenol + eo, std 3	+6 EO	+6 EO	0.6667	+8 EO	163.2756	268.6611	0.6667
4	nonyl phenol + eo, std 4	+6 EO	+6 EO	0.8138	+8 EO	176.9677	249.4082	0.8138
5	nonyl phenol + eo, std 5	+6 EO	+6 EO	0.6666	+8 EO	199.8097	228.6683	0.6666
6	nonyl phenol + eo, std 6	+6 EO	+6 EO	0.6665	+8 EO	188.7124	236.3641	0.6665
7	nonyl phenol + eo, std 7	+8 EO	+8 EO	1.2306	+10 EO	26.2442	857.1513	191.3353

Calibration Results Table

If you want to see the distance value between a standard and one of the other classes in the method, use the Back or Next button in either the Select X-axis or Select Y-axis group to select the class. When you select a new class, a column for the selected class appears in the table showing the distance between each standard and the selected class.

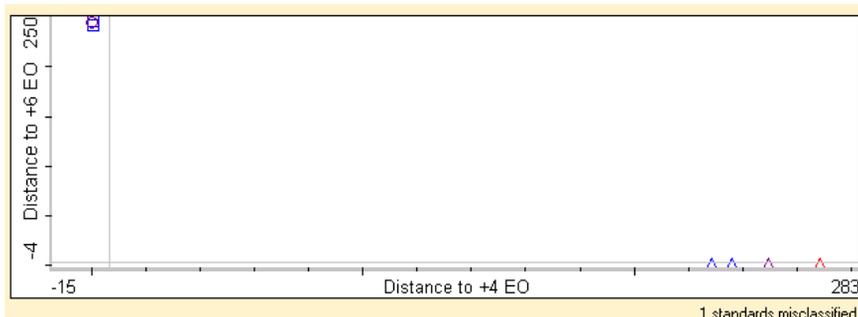
Select X-Axis

1 of 5 +4 EO

Select Y-Axis

2 of 5 +6 EO

The pairwise distance plot is shown at the top of the task window.



Pairwise Distance Plot

The plot shows graphically the *Mahalanobis distance* between each standard and the two classes that are selected for the X- and Y-axis of the plot. Mahalanobis distance is an algorithm for calculating the distance of a *sample* from the mean of a set of standards.

Data points that correspond with the calibration and validation standards for the class that is selected for the Y-axis are represented with triangle symbols. Squares are used to represent the standards that define the class that is displayed on the X-axis. If data points for other standards fall within the displayed range for the X- or Y-axis, they will be displayed as circles.

Data points for validation standards are displayed in a different color than the points for the *calibration standards* so you can distinguish them. You use the Usage parameter on the Standards tab to set the usage of each standard.

Use the Back and Next buttons in the Select Y-axis and Select X-axis groups to select which class is assigned to each axis in the plot. When you select a new class, the name (or abbreviated name) and *index number* of the new class are displayed below the group name.

TQ Analyst calculates boundaries for each class based on the number of standards in the class. The boundaries, displayed as perpendicular gray lines in the pairwise distance plot, show the 95% confidence intervals for the two classes that are selected for the X- and Y-axis.

Use the Pairwise Distance plot to compare the standards for all of the classes in your method. If all of the calibration and validation standards for the class that is selected for the X-axis of the pairwise distance plot are similar to each other, the data points for those standards will be clustered in the upper left corner of the plot. Similarly, if the standards for the class that is selected for the Y-axis are well defined, the data points for those standards will be clustered in the lower right corner of the plot. The greater the distance between a data point and the other data points in the cluster, the greater is the difference between the corresponding standard and the other standards in the class.

If data points for standards from other classes are clustered with the calibration and validation standards for one of the classes in your method, especially if the data points fall inside the boundary (gray line) for the selected class, the two classes are similar and the method may not be able to distinguish them.

If one or more standards were assigned to the wrong class, a message will also appear indicating the number of standards that were misclassified. If a standard was classified incorrectly, check that the class was specified correctly in the Standards table (see the Standards tab). Then display the spectrum of the misclassified standard. If the quality of the spectrum is poor, replace the spectrum with a new one.

Each data point in the plot is linked to a standard in the table. To identify the standard that is associated with a data point in a plot, click the data point. The corresponding standard will be highlighted in the table. To find the data point that corresponds to a standard in the table, click anywhere in the row. The associated data point will be highlighted in the plot.

The number of *principal components* that were used to *calibrate* the method is shown above the Calibration Results table. If the % Variability Described readout appears in the task window, the principal components used in the current calibration do not describe all the variability in the calibration spectra. You may want to increase the setting for the maximum number of principal components to calculate. You can edit this value from the Principle Components group on the Other tab.

If you edit the maximum number of principal components to calculate, calibrate the method again to see if the % Variability Described value increased. If the % Variability Described readout is no longer displayed in the Calibration Results task window, the value has reached 99.9%.

If a Discriminant Analysis method includes at least one validation standard, TQ Analyst calculates a *performance index* which appears on the TQ Analyst toolbar. The performance index indicates how accurately a calibrated method can *classify* the validation standards.

When you are finished viewing the calibration results, click the Close button on the TQ Analyst toolbar to close the Calibration Results window.

Validating the method

The final step in developing a *classification method* is to validate the method. Validation simply means using the method to analyze a few real world *samples* in order to test the method's performance.

There are several ways to validate a method using TQ Analyst software. For example, if method includes *validation standards*, you can use the *performance index* and the validation data displayed in the Calibration Results task window to evaluate performance. If you did not include validation standards in the method or if the method doesn't require them, you can prepare a few additional standards, analyze them with

the completed method and use the results to validate the method. Both of these options are discussed in the sections that follow.

Performance index If the method includes at least one *validation standard*, TQ Analyst calculates a *performance index* during calibration. The performance index is a number that indicates how accurately a *calibrated* method can *classify* the validation standards. The performance index appears on the TQ Analyst *toolbar*.

Note See the performance index entry in the glossary at the end of this document for information on the algorithm used to calculate the performance index for classification methods. ▲

If you calibrate the method more than once, the performance value from the previous calibration is retained. This allows you to determine whether the changes you make to a method actually improve its performance. It's up to you to decide what is an acceptable performance value.

Validation data The calibration data from the *validation standards* are also important indicators of performance. If all of the validation standards were classified correctly, your method is complete and ready for repeated *sample* analysis.

If you did not include validation standards, you can still validate the method by preparing a few additional standards and using the completed method to analyze them.

Note We do not recommend using *calibration standards* to validate a method. Since calibration standards are included in the distribution model, their results would be significantly biased. ▲

You can run the validation standards individually or use the External Validation diagnostic routine to run them automatically. External Validation can be used to analyze a large group of validation standards that are listed in a spreadsheet or text file. The validation data are placed in a spreadsheet (Microsoft® Excel®) or text file. See the chapter on “Method Diagnostics” in this document for more information on External Validation.

If the method classifies the validation standards correctly, your method is complete and ready for repeated sample analysis.

Sources of error

If one or more *standards* were classified incorrectly, you must identify what caused the error and try to fix it. Some typical sources of error are listed below:

- The standard is assigned to the wrong class.
- The *region*, region type or baseline treatment is inappropriate for the *analysis region* or regions.
- The standards don't accurately describe the *class* (i.e., there are too few standards or the standards don't adequately represent the variation in the class).
- The classes are too similar.
- Spectral errors caused by sampling, instrument *noise*, or strong absorptions are present in the calibration spectra.
- The selected region or regions don't include enough or appropriate spectral information.
- Other *components* in the standards are producing interfering *peaks* in the *analysis region* or regions.
- An inappropriate pathlength treatment was chosen. See “Choosing a Pathlength Option” in this chapter for more information.

Where to go from here

Continue checking for errors and recalibrating the method until the validation results are acceptable.

Method development is an iterative process. Take time to try the options available for developing a *classification method* to see if there is room for further improvement. We also recommend reviewing the information on classification methods in the chapter called “Principles of TQ Analyst” at the beginning of this document.

Each time you make a significant change to the method, calibrate the method and look at the performance indices for the *component* you are trying to improve. If the new *performance index* is higher than the previous one, keep going. If the performance index goes down, undo the changes you made to the method since the last calibration.

TQ Analyst also provides a collection of useful diagnostic routines for *classification methods*. See the chapter called “Method Diagnostics” in this document for more information. Detailed explanations of the diagnostic displays, including tips on how to interpret the diagnostic results, are also available on-line by pressing the *Explain button* when the diagnostic window is open.

If you need information on updating the calibration data or setting up sample reports, individual chapters on those topics are provided later in this document.

10 Creating a Spectral Measurement Method

You can use TQ Analyst to set up methods that simply measure spectral features and report the measured values. These “measure only” methods can be used to determine *peak heights* or areas, peak locations and even the *noise* in a given *spectral region*.

Read this chapter to learn the key steps in creating a *spectral measurement method* using TQ Analyst software. The following topics are covered:

- Defining the problem
- Choosing a sampling technique
- Creating a new method file
- Giving the method a title
- Selecting a measurement technique
- Choosing a pathlength option
- Defining the measurements
- Collecting representative sample spectra
- Selecting the analysis regions
- Setting up a denominator peak or region
- Customizing the method
- Saving the method
- Calibrating the method
- Validating the method

- Where to go from here.

Most of the operations described in this chapter are carried out in a *method window*. The method window is broken into a series of tabs. The tabs are arranged in an order that is convenient for creating a spectral measurement method. If you select the tabs in sequence from left to right, starting with the Description tab, they will lead you step by step through the method development process.

If you need information on running diagnostics or setting up sample reports, individual chapters on those topics are provided later in this document.

If you want to create a quantitative or *classification method*, skip this chapter. Instructions for creating quantitative and classification methods are provided in the previous two chapters of this document.

Defining the problem

The first step in creating a *spectral measurement method* is to define the analytical problem by answering the following question:

How much information do you have about the samples you want to measure?

What spectral information do you want to measure or report?

For example, are you simply trying to consistently measure a certain *peak* in a series of similar spectra or are you interested in defining a process for producing validation reports? The answers will help you choose an appropriate *sampling technique* and define the experimental conditions that will be used for the analysis.

Choosing a sampling technique

Once you've defined the problem, the next step is to choose a *sampling technique* and define the experimental conditions for the analysis. In many cases, the physical characteristics of the *sample* dictate the sampling technique. For example, if you are analyzing polymer beads, you might use a single-bounce ATR (Attenuated Total Reflectance) sampling accessory with a built in pressure device. Other samples, such as gas mixtures, might require a multipath gas cell.

Creating a new method file

The first step in creating a method using TQ Analyst is to create a new method file. A method file contains all of the parameters that define how your method will operate. Your method must contain valid settings for all of the *method parameters* before it can be *calibrated* or run.

To create a new method file:

1. Choose New Method from the File menu.

The method parameters are displayed in a new *method window*. The Description tab, which contains the first group of method parameters, is already open.

2. Choose Save Method As from the File menu.

The Save Method As dialog box is displayed.

3. Type a file name for the new method in the File Name box and select the directory and disk where you want the method saved.

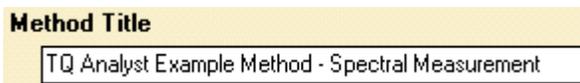
4. Choose OK.

When you create a new method file, the method parameters are automatically set to their default values. You can change any of the parameter settings or use the *default settings*.

Note Save your method frequently while creating the method or editing the method parameters to avoid losing data in the event of a computer problem or power failure. ▲

Giving the method a title

Use the Method Title box on the Description tab to enter a title for your new method.



You will use the *method titles* along with their file names to select a method to open. The title can also be displayed or printed with the *analysis results*.

You may enter a longer description of the method in the Method Description box. You may also enter your name in the Developer's Name box so people know who created the method.

The Method Description and Developer's Name parameters are also on the Description tab. Use the *scroll bar* to bring these parameters into view.

Selecting a measurement technique

Another important step in developing a *spectral measurement method* is choosing what you want to measure. A spectral measurement method can be configured to do any of the following:

- Measure the height or area of a *peak* or *region*.

- Measure the ratio of two spectral peaks.
- Measure the random *noise* in a given region.
- Measure the width at half maximum of the largest peak in a region.
- Locate the largest peak in a specified region.
- Find where a peak is reduced to 1%, 2%, 5% or 10% of its maximum height.

You can measure up to 50 peaks or regions in a spectral measurement method.

Note You can also design a method that calculates the ratio of two spectral peaks by setting the TQ Analyst Pathlength Type option to Peak Ratio. See “Choosing a Pathlength Option” in this chapter for more information. ▲

Methods that report peak heights *or areas* are typically used to consistently measure a peak or area in a series of spectra. The *absorbance* values could then be used to plot a *calibration curve* by hand or on a computer that can calculate spectral plots.

An optional linear function can be applied to the absorbance values from each measured peak for simple mathematical corrections. Each function is defined by its *slope* and *intercept* values. If a linear correction is specified for a given peak, the corrected absorbance value is reported instead of the measured value.

People use methods that measure *spectral noise* to test spectrometer performance or to validate a specific analysis. You can configure a method to measure either *RMS noise* or *peak-to-peak noise* in a specified region.

Some people need to consistently measure the *width of a peak* in a *spectral region* or simply report the peak's exact *location*. These measurements are often useful for validation purposes or to detect changes in the location or shape of a peak. Peak width values and peak locations are reported in the X-axis unit of the corresponding spectrum.

Others simply want to report the location where a peak is reduced to a certain percentage of its maximum value. Percent-of-maximum measurements are typically used for instrument validation. For example, you can use them to report the X-axis location where the energy in a *single-beam spectrum* is reduced to a level that is no longer useful. You can also use percent-of-maximum measurements to monitor the *components* of a chemical reaction.

At this point, you only need to specify that you are creating a spectral measurement method. To do this, set the Analysis Type parameter on the Description tab to "Measurement Only."

To see a detailed description of the Measurement Only option, click the Explain button on the toolbar to open the Explain help window and then click the option in the software.

Analysis Type

Quantitative analysis

- Simple Beer's law
- Classical least squares (CLS)
- Stepwise multiple linear regression (SMLR)
- Partial least squares (PLS)
- Principal component regression (PCR)
- Undecided

Classification

- Similarity match
- Distance match
- Discriminant analysis
- Search standards
- QC Compare search

Measurement

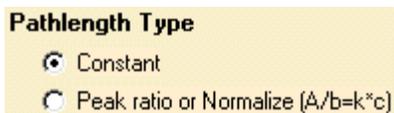
- Measurement only

You will have an opportunity to specify the measurement technique later in this chapter.

Choosing a pathlength option

You can design a method that calculates the ratio of two spectral *peaks* by setting the TQ Analyst Pathlength Type to “Peak Ratio” and specifying the denominator peak or *region* as the *pathlength peak*. See the section on “Selecting the Analysis Regions” in this chapter for instructions. If you specify a denominator peak, the software will apply it to all of the measurements specified in the current method.

To see descriptions of these pathlength options, click Explain and then click each option in the software.



Note

If you don't want to use a denominator peak for any of the measurements in your method, set the Pathlength Type to “Constant.” When Pathlength Type is set to Constant, the software makes no mathematical pathlength correction for any of the measurements specified in the method. ▲

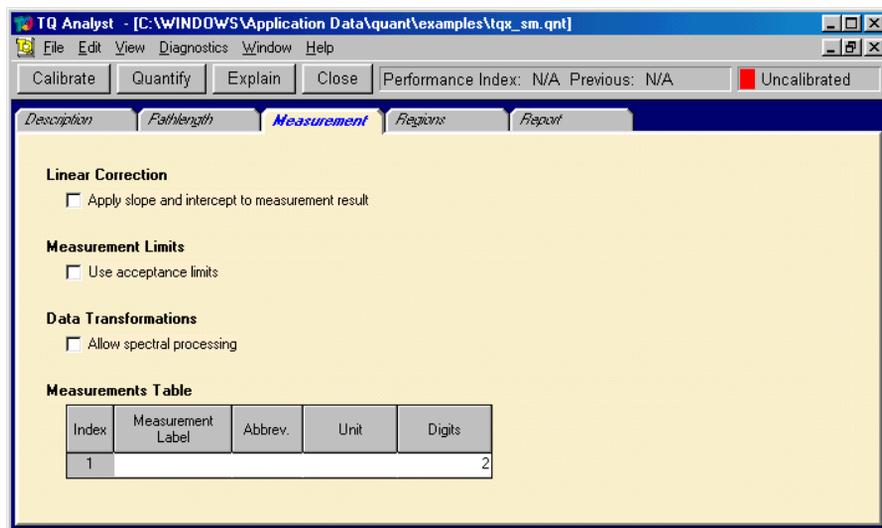
Defining the measurements

The next step in developing a *spectral measurement method* is to define each measurement. Use the Measurements tab to enter information about the measurements in a Measure Only method, such as the label and unit that will be reported. You can also specify a *multiplicative factor* or *offset value* for each measurement.

Entering labels

You can make up to 50 measurements in a *spectral measurement method*. A unique name or label must be specified for each measurement. Enter the labels in the Measurements table on the Measurements tab. When you use the method to analyze a *sample spectrum*, each label and unit will be displayed or printed next to the corresponding measurement result.

To see descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.



Measurements Tab

To label the measurements:

- 1. Select the Measurements tab.**
- 2. Click in the first row of the Measurement Label column, type a label for the first measurement and press Enter.**

When you press Enter, the software adds a blank row to the end of the table.

- 3. Continue entering labels until all of the measurements have been specified.**
- 4. Use the Abbrev column to enter abbreviated labels for each measurement.**

The abbreviated labels will be used whenever the full label doesn't fit in the available space. If no abbreviated labels are entered, the default abbreviations are used.

- 5. Use the Unit column to enter the unit that will be used to report each measured value.**
- 6. Use the Digits column to specify the number of digits after the decimal symbol that will be used to report each measured value.**

Note The digits parameter defines the number of digits that are displayed in TQ Analyst or printed with the *analysis results*, not the number of significant digits that are used in the calculations. ▲

When you use this method to analyze a sample spectrum, the measured value for each *peak* or *region* that is specified in the Measurements table will be reported in the Quantify dialog box, using the specified label, unit and digits.

Applying a multiplicative factor or offset value

You can do simple calculations with a measured value by defining a *multiplicative factor* or *offset value*. When you use the method to measure a *sample spectrum*, the specified corrections will be applied to each measured value according to the following equation:

$$\text{<Corrected value>} = \text{slope} * \text{<measured value>} + \text{intercept}$$

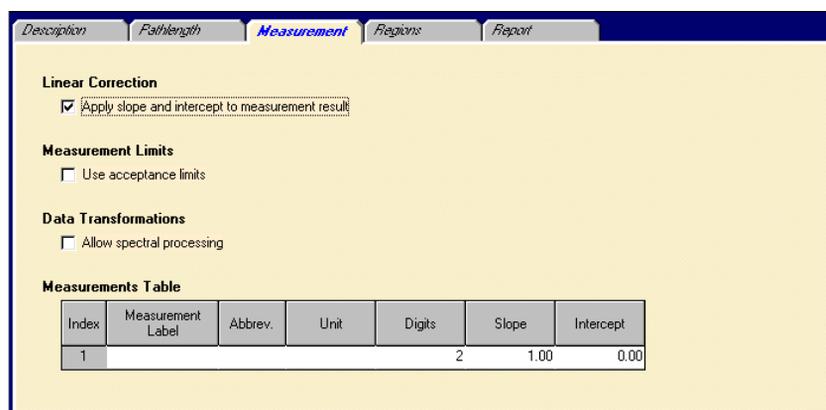
Only the corrected values will be reported.

Note If you are applying linearity corrections to a measurement that is the ratio of two spectral *peaks*, the correction is applied to the calculated ratio instead of the measured value. See the section called “Choosing a Pathlength Option” in this chapter for information on setting up methods that take the ratio of two spectral peaks. ▲

To specify factors or *offset values* for the measurements in your method:

1. Turn on the Linear Correction check box on the Measurements tab.

When the Linear Correction check box is on, TQ Analyst adds a Slope column and an Intercept column to the Measurements table.



The screenshot shows the 'Measurement' tab in the TQ Analyst software. The 'Linear Correction' checkbox is checked, indicating that slope and intercept will be applied to measurement results. Below this, there are sections for 'Measurement Limits' (unchecked) and 'Data Transformations' (unchecked). At the bottom, the 'Measurements Table' is displayed with the following columns and values:

Index	Measurement Label	Abbrev.	Unit	Digits	Slope	Intercept
1				2	1.00	0.00

- 2. In the column labeled “Slope,” enter a multiplicative factor for each measurement.**

If you don't want to apply a factor to a measurement, enter “1” in the Slope field.

- 3. Use the column labeled “Intercept” to specify offset values for the spectral features to be measured.**

Enter “0” in the Intercept field if you don't want to use an offset.

The specified linear corrections will be applied to each measured value. Only the corrected values will be reported.

Collecting representative sample spectra

When you are finished defining the measurements and selecting a pathlength option, you are ready to collect the spectra of a few representative *samples*. You will use the representative spectra to set up the *analysis regions* for the method. This section explains how to choose representative samples and collect their *infrared* spectra.

Choosing representative samples

Choosing typical *samples* is probably the most critical factor in making accurate measurements of spectral data. For example, if you specify a complex baseline correction for a *peak* or *region*, you must be sure the peak or region looks similar in all of the spectra that will be measured.

When selecting representative samples for a *spectral measurement method*, consider the following guidelines.

- **Use sufficient quantity.**

To determine the number of samples required, consider the type of measurement you want to make. If you are measuring a *peak height*, width, or area with a simple baseline, two or three samples should

be sufficient. Two or three samples should also be enough for measuring the *noise* in a *spectral region* or finding where a peak is reduced to a percentage of its maximum value. If you want to measure a complex peak with overlapping *bands* or specify a complex baseline correction, such as quadratic removed, make sure you collect enough sample spectra to be sure the peak has a relatively consistent shape and location.

The key point is to make sure you include enough samples to adequately represent the variation you expect to see in the samples the method will measure. In general, the more samples used to set up the regions the better they will represent “real world” samples.

- **Use representative mixtures.**

The composition of the representative samples should be similar to the composition of the samples you want to measure. This insures that spectral *baselines* are handled properly and that all substances in the sample are represented.

Make sure you include examples of any variation you expect to see in the samples the method will measure. If you consider the variations when selecting the *analysis regions*, the method will measure the unknown samples with greater accuracy.

Optimizing the data collection parameters

For best results, the representative *samples* should be collected in the same way you will collect the spectra of the unknown samples, using the same settings for the *data collection parameters*. The spectra of the representative samples should be of the highest achievable quality. Assuming that the *sampling technique* is reproducible, spectral quality is determined mainly by the *resolution* and *signal-to-noise ratio* of the spectral data. The parameters that define resolution and signal-to-noise ratio are part of the data collection parameter set.

See your Thermo Scientific spectral analysis software for details on setting the data collection parameters. Once the parameters have been set, we recommend using the chosen settings to collect the representative samples as well as every unknown sample you use the method to measure.

Collecting the sample spectra

Once the *samples* are prepared and the optimal parameter settings defined, the spectra must be carefully collected and stored on a disk.

Use another spectral analysis package, such as Thermo Scientific OMNIC or RESULT software, to collect the sample spectra. Refer to the documentation that came with that software for step-by-step instructions. Be sure to name and save each spectrum in a file when you are finished collecting it.

Selecting the analysis regions

The final step in building a method is to select the spectral *peaks* or *regions* and specify how they will be measured. If you want to divide the measurement peak by another peak, you must also specify the denominator peak. This feature could be used to calculate the *signal-to-noise ratio* of a spectrum, for example, by measuring the signal in the *analysis region* and the *noise* in the pathlength region.

Selecting the spectral peaks or regions

Use the Regions tab to select the spectral *peaks* or *regions* and specify how they will be measured.

To specify the peaks or regions to be measured:

- 1. Click the Regions tab.**

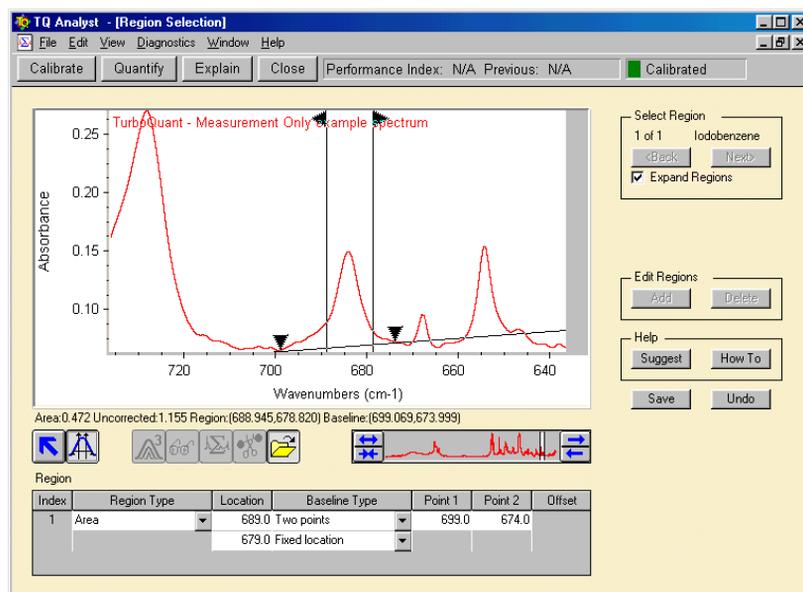
2. Click the Edit Regions button on the Regions tab.

The Open Spectrum dialog box is displayed.

3. Select a few spectral data files that are similar to the spectra you want to measure with this method and choose OK.

The Region Selection task window is displayed.

To see detailed descriptions of the items in this task window, click the Explain button on the toolbar to open the Explain help window and then click the item. For step-by-step instruction on editing regions, click the How To button in the task window.



4. Use the tools in the task window to specify the X-axis limits, Region Type, and baseline of the first peak or region that will be measured.

- Click the Region Type list box and select the type of measurement you want to make. The options are described briefly below. For detailed descriptions of these Region Type options, click the How To button in the Region Selection task

window and display the topic on “Selecting the Region Measurement Type.”

<i>Region Type options</i>	<i>Description</i>
Fixed location height	Finds the nearest data point to the specified X-axis location. Uses that point and the two points on either side to find the interpolated maximum. Then measures the intensity at that location. (Same as OMNIC Peak Height tool.)
Average ht in range	Reports the average intensity value in the specified X-axis range. Useful for measuring <i>noise</i> levels or <i>baseline</i> drift rather than a peak maximum.
Maximum ht in range	Reports the maximum intensity value in the specified X-axis range. Useful for measuring peaks that tend to shift position slightly.
Minimum ht in range	Reports the minimum intensity value in the specified X-axis range. Useful for measuring peaks that point downward and may shift slightly along the X-axis.
Absolute max in range	Reports the maximum intensity value, positive or negative, in the specified X-axis range.
Area	Calculates the sum of the intensity values in the specified X-axis range.
Computed area	Calculates the area of the spectral region that falls between the specified baseline points (or the specified baseline range).

Continued on next page

<i>Region Type options</i>	<i>Description</i>
RMS noise	Measures RMS noise in the specified X-axis range. Useful for testing spectrometer performance or validating a specific analysis.
Peak-to-peak noise	Measures <i>peak-to-peak noise</i> in the specified X-axis range. Useful for testing spectrometer performance or validating a specific analysis.
Interpolated ht at exact loc	Uses the two nearest data points to measure interpolated intensity at the specified X-axis location. Good for measuring baseline intensity or producing the numerator value for signal-to-noise measurements.
Peak location (interpolated)	Reports the interpolated location of the largest peak in the specified X-axis range. Useful for detecting changes in peak location for validation.
Peak height (interpolated)	Finds the interpolated location of the largest peak in the specified X-axis range and measures the intensity at that location. Ideal for measuring <i>peak height</i> .
Peak width (at half max)	Measures the width at half maximum of the largest peak in the specified X-axis range. Useful for detecting changes in peak shape for validation.
Location at x% of peak max	Reports the X-axis location where the largest peak in the specified X-axis range is reduced to 1, 2, 5, or 10 percent of its maximum height. If the peak has two locations that match these criteria, the software reports the location that is nearest the peak maximum. Useful for finding where the energy levels off in a <i>single-beam spectrum</i> .

- When you are finished setting the Region Type, use the Location column to specify the X-axis location or region to be measured. The Fixed Location Height Region Type requires one X-axis value. All of the others except Computed Area require two X-axis values. Use the first Location box to specify the first data point to be included in the

region. Use the second Location box to specify the last data point to be included. Location values are not used for the Computed Area Region Type.

- Now define the spectral *baseline*. Use the first list box in the Baseline Type column to specify a baseline type for the corresponding region. The options are described briefly below. For detailed descriptions of these Baseline Type options, click the How To button in the Region Selection task window and display the topic “Selecting the Baseline Measurement Type.”

<i>Baseline Type options</i>	<i>Description</i>
One Point	Calculates a horizontal line that passes through the spectrum at the specified baseline point and subtracts its absorbance from the peak or region. Use the Point 1 column to specify the baseline offset.
Two point	Calculates a line that passes through the spectrum at two baseline points and subtracts its absorbance from the peak or region. Use the Point 1 and Point 2 columns to specify the baseline points.
Baseline offset	Subtracts a constant from a measured peak or each point in a spectral region. Use the Offset field to specify the constant. Useful for subtracting a constant value that is not a data point in the spectrum.
Linear removed	Calculates a linear least squares fit over the specified region and subtracts it from the region. Useful for correcting baselines that vary.
Quadratic removed	Calculates a 2 nd order polynomial over the specified region and subtracts it from the region. Useful for removing baseline curvature.
None	Measures the peak or region from zero absorbance units

- If you selected a one-point or two-point baseline, use the lower list box in the Baseline Type column to specify how each baseline point will be chosen. The techniques available for choosing baseline points are summarized below. For detailed descriptions of these Baseline Point options, click the How To button in the Region Selection task window and display the topic on “Selecting the Baseline Measurement Type.”

<i>Baseline Point options</i>	<i>Description</i>
Fixed location	Uses the intensity value at the specified X-axis location to define each baseline point. (Allows you to pick a specific baseline point.)
Average in range	Uses the average intensity value in the specified baseline range to define each baseline point. (Useful for picking a baseline point in a noisy region.)
Maximum in range	Uses the maximum intensity value in the specified baseline range to define each baseline point. (Useful for picking the highest baseline point in the range.)
Minimum in range	Uses the minimum intensity value in the specified baseline range to define each baseline point. (Useful for picking the lowest baseline pint in the range.)

- If you selected the One Point, Fixed Location combination, use the Point 1 entry box to specify the X-axis location where you want to draw the horizontal baseline. The Two Point, Fixed Location pair lets you define two points (Point 1 and Point 2) for drawing the baseline.

The remaining options require two baseline points (Point 1 and Point 2) but use two values to define each of them. The software selects each baseline point from the specified baseline

range using the selected technique.

If you selected the Baseline Offset Baseline Type, use the Offset column in the Regions table to define the constant.

You can also specify the *analysis regions* and *baseline points* graphically, by moving the markers in the graphical window. For information on specifying regions graphically, click the How To button in the Region Selection task window.

- 5. For each additional region you want to measure, click the Next button in the Select Region box to select the next region and then repeat step 4 to specify the X-axis limits, Region Type, and baseline for that region.**
- 6. When you are finished specifying regions, click the Close button on the TQ Analyst toolbar to close the Region Selection task window.**

The specifications for the measured regions will be saved automatically and displayed in the Regions table on the Regions tab.

Setting up a denominator peak or region

You can design a method that calculates the ratio of two spectral *peaks* by setting the TQ Analyst Pathlength Type to “Peak Ratio” and specifying the denominator peak or *region* as the *pathlength peak*. This feature can be used to calculate a *signal-to-noise ratio*, for example, by setting up the *noise* measurement as the denominator peak. If you specify a denominator peak, the software will apply it to all of the measurements specified in the current method.

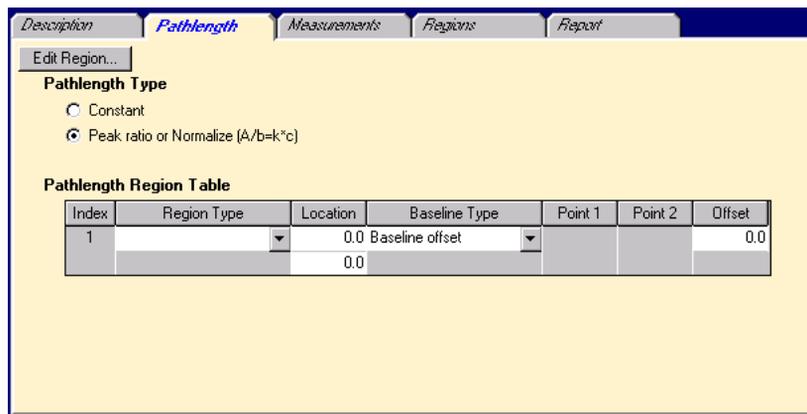
Note If you don't want to use a denominator peak for any of the measurements in your method, set the Pathlength Type to "Constant" and skip this section. When Pathlength Type is set to Constant, the software makes no mathematical correction for any of the measurements specified in the method. ▲

To define a denominator peak or region for all of the measurements in a *spectral measurement method*:

1. **Click the Pathlength tab.**
2. **Set the Pathlength Type parameter to "Peak Ratio."**

The Pathlength Region table appears on the Pathlength tab.

To see detailed descriptions of the pathlength options for spectral measurement methods, click the Explain button on the toolbar to open the Explain help window and then click each option in the software.



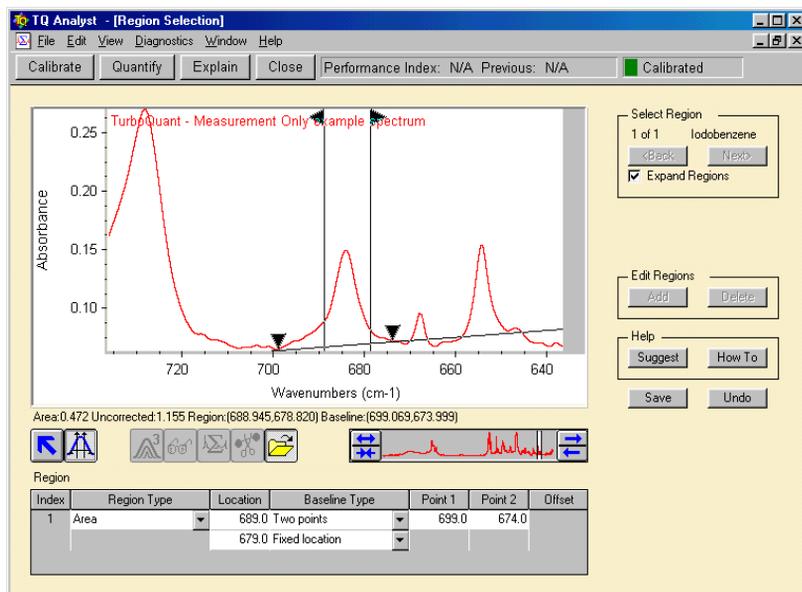
3. **Click the Edit Region button on the Pathlength tab.**

The Open dialog box is displayed.

4. **Select a few spectral data files that are similar to the spectra you want to measure with this method and choose OK.**

The Region Selection task window is displayed.

To see detailed descriptions of the items in this task window, click the Explain button on the toolbar to open the Explain help window and then click the item. For step-by-step instruction on editing regions, click the How To button in the task window.



5. Use the tools in the task window to specify the X-axis limits, Region Type, and baseline for the denominator peak.

See “Selecting Spectral Peaks or Regions” in the previous section for instructions.

6. Click the Close button on the TQ Analyst toolbar to close the Region Selection task window.

The specifications for the *pathlength peak* will be saved automatically and displayed in the Pathlength Region table on the Pathlength tab. When you use this method to analyze a *sample spectrum*, each reported value will be the ratio of the measured peak and the denominator peak.

Note For more information about setting pathlength options and defining the region for the Peak Ratio pathlength setting, click the *Explain*

Customizing the method

button on the TQ Analyst *toolbar* to open the Explain help window and then click a feature on the Pathlength tab. ▲

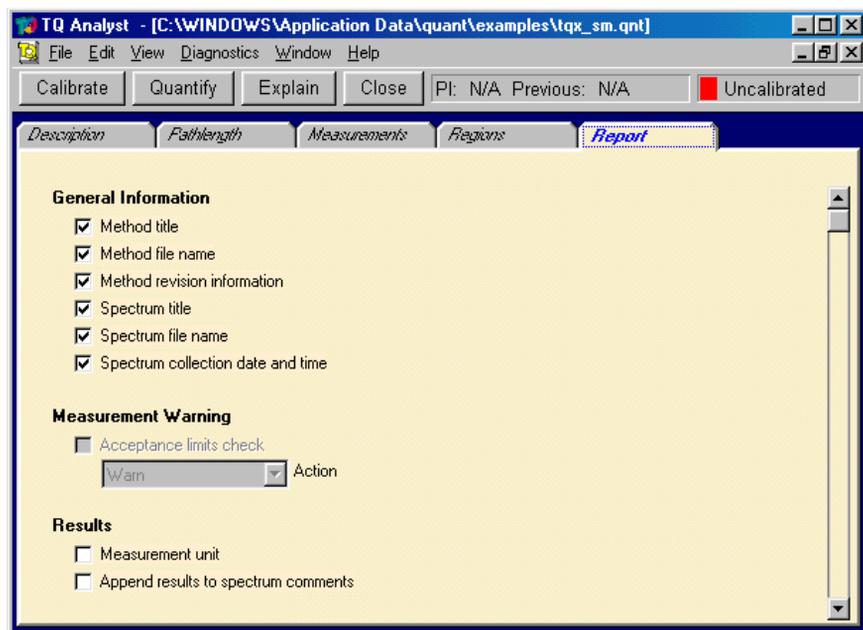
If you have been following the guidelines provided in this chapter, your method contains all of the information needed for calibration, including:

- Descriptions of the measurements.
- Representative *samples* measured using reproducible *sampling technique*.
- *Spectral regions* defined.

There are other features and settings in the software that may be used to customize your method. For example, if you want to calculate *derivative* spectra or apply smoothing or baseline correction, turn on the *check box* for Allow Spectral Processing on the Standards tab. (See the chapter called “Processing Spectral Data” in this document for more information.)

Use the parameters on the Report tab to configure the sample reports. See the section on “Setting Up Sample Reports” in the chapter called “Preparing a Method for Sample Analysis” for details.

To see detailed descriptions of the software features in a spectral measurement method, click the Explain button on the toolbar to open the Explain help window and then click the feature.



Report Tab for Measure Only Method

Feel free to consider or try all of the features included in your method. If you don't have time to review them or don't need special enhancements, leave the *default settings* provided in the software. TQ Analyst is designed to provide a working method with a minimum of effort.

The method should produce acceptable results for most sample types when the *method parameters* are set to their default values. To see the default settings for a *spectral measurement method*, choose New Method from the File Menu and set the Analysis Type to "Measurement Only."

If you prefer using other method specifications or have specifications you need to follow, you may change the settings for any *method parameter* at any time.

Saving the method

We recommend that you save your work regularly when creating or editing methods. Be sure to save the method again before you *calibrate* it.

To save your method:

- 1. Select the method you want to save by clicking the method window or by choosing the method name in the Window menu.**

- 2. Choose Save Method from the File menu.**

If the method does not have a file name, the Save Method As dialog box appears.

- 3. If the Save Method As dialog box appears, type a file name and select the directory and disk where you want the method saved.**

- 4. Choose OK.**

Calibrating the method

When you are finished choosing a pathlength option, defining the measurements, collecting representative *sample* spectra, selecting the *analysis regions* and saving, your method is ready for calibration.

Spectral measurement methods do not require calibration in the same sense that quantitative and discriminant analysis methods do. These methods are intended for reporting information about or simple measurements from spectral data, rather than predicting behavior in unknown samples, so they don't require a *calibration model*. However, TQ Analyst uses the "calibration" step to test a spectral measurement method for consistency and experimental design.

To calibrate a spectral measurement method, click the Calibrate button on the TQ Analyst *toolbar*. Calibration takes only a second. When calibration is completed, the calibration *readout* on the TQ Analyst toolbar changes from red to green and the “Uncalibrated” message changes to “calibrated.”

Validating the method

The final step in developing a *spectral measurement method* is to validate the method. Validation simply means using the method to analyze a few real world *samples* in order to test the method’s performance.

You can run the samples individually or use the External Validation diagnostic routine to run them automatically. External Validation can be used to analyze a large group of validation samples that are listed in a spreadsheet or text file. The validation data are placed in a spreadsheet (Microsoft® Excel®) or text file. See the chapter on “Method Diagnostics” in this document for more information on External Validation.

If the validation results are acceptable, your method is complete and ready for repeated sample analysis. If the method measures or reports incorrect values for the validation samples, you must identify what caused the error and try to fix it.

Some typical sources of error are listed below:

- The *region*, region type or baseline treatment is inappropriate for the spectral featured that is measured. See “Selecting the Analysis Regions” in this chapter for more information.
- The spectral information from the unknown samples is different from the samples that were used to select the analysis regions. See “Choosing Representative Samples” in this chapter for more information.

- Spectral errors caused by sampling, instrument *noise*, or strong absorptions are present in the sample spectra. See “Replacing a Standard” in the chapter called “Working With Standards” for more information.
- Other components in the samples are producing interfering *peaks* in the selected *analysis region*. See “Choosing Standards for Spectral Measurement Methods” in this chapter for more information.
- An inappropriate pathlength treatment was chosen. See “Choosing a Pathlength Option” in this chapter for more information.

Continue checking for errors and recalibrating the method until the validation results are acceptable.

Where to
go from here

Method development is an iterative process. Take time to try the options available for developing a *spectral measurement method* to see if there is room for further improvement. We also recommend reviewing the information on spectral measurement methods in the chapter called “Principles of TQ Analyst” at the beginning of this document.

Remember to *calibrate* the method each time you change a *method parameter*.

TQ Analyst provides a few diagnostic routines for spectral measurement methods. See the chapter called “Method Diagnostics” in this document for more information. Detailed explanations of the diagnostic displays, including tips on how to interpret the diagnostic results, are also available on-line by pressing the *Explain button* when the diagnostic window is open.

If you need information on setting up sample reports, see the chapter titled “Preparing a Method for Sample Analysis.”

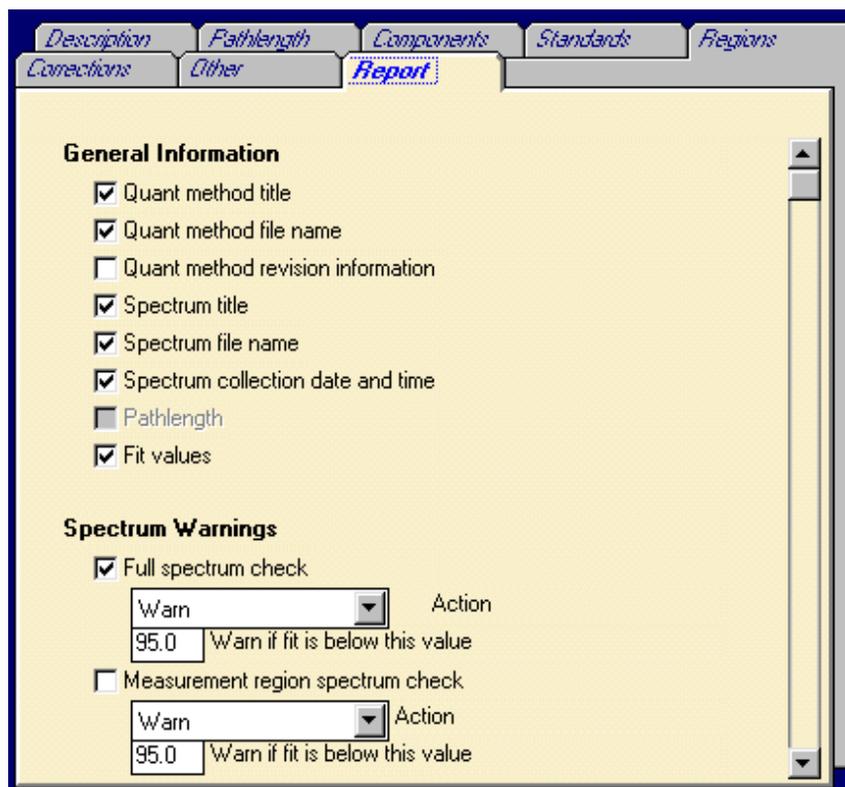
11 Preparing a Method for Sample Analysis

If your *calibrated* method is performing acceptably, you are ready for the final step in the method development process. Read this section to learn how to set up the sample reports, run a test sample, and set security options on your completed method.

Setting up the sample reports

The parameters on the Report tab allow you to choose the information you want to include in your sample reports, including any spectrum or result warnings.

To see detailed descriptions of the items in this window, click the Explain button on the toolbar to open the Explain help window and then click the item.



The screenshot shows a software window with several tabs: Description, Pathlength, Components, Standards, Regions, Corrections, Other, and Report. The Report tab is selected and highlighted. The window contains two sections: General Information and Spectrum Warnings. In the General Information section, there are seven checkboxes: Quant method title (checked), Quant method file name (checked), Quant method revision information (unchecked), Spectrum title (checked), Spectrum file name (checked), Spectrum collection date and time (checked), Pathlength (unchecked), and Fit values (checked). In the Spectrum Warnings section, there are two checkboxes: Full spectrum check (checked) and Measurement region spectrum check (unchecked). Each checked checkbox has a dropdown menu set to 'Warn' and a text input field set to '95.0', with the text 'Warn if fit is below this value' to the right of the input field. The word 'Action' is positioned to the right of the dropdown menu for each warning type.

Report Tab for Measurement Only Method

Adding and removing report features

You can specify the information you want included in your sample reports by clicking the desired options on the Report tab. For example, if you want the *method title* displayed or printed in each sample report, make sure the *check box* for that feature is on. If you don't want to include an item, turn off the corresponding check box.

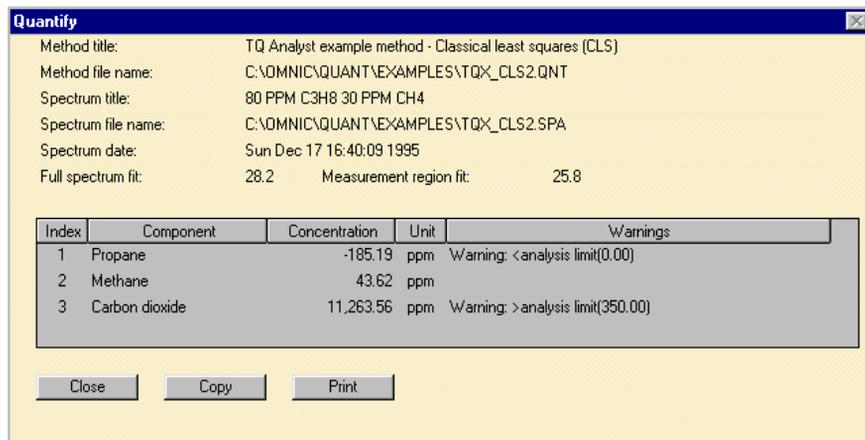
Note *Uncertainty values* for PLS and PCR methods are calculated and reported only if the method includes *validation standards* and the Uncertainty Value check box on the Report tab is on. ▲

Turning sample checking options on and off

You can use the software's built-in *sample checking* features to monitor the *samples* you analyze with a method for possible problems. TQ Analyst can catch three types of problems during sample analysis:

- Problems with the collection parameters
- Problems with the sample spectra, and
- Problems with the *analysis results*.

If a sample checking feature is active and a sample fails the check, a warning appears on the *sample report* as shown in the example below.



The screenshot shows a window titled "Quantify" with a yellow background. It displays the following information:

Method title: TQ Analyst example method - Classical least squares (CLS)
Method file name: C:\DMNIC\QUANT\EXAMPLES\TQX_CLS2.QNT
Spectrum title: 80 PPM C3H8 30 PPM CH4
Spectrum file name: C:\DMNIC\QUANT\EXAMPLES\TQX_CLS2.SPA
Spectrum date: Sun Dec 17 16:40:09 1995
Full spectrum fit: 28.2 Measurement region fit: 25.8

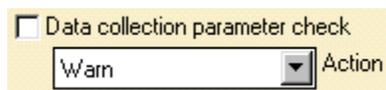
Index	Component	Concentration	Unit	Warnings
1	Propane	-185.19	ppm	Warning: <analysis limit(0.00)
2	Methane	43.62	ppm	
3	Carbon dioxide	11,263.56	ppm	Warning: >analysis limit(350.00)

At the bottom of the window are three buttons: Close, Copy, and Print.

Parameter check The parameter check compares the parameter settings that were used to collect each *unknown sample spectrum* to the settings used to collect the calibration spectra for the current quantitative or *classification method*. If the settings for certain key collection parameters (*resolution* and Y-axis unit) don't match, TQ Analyst can display or print a warning on the *sample report*.

Note The data collection parameter check is not available for *spectral measurement methods*. ▲

To turn on the parameter checking feature, set the check box for Data Collection Parameter Check on the Report tab.



Then specify what action TQ Analyst should take if a spectrum fails the parameter check by choosing an option in the corresponding *Action list box*.

If Action is set to “Warn” and a sample you *quantify* with the active method fails the Data Collection Parameter Check, TQ Analyst displays or prints the quantitative or qualitative results for that spectrum along with the following message: “Warning: Data collection parameter settings don't match those in method.”

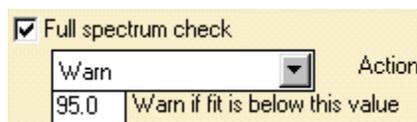
If Action is set to “Warn and don't report values,” TQ Analyst displays or prints the warning message but not the quantitative or qualitative results.

Spectrum checks

The spectrum checks, such as the *full spectrum check* and the *measurement region spectrum check*, compare each *unknown sample spectrum* to the calibration spectra in the current method. The full spectrum check uses all of the spectral data in the entire X-axis range of the calibration spectra for the comparison. The measurement region spectrum check compares the spectral information in the specified *analysis regions*.

The sample spectrum is then assigned a fit value that is scaled to 100, where 100 is a perfect match. If the fit value falls below a predefined threshold in the current method, the sample is flagged and the specified action is taken.

The spectrum checks can be turned on in three steps. First turn on the checking feature by selecting Full Spectrum Check or Measurement Region Spectrum Check.



The screenshot shows a configuration window for the 'Full spectrum check'. It features a checked checkbox labeled 'Full spectrum check'. Below this, there is a dropdown menu currently set to 'Warn' with the label 'Action' to its right. Underneath the dropdown is a text input field containing the value '95.0' and a label 'Warn if fit is below this value'.

Then enter a threshold (0 to 100) for the spectrum's fit value. Finally, select what action will be taken if a spectrum fails the check by choosing an option in the *Action list box*.

If Action is set to "Warn" and a sample you *quantify* with the active method fails the full spectrum check, TQ Analyst displays or prints the quantitative or qualitative results for that spectrum along with the following message: "Warning: full spectrum check indicates a significant difference from *standards*."

If Action is set to "Warn and don't report values," TQ Analyst displays or prints the warning message but not the quantitative or qualitative results.

Note If the Fit Values *check box* on the Report tab is on, the *full spectrum fit value* and the *measurement region fit value* for each unknown sample spectrum will be reported with the *analysis results*. ▲

Result checks The result checks compare the *analysis results* for each *component* to a predefined threshold or limits in the current method. If a measured *peak height* or area, or a calculated concentration value or *match value* falls outside the specified limits or below the threshold, TQ Analyst flags the result and takes the specified action.

The result checks can be turned on in three steps. First, turn on the checking feature by setting the appropriate *check box* (*Analysis Limits Check*, *Acceptance Limits Check*, *uncertainty value check*, *Standard Error of Measurement Check*, *Match Value Check*, or *Distance Value Check*).



Then enter a threshold or limits for the analysis result. You can set the limit for the *Distance Value Check* in a *Discriminant Analysis* method or the *Match Value Check* in the other classification methods directly from the Report tab.

The limits for the *Analysis Limits Check*, *Acceptance Limits Check*, *uncertainty value check* and *Standard Error of Measurement Check* must be specified on the *Components* tab for quantitative methods or the *Measurements* tab for *Measurement Only* methods.

Note The *uncertainty value sample checking* feature is available only for *PLS* and *PCR* methods and works only if the method includes *validation standards*. ▲

Note Standard error of measurement sample checking is available only for CLS methods and works only if the method includes validation standards. ▲

Finally, select what action will be taken if a spectrum fails the check by choosing an option in the *Action list box*.

If Action is set to “Warn” and a sample you *quantify* with the current method fails the corresponding check, TQ Analyst displays or prints the result along with a warning that the result is above or below the specified limit or threshold.

If Action is set to “Warn and don’t report values,” only the warning appears on the report.

Running a test sample

It is a good idea to retest a new method by using it to analyze a few known *samples* before distributing the method to your labs for repeated sample analysis. Testing the method also allows you to check out the *sample reports* the method will produce.

The *sample spectrum* must be stored in a spectral data file before it can be analyzed.

To analyze a sample from TQ Analyst:

- 1. Select the method you want to run by clicking the method window or choosing the method name in the Window menu.**
- 2. Click the Quantify button on the TQ Analyst toolbar.**

The Open Spectrum dialog box is displayed.

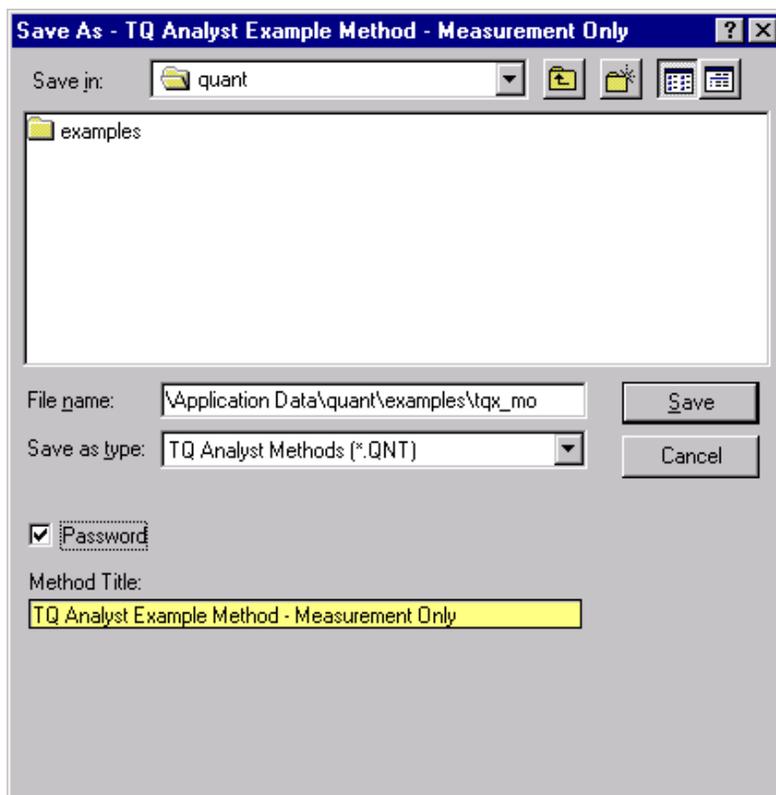
3. Select a spectral data file and choose OK.

You can select spectra that were saved using any Thermo Scientific software or spectra that are in another format, or “file type,” such as GRAMS386 and CSV. Use the Files of Type list box to select a specific file type, or choose “All Files” to see all the files in the current directory.

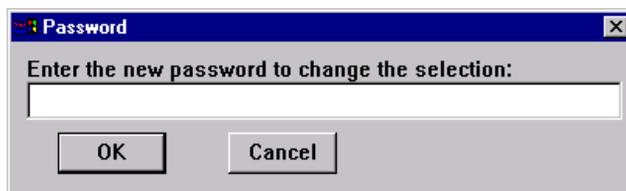
For more information on analyzing samples from TQ Analyst, refer to the sections titled “Analyzing a sample from TQ Analyst,” and “Analyzing multiple samples from TQ Analyst” in this document.

Saving and protecting your method

To save your completed method, choose the Save Method As command from the File menu. You can set a security code in the Save Method As dialog box so others can’t open or change your method file without your permission. To protect the current method, turn on the Password *check box* in the Save Method As dialog box.



When you choose OK to close the dialog box, another dialog box appears asking you to type a password for the method.



Type a password and choose OK. Then retype the password to confirm it and choose OK again.

If you decide to protect a method, make sure you write down the password and store it in a safe place. You won't be able to reopen or delete the method without it.

Note You don't have to enter a password to run a protected method from another Thermo Scientific application, such as OMNIC or RESULT. ▲

12 Updating the Calibration Data for a Method

You may find it necessary to update the calibration data for a method, either on a development system or on a system that is located in a process or other laboratory environment. This process typically involves adding, replacing or deleting one or more *standards* and then recalibrating the method.

See the chapter on “Working With Standards” for information on adding, replacing and deleting standards in a TQ Analyst method. The spectrum for a new standard can come from a file or the operator may collect the standard directly in TQ Analyst. Collecting new standards in TQ Analyst ensures that the new standards will match the other standards in the method (TQ Analyst automatically uses the same settings for the data collection parameters). If you add or replace standards, make sure you update the component concentration values associated with those standards.

When you are finished updating the standards, click the Calibrate button on the TQ Analyst *toolbar* to calibrate the new method. See the sections called “Calibrating The Method” in the individual chapters in creating a quantitative, classification or *spectral measurement method* for details on interpreting the calibration results.

Note If you update the calibration data, make sure you check whether any corrections were specified for the calibrated method. If corrections were specified, check whether the corrections are still valid and update or delete them as necessary. ▲

When you are satisfied with the calibration results, choose Save Method from the File menu to save the updated method.

Each time you calibrate and save a method, the Revision code on the Description tab increments. This will help you track changes made to a method when the initial development has been completed.

13 Method Diagnostics

TQ Analyst provides a collection of useful diagnostics for each of the analysis types. If you use the wizards and other automatic features of TQ Analyst software, most of these diagnostic routines won't be necessary. Whenever possible, the software runs statistical and other analyses on your data and uses the results to provide default values and recommendations during method development. In most cases, the default values and recommendations will be the optimum settings for your analysis

If you are experienced in chemistry and statistical analysis or have favorite diagnostic routines you typically run on new methods or use to solve specific problems, read this chapter. Our software provides many of the diagnostic routines commonly used for spectroscopic analysis, including interactive displays of the diagnostic results. Use them to:

- Identify when too much or too little data are used to create the method calibration model.
- Look for inaccuracies in the data that affect calibration.
- Help you make adjustments to improve the method's performance.

Detailed explanations of the diagnostic displays, including tips on how to interpret the diagnostic results, are available for each *diagnostic task window*. To display information about a diagnostic task window, click the *Explain button* on the TQ Analyst *toolbar* while the task window is open and selected.

The on-line help also includes information on each diagnostic, including examples of typical diagnostic results. Look for them in the Diagnostics section under the TQ Analyst Help Topics item in the Help menu.

All of the diagnostic routines provided in the software are listed in the Diagnostics menu. Many of the diagnostics are available only for certain method types or certain stages of development. For example, you can run the Principal Component Spectra diagnostic on any quantitative or discriminant analysis method but you must collect or open the spectral data files for the *standards* before the diagnostic can be used.

To see the diagnostic commands that are available for the current method, select the method by clicking the *method window* or selecting its name in the Window menu. Then click the Diagnostics *menu name* at the top of the *TQ Analyst window*.

Diagnostic routines that are currently available for the selected method are listed on the menu in black type; routines that are unavailable appear in gray and cannot be selected, either because they don't apply to the method type or the method doesn't include enough information to run them.

The following table provides a list of the diagnostic routines available in TQ Analyst and the compatible method types.

<i>Name</i>	<i>Available for...</i>
Eigenanalysis	All quantitative methods, Discriminant Analysis, Distance Match
Principal Component Spectra	All quantitative methods, Discriminant Analysis, Similarity Match, Distance Match
Pairwise Concentration	All quantitative methods
Spectrum Outlier	All quantitative methods
Pure Component Spectra	All quantitative methods
Statistical Spectra	All quantitative methods, Discriminant Analysis, Distance Match
Cross Validation	All quantitative methods
External Validation	All method types
Residual Spectra	CLS, PLS, PCR, Similarity Match and Distance Match methods
Multiple Quantify	All method types
PRESS	PLS methods
Principal Component Scores	PLS, PCR, Discriminant Analysis and Distance Match methods
Factor Loading	PLS methods
Leverage	PLS methods
Loading Spectra	PLS methods

Eigenanalysis diagnostic

The Eigenanalysis diagnostic determines the distribution of spectral variation and concentration (or class) in the *principal components* of a *calibration set*. The diagnostic results can help you determine if you have enough independent sources of spectral variation relative to the number of *components* or *classes* in a quantitative or *classification method*. For example, if your method is trying to *quantify* seven components, or to distinguish between 7 classes, the spectral data for the *standards* must contain at least seven independent sources of variation for the method to work.

You can run the Eigenanalysis diagnostic on any quantitative, Discriminant Analysis or Distance Match method. You must fill in the Standards table on the Standards tab and the Components table on the Components tab (quantitative methods only) or the Classes table on the Classes tab (Discriminant Analysis and Distance Match methods only) before the Eigenanalysis diagnostic can be used.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Eigenanalysis command from the Diagnostics menu. The diagnostic results are displayed in a task window. The data show how the variation in the *calibration standards* is distributed over the number of principal components that are found.

TQ Analyst - [Eigenanalysis]

File Edit View Diagnostics Window Help

Calibrate Quantify Explain Close Performance Index: 92.2 Previous: N/A ■ Calibrated

There is sufficient variability for this method

Principal Component	Concentration Contribution	Full Spectrum Contribution	Analysis Region Contribution
1	44.5306	43.4805	61.6064
2	76.8456	60.7268	93.4434
3	98.3899	70.2599	97.4975
4	100.0000	75.0156	98.8914
5	100.0000	78.3917	99.5050
6	100.0000	81.0869	99.6250
7	100.0000	83.4931	99.7011
8	100.0000	85.6180	99.7517
9	100.0000	87.6716	99.7844

The Concentration Contribution column indicates the percentage of the variation in concentration that is described by each principal component. The Full Spectrum Contribution column shows how the variation in the entire spectral range of the standards is distributed among the principal components. The Analysis Region Contribution column shows the variation in the *analysis region* or regions. This column appears in the Eigenanalysis task window only if the method is calibrated.

A statement at the top of the diagnostic window indicates whether there is sufficient variability in the standards to analyze the specified number of components or to distinguish the specified number of classes in the method.

Principal Component Spectra diagnostic

The Principal Component Spectra diagnostic displays the principal component spectra for the following method types:

- All quantitative methods
- Discriminant Analysis
- Similarity Match
- Distance Match.

A *principal component spectrum* is the orthogonal spectrum that represents the amount of variability described by a *principal component*. These spectra can help you determine the proper number of principal components or *factors* to use for the analysis (look for prominent spectral features that appear in the first few principal component spectra).

One principal component spectrum is included for each principal component that is needed to describe 99.9% of the spectral variation in the *standards*. At least one principal component spectrum is created for each *component* or *class* that is specified in the method (or a minimum of 10 principal component spectra).

The principal component spectra show how the spectral information in a *calibration set* is represented by the principal components and how much of the total spectral variation each principal component describes. This information can help you determine if there are enough sources of variation in your data. You can also use this diagnostic to verify the number of principal components required to describe the spectral variation in a calibration set.

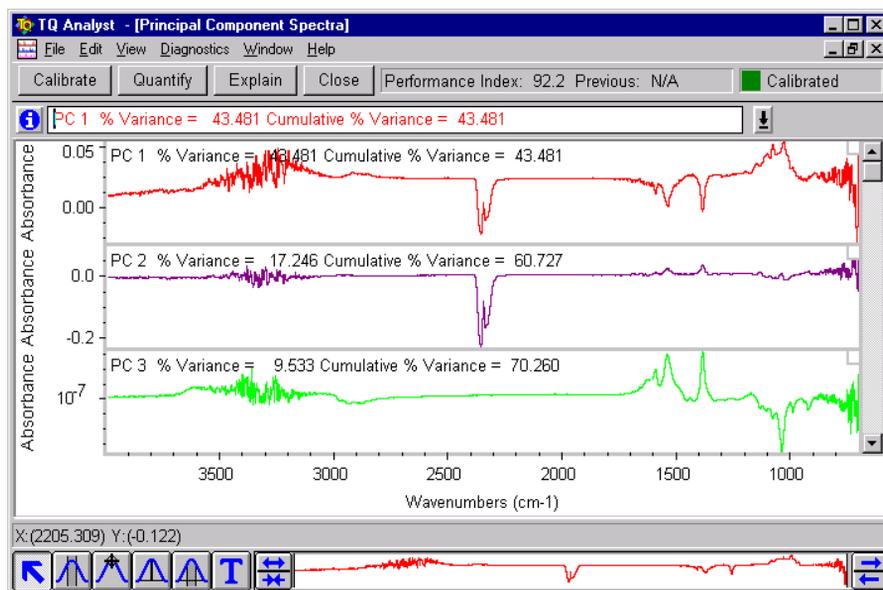
Sources of variation in the calibration spectra will produce distinct features in the principal component spectra. The features may look like a spectral *peak* or a *derivative* peak. A noisy or featureless principal component spectrum indicates that the corresponding (and any subsequent) principal component contributes little useful information to the *calibration model*.

Note This same information is provided in tabular form in the Full Spectral Contribution column of the Eigenanalysis diagnostic results. ▲

You can run the Principal Component Spectra diagnostic on any quantitative or discriminant analysis method. You must specify the spectral data files for the standards (see the Standards tab) before using this diagnostic. All of the data in the full spectral range of the calibration and validation spectra are used to calculate the principal component spectra. Only the spectral information is examined; any concentration data that is specified in the method will not be used.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Principal Component Spectra command from the Diagnostics menu.

The diagnostic results are displayed in a *spectral window*. The selected spectrum is displayed in red.



Each principal component spectrum appears in a separate *pane*. The spectra are labeled “PC 1,” “PC 2,” etc. followed by the percentage of the spectral variance described by that principal component and the cumulative percent variance described by all of the principal components combined.

Use the Title box in the spectral window to display a list of the titles of the principal component spectra. To select another spectrum, click its title in the list of titles or click the displayed spectrum.

You can use the View Finder and any of the commands in the View menu to change the limits of the spectral window or to rearrange the spectra in the window. You can also use the tools on the palette, such as the selection tool or the annotate tool, to interact with the displayed spectra.

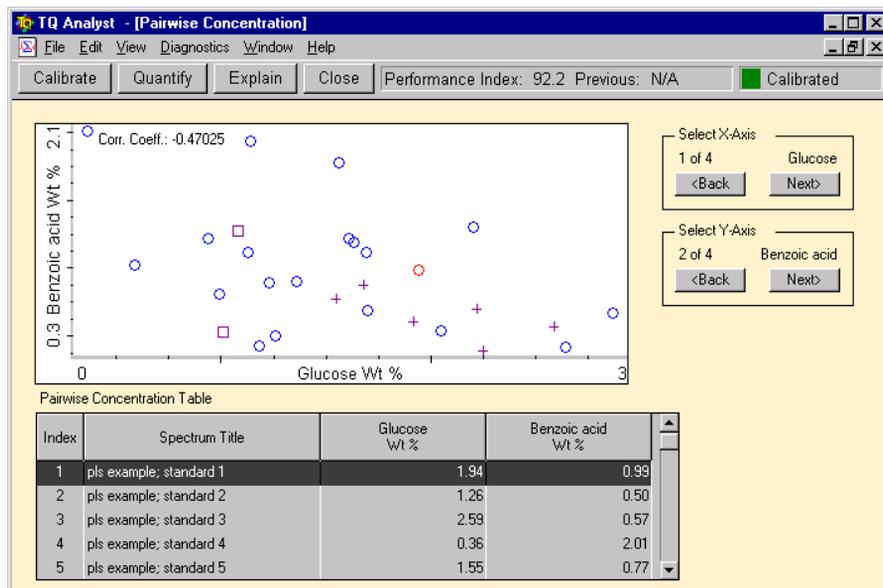
Pairwise Concentration diagnostic

The Pairwise Concentration diagnostic can help you evaluate your *calibration set* for the active quantitative method. The Pairwise Concentration diagnostic allows you to compare the concentration of each *component* in each *standard* to the concentrations of the other components that are present in the standard. The diagnostic results can help you verify that the component concentrations of your standards are distributed evenly over the *analysis range* for each component in the method. The results will also indicate whether the component concentrations of the standards vary independently.

The Pairwise Concentration diagnostic is available only for quantitative methods. You must fill in the Components table on the Components tab and enter the titles and concentration data for the standards in the Standards table (Standards tab) before using the Pairwise Concentration diagnostic (spectral data are not required).

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Pairwise Concentration command from the Diagnostics menu.

The diagnostic results are displayed in a task window. The data show how the concentration of each component in each standard relates to the concentrations of every other component in the standard.



The concentrations of components 1 and 2 in each standard are displayed in a table at the bottom of the window. The plot shows the concentration of component 1 in each standard along the X-axis and the concentration of component 2 along the Y-axis.

Data points in the plot that correspond with *calibration standards* are represented with circles. Plus signs (+) are used to represent *validation standards* and triangles are used for *correction standards*. The Usage parameter on the Standards tab defines how each standard is used in a method.

The ideal pairwise concentration plot will have data points that are distributed randomly over all areas of the plot. The limits of the X- and Y-axis should cover the specified *analysis range* for the selected component. Data points that are clustered or that fall in a line indicate that the two components do not vary independently.

Each concentration value in the table is linked to a data point in the plot. To see the data point that corresponds with a concentration value

in the table, click anywhere in the corresponding row. The data point will be highlighted in the plot.

To see the standard that is linked to a data point in the plot, click the data point. The title of the standard will be highlighted in the table.

Spectrum Outlier diagnostic

The Spectrum Outlier diagnostic finds the spectra of the *standards* which are most unlike the spectra of the other standards and uses either the Dixon or *Chauvenet test* for outliers to determine whether the difference is significant. The diagnostic results can help you identify standards that are *outliers* based on the spectral information for all of the standards. Since the diagnostic ranks each standard based on its distance from the mean, the results are also useful for selecting standards.

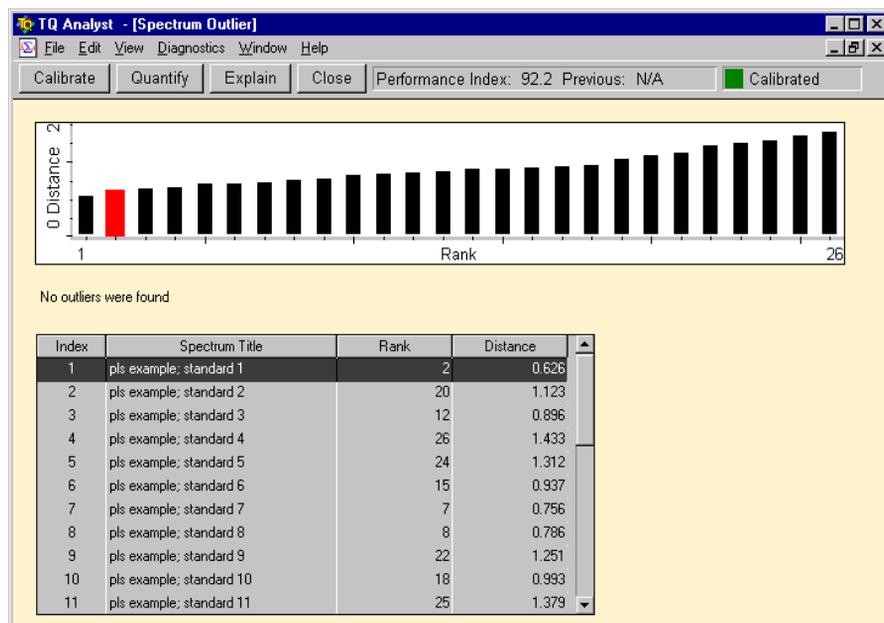
The Spectrum Outlier diagnostic is available only for quantitative methods. You must fill in the Components table on the Components tab, the Standards table on the Standards tab and the Regions table on the Regions tab before the diagnostic can be used.

The diagnostic calculates the *mean spectrum* for all of the standards in the method and then measures the distance between the mean spectrum and the spectrum of each standard. The mean spectrum is a calculated spectrum that shows the average of the spectral features that are present in the spectra of all of the standards.

A test is applied to the standard with the highest rank to see if it is statistically different from the standard with the next highest rank. If the number of standards in the method is less than 30, the *Dixon test* for outliers is used. If there are 30 or more standards, the *Chauvenet test* is used. If the standard fails the test, the standard is considered an outlier.

If the highest ranked standard is identified as an outlier, the standard with the next highest rank is tested. This process continues until a standard passes the outlier test.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Spectrum Outlier command from the Diagnostics menu. The diagnostic results are displayed in a task window.



The histogram displayed shows the *Mahalanobis distance* values of the standards ranked from smallest (closely clustered) to largest (most unlike the others). A vertical gray line is used in the histogram to separate standards that passed the *Dixon test* (left side of the separator) from standards that failed (right side of the separator). A summary of the results is displayed below the histogram.

Note Although Mahalanobis distance values are used in discriminant analysis, this diagnostic routine cannot be used to evaluate the standards in a discriminant analysis method. ▲

The distance value and rank of each standard is also displayed in a table at the bottom of the task window. Each standard in the table is linked to a bar in the histogram. To see the bar that corresponds with a standard in the table, click anywhere in the corresponding row. The bar will be highlighted in the histogram.

To see the standard that is linked to a bar in the histogram, click the bar. The title of the spectrum for the standard will be highlighted in the table.

If an outlier is found, make sure the component concentration values that were entered for the outlier standard (see Standards tab) are correct. You should also display the spectrum of the outlier standard. If the quality of the spectrum is poor, replace the spectrum with a new one. When you are finished, rerun the Spectrum Outlier diagnostic.

Pure Component Spectra diagnostic

The Pure Component Spectra diagnostic displays the calculated pure component spectrum for each *component* in a quantitative method and one pathlength spectrum. Each pure component spectrum is composed of the spectral information that correlates with the concentration information for a given component in all of the *standards*.

The following assumptions are made:

- All of the components that are present in each standard are included as components in the method.
- There are no chemical interactions between components that affect the absorbance in the *analysis region* or regions.

Note If the method is configured to measure *sample* properties rather than component concentrations (see Component Interactions *check box*), the “pure component spectra” are actually “single component spectra,”

which means they are handled as if no other “components” (properties, really) are contained in the sample. ▲

The calculated pure component spectra can help you evaluate the spectral information that will be used to measure each component in a quantitative method. Compare the calculated pure component spectra to the real spectra of the individual components, if those spectra are available. The spectral information in each calculated pure component spectrum should be unique. If the calculated pure component spectra for two or more components in your method look the same or very similar, correlations between spectral and concentration information will be difficult to distinguish and the quantitative results will not be accurate.

If you are creating a CLS method, you can also use the pure component spectra to determine if there are other sources of variation in the *analysis region* or regions of the standards that should be included in the *calibration model*. To determine if additional components are present, compare the calculated pure component spectra created by this diagnostic to the real pure component spectra. To do this, use your Thermo Scientific spectral analysis software to display the real spectrum. Then click the calculated spectrum in this task window to select it and use the Copy command in the Edit menu to copy the spectrum to the Clipboard. Use the Paste feature in your Thermo Scientific spectral analysis software to paste the spectrum into the selected *spectral window*.

When calculating pure component spectra, the software considers *pathlength* as an additional component. Any spectral information that correlates with differences in the pathlengths of the standards is placed in the calculated pathlength spectrum.

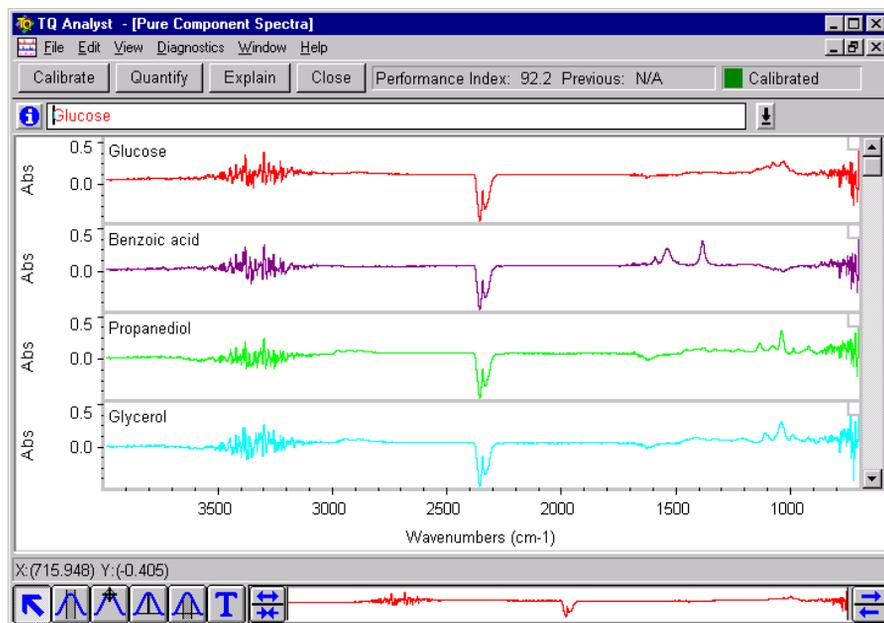
The pathlength spectrum shows the spectral information in the standards which the model attributes to pathlength. When the Pathlength Type (see Pathlength tab) is set to Constant, the pathlength

spectrum may not represent true pathlength but the interpretation is the same. The spectrum represents those spectral features which are constant in all of the standards.

The Pure Component Spectra diagnostic is available only for quantitative methods. You must fill in the Components table on the Components tab and the Standards table on the Standards tab before the diagnostic can be used. All of the data in the full spectral range of the calibration and validation spectra are used to calculate the pure component spectra.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Principal Component Spectra command from the Diagnostics menu.

The diagnostic results are displayed in a spectral window. The selected spectrum is displayed in red.



Each pure component spectrum is displayed in a separate *pane*. The spectra are labeled with the component names from the Components table (Components tab). The pathlength spectrum is labeled “Pathlength.”

Use the Title box in the spectral window to display a list of the titles of the pure component spectra. To select another spectrum, click its title in the list of titles or click the displayed spectrum.

You can use the View Finder and any of the commands in the View menu to change the limits of the spectral window or to rearrange the spectra in the window. You can also use the tools on the palette, such as the selection tool or the annotate tool, to interact with the displayed spectra.

Note The information provided in the pure component spectra can help you select *analysis regions* for the components in your method. To display the pure component spectra in the spectral display area of the Region Selection task window, click the  tool on the View palette in the task window. The pure component spectra will replace whatever spectrum or spectra that were previously displayed in the window. ▲

Statistical Spectra diagnostic

The Statistical Spectra diagnostic generates a series of calculated spectra that illustrate the variation present in the *standards* and, for quantitative methods, the *spectral regions* that correlate with changes in *component* concentration.

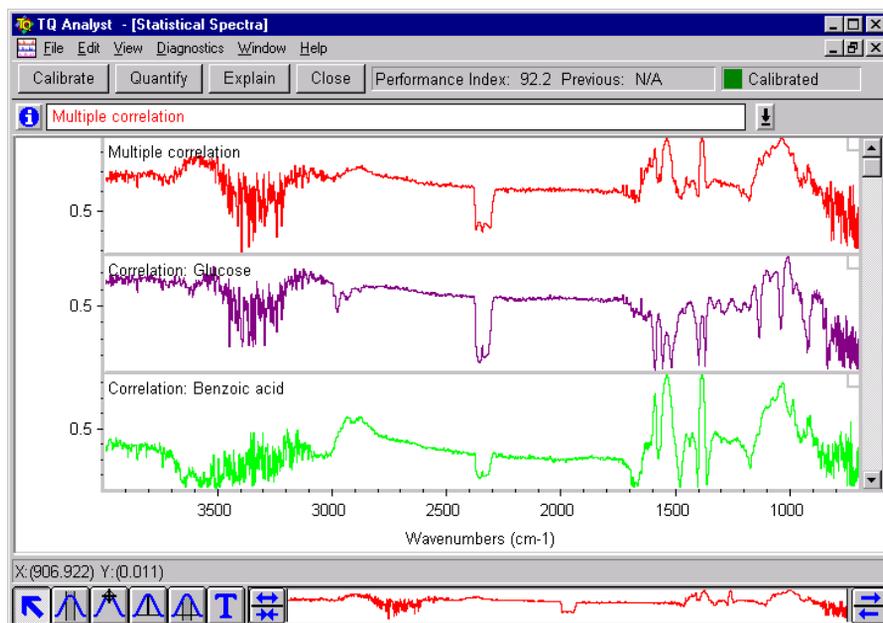
You can run the Statistical Spectra diagnostic on any quantitative, Discriminant Analysis or Distance Match method. You must fill in the Components table on the Components tab (or the Classes table on the Classes tab) and the Standards table on the Standards tab before this diagnostic can be used. All of the data in the full spectral range of the calibration and validation spectra are used to calculate the statistical spectra.

For quantitative methods, the diagnostic uses the spectral and concentration information for all of the standards to generate a *mean spectrum*, a *variance spectrum*, a *multiple correlation spectrum*, and a separate *correlation spectrum* for each component in the method, including the pathlength component. The mean spectrum shows the dominant features that are present in all of the standards. The variance spectrum shows the spectral information that varies in the standards (independent of concentration). The correlation spectra show the spectral regions that correlate with changes in component concentration. This information can help you choose *analysis regions* for your method or understand why specific regions are suggested. The statistical spectra can also be useful for choosing the proper analysis type. For example, if nonoverlapping regions of high correlation exist for each component, a simple calibration model such as SMLR may work well for the analysis.

Note If you run the Statistical Spectra diagnostic on quantitative method that is set up to measure *sample* properties, rather than component concentrations (see Component Interactions *check box*), a *multiple correlation spectrum* will not be generated. ▲

If you run the diagnostic on a discriminant analysis method, it calculates a variance spectrum and a *mean spectrum* for each class.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Statistical Spectra command from the Diagnostics menu. The diagnostic results are displayed in a *spectral window*. The selected spectrum is displayed in red.



Each statistical spectrum is displayed in a separate *pane*. For quantitative methods, the *multiple correlation spectrum* appears first, followed by the *correlation spectrum* for each component in the method, the pathlength correlation spectrum, the *mean spectrum*, and the variance spectrum.

If you run the diagnostic on a discriminant analysis method, the mean spectrum for each *class* appear first, followed by the variance spectrum (labeled “Within Class Standard Deviation”).

The mean spectrum shows the average of the spectral features that are present in all of the standards. The variance spectrum shows the spectral variance in the standards. It is produced by calculating the square root of the spectral variance at each X value over all of the standards. The *multiple correlation spectrum* shows the correlation coefficient of the combined standards at each X-axis value. Each component *correlation spectrum* provides the correlation coefficient of the selected component at each X-axis value.

The component correlation spectra can help you choose the *analysis regions* for the components in your method. Try to find at least one unique region that shows high correlation in each component *correlation spectrum*. If unique regions do not exist for one or more components, choose the regions that have the highest correlation in the *multiple correlation spectrum*. If a component correlation spectrum shows little or no correlation, the component may be difficult to *quantify* because there is little unique spectral information that correlates with changes in component concentration.

Note To display a statistical spectrum in the spectral display area of the Region Selection task window, click the  tool on the View palette in the task window. The statistical spectra will replace whatever spectrum or spectra that were previously displayed in the window. ▲

Compare each component *correlation spectrum* to the variance spectrum to determine the significance of any highly correlated spectral regions. If a spectral region shows high correlation but low variance, the region is important to the analysis but the magnitude of the variation is small. The best regions will have high correlation and high variance.

If one or more unique regions exist for each component in the method, use the Simple Beer's Law analysis type. If the component correlation spectra show regions of high correlation but the regions overlap somewhat, try using the CLS analysis type. If no unique features exist or some of the component correlation spectra show low correlation in all regions, try using the PLS analysis type.

Use the Title box in the spectral window to display a list of the titles of the statistical spectra. To select another spectrum, click its title in the list of titles or click the displayed spectrum.

You can use the View Finder and any of the commands in the View menu to change the limits of the spectral window or to rearrange the

spectra in the window. You can also use the tools on the palette, such as the selection tool or the annotate tool, to interact with the displayed spectra.

Cross Validation diagnostic

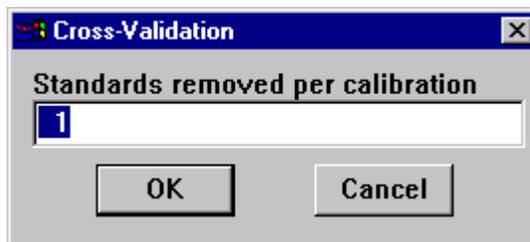
The Cross Validation diagnostic shows how well a *calibrated* quantitative method performs by *quantifying* each *calibration standard* as if it were a *validation standard*. The diagnostic results can help you identify standards that may be *outliers*. You may also use Cross Validation to validate a method that does not include validation standards.

The Cross Validation diagnostic is available only for quantitative methods. The method must be calibration before Cross Validation can be used.

When you run Cross Validation, the software quantifies each calibration standard in the method as if it were a validation standard. This is accomplished by sequentially removing the specified number of standards from the *calibration set*, calibrating the method and using the new *calibration model* to quantify the standards that were removed from the calibration set. The process is repeated until all of the standards in the calibration set have been quantified as validation standards.

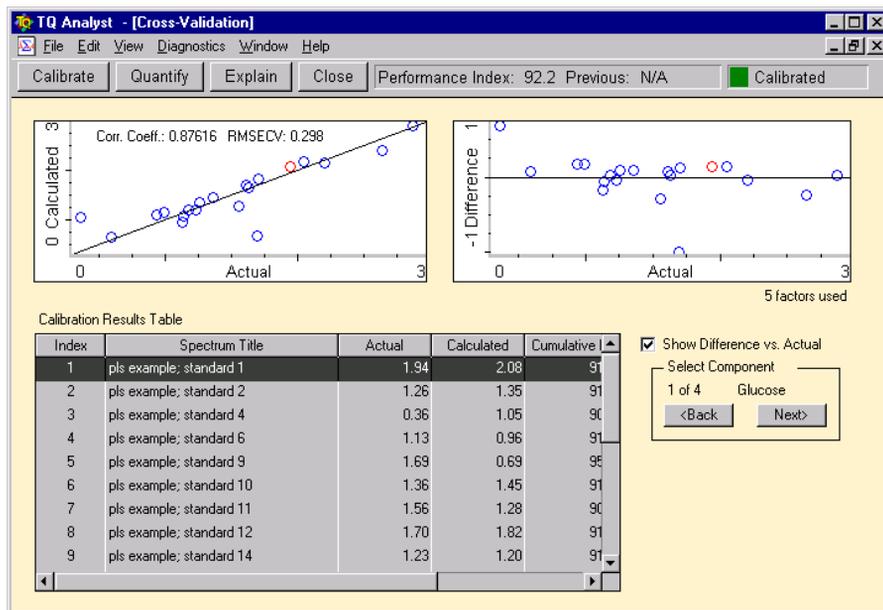
Note If duplicate standards are present in the method, the software will remove and analyze all of them during the same cross validation iteration. We define “duplicate standard” as any standard that has exactly the same concentration values as another standard for all components in the method. ▲

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Cross Validation command from the Diagnostics menu. The following dialog box is displayed.



Enter the number of calibration standards you want to remove for each calibration iteration or use the suggested value. If the method includes many standards, you may want to remove more standards per iteration to reduce the calculation time.

The diagnostic results are displayed in a task window.



The table in the lower half of the task window shows the calculated and actual concentration values and the % difference value for each *cross validation standard* that contains the selected component. A

cumulative and component *performance index* for each cross validation calibration are also provided in the table.

Note These performance values will only be provided if validation standards are specified in the Standards table (see Standards tab). ▲

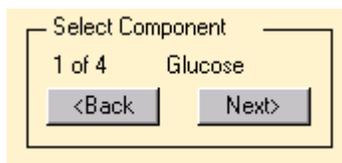
Two plots are shown at the top of the task window. The Calculated versus Actual plot compares the calculated concentration value of each standard in the *validation set* to its actual concentration value. The calculated values are the concentration values that were calculated using the calibrated method that excludes that standard. The actual values are the known concentration values that were entered in the Standards table (see Standards tab).

The % Difference plot shows the differences between the calculated and the actual concentration values relative to the actual values.

If a pathlength column is displayed in the table, the concentration values shown in the plots have been adjusted for differences in *pathlength*. The pathlength adjusted concentration values are the calculated and actual concentration values displayed in the table multiplied by the appropriate pathlength value.

Each data point in the plots is linked to a standard in the table. To identify the standard that is associated with a data point in a plot, click the data point. The corresponding standard will be highlighted in the table. To find the data point that corresponds to a standard in the table, click anywhere in the row. The associated data point will be highlighted in both plots.

To select the next or previous component in the method, click the Next or Back button in the Select Component group.



The name (or abbreviated name) and *index number* for the selected component are displayed above the buttons. When you select another component, the cross validation data for the new component are displayed in the cross validation table and plots.

Use the cumulative and component performance indices listed in the Cross Validation Results table to evaluate how the method will perform when the corresponding standard is excluded from the *calibration set*.

Note If you removed two or more standards for each cross validation iteration, the performance indices for the standards in each group that was removed will be identical. ▲

If a method calculates concentration values perfectly (i.e., the calculated value for every standard matches its actual value exactly), the data points in the Calculated vs. Actual plot will form a line exactly 45° from both axes. The greater the distance between a data point and this ideal line, the greater is the difference between the calculated and the actual value for the corresponding standard.

A typical % Difference plot will show data points distributed randomly above and below the zero line within a narrow concentration range.

If you suspect that a standard is an outlier, check that the concentration and pathlength values were entered correctly in the Standards table (see the Standards tab). You should also display the spectrum of the outlier standard. If the quality of the spectrum is poor, replace the spectrum with a new one.

If the data look okay, consider the chemical makeup of the standard and how the standard is used in the method. *Cross validation* data provide a true measure of accuracy because only the validation data are included in the displayed results. Validation data are better indicators of the accuracy of a *calibration model* than calibration or correction data because calibration and *correction standards* are used to generate the model while validation standards are used only for validation. It is up to you to decide whether or not an outlier standard should be included in the calibration or validation set.

External Validation diagnostic

External Validation allows you to *quantify* a large group of validation spectra using any TQ Analyst method and store the data in a format that is accessible to other applications.

The validation results include the calculated concentration value, the actual concentration value and the % difference ($((\text{calculated} - \text{actual}) / \text{actual}) * 100$) for each component in each validation sample. A *performance index* is also provided for each component in the method as well as the *slope* and *intercept* of the line that represents the calculated versus actual plot for each component. The slope and intercept values are used to measure linearity.

An input file must be used to specify the file names and component concentrations of the spectra to be quantified. You can use any spreadsheet or text editor application to create the input file. If you use a spreadsheet application to create the input file, you must save the file as a tab delimited text file in order to use it with External Validation.

Note The input file must be formatted properly for use with External Validation. For more information, refer to the next section. ▲

The validation data are placed in a spreadsheet (Microsoft® Excel®) or text file.

You can run External Validation on any TQ Analyst method. The method must be *calibrated* before External Validation can be used.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the External Validation command from the Diagnostics menu. The standard Windows Open dialog box appears listing files of the text (*.TXT) file type. To list all the files in the indicated directory, select All Files (*.*) from the Files of Type drop-down list box. Select the file that contains the list of validation spectra you want to quantify and click OK to begin the analysis.

When the analysis is finished, the results are displayed in an application window. If TQ Analyst locates the Microsoft® Excel® spreadsheet application on your computer, the validation data are displayed in an Excel application window. If Excel is not found, the data appear in a window for a text processing application, such as NotePad or Write.

The validation results include the calculated concentration value, the actual concentration value and the % difference ($((\text{calculated} - \text{actual} / \text{actual}) * 100)$) for each component in each validation sample. A performance index is also provided for each component in the method as well as the slope and *intercept* of the line that represents the calculated versus actual plot for each component.

If you close the result window, the data will be saved in the file VALID.TXT (or VALID.XLS).

Notice The contents of the file VALID.TXT (or VALID.XLS) will be overwritten the next time you use External Validation. If you want to keep your *analysis results*, use the Save As command to save them with a new file name. ▲

Formatting an input file

In order to use the External Validation diagnostic to *quantify* a group

for External Validation

of validation spectra, the file names and component concentrations of the validation spectra must be specified in an input file. You can use any spreadsheet or text editor application to create the input file. If you use a spreadsheet application to create the input file, the file must be saved in text format with tab delimiters.

The input file must be formatted according to the sample and specifications provided below.

Example

file header information

Index	File name	Pathlength	C1	C2	C3
1 {tab}	PLS01.SPA {tab}	1.0 {tab}	1.94 {tab}	0.99 {tab}	1.92 {CR} {LF}
2 {tab}	PLS02.SPA {tab}	1.0 {tab}	1.26 {tab}	0.5 {tab}	4.01 {CR} {LF}
3 {tab}	PLS03.SPA {tab}	1.0 {tab}	2.59 {tab}	0.57 {tab}	1.49 {CR} {LF}

where: CR = carriage return

LF = line feed

You can include any number of lines in the file header information or use different column headings in the table. TQ Analyst starts reading the first line that begins with a “1”. Any information that is placed at the top of the file will be ignored.

Make sure you use tabs to separate the entries in each column of the table.

The pathlength values are required only when the Pathlength Type parameter in the current method is set to “Known” and the method is set up to prompt the operator to provide a pathlength value for each *sample* that is quantified (Pathlength Prompt *check box* is on). If one of the other pathlength settings is specified in the method, the pathlength information in the input file will be ignored.

Note You may also use an External Validation input file with the Multiple Quantify command in the Diagnostics menu. Any additional information that is present in an External Validation input file will be ignored when the file is used with Multiple Quantify. ▲

Residual Spectra diagnostic

The Residual Spectra diagnostic quantifies a spectrum using the active method and then displays the *residual spectrum* or spectra for the spectrum that was quantified.

The Residual Spectra diagnostic is a handy tool for troubleshooting problems with the unknown samples. For example, if the *uncertainty values* for one or more spectra that are quantified with a method are significantly higher than usual or the calculated concentration values are much higher or lower than expected, the residual spectra can help you determine the source of the problem (the residual spectrum represents graphically the prediction error or uncertainty). If you know that your spectrometer or the *samples* you are measuring have changed, you can also use the Residual Spectra diagnostic to find out if the method is still valid.

The Residual Spectra diagnostic can be run on any CLS, PLS, or PCR method using any spectrum that is compatible with TQ Analyst and the selected method (i.e., any spectrum that has the same data spacing and Y-axis unit as the method's calibration spectra). This diagnostic may also be used with the *classification methods* that calculate residual spectra (Similarity Match and Distance Match). The method must be *calibrated* before the Residual Spectra diagnostic can be used. Only the spectral information in the specified *analysis region* or regions are used to calculate the residual spectrum or spectra.

If you run the diagnostic on a CLS or PCR method, the software calculates the following two spectra:

- The *unknown sample spectrum* that the method was used to *quantify*.

- The *cumulative residual spectrum*.

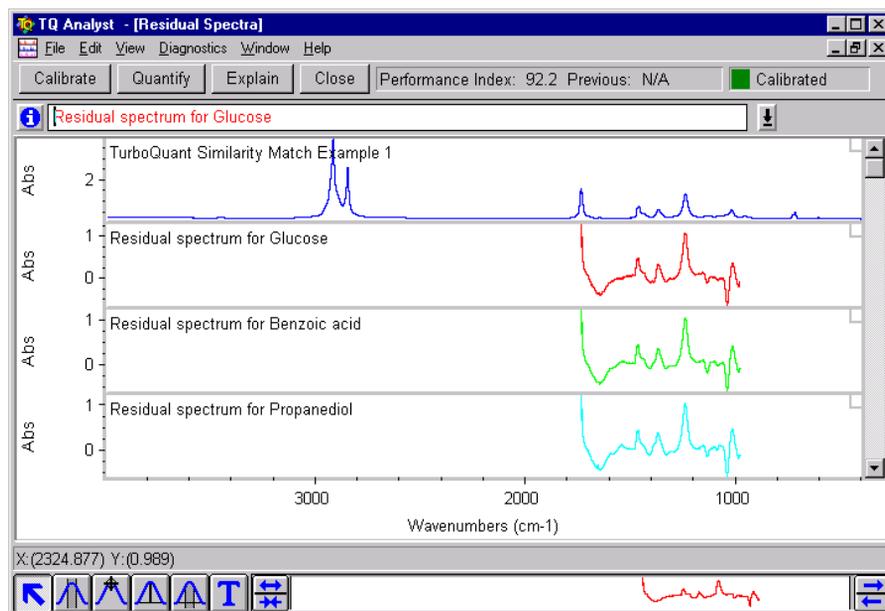
If you run it on a PLS method, the following spectra are created:

- The unknown sample spectrum that the method was used to quantify.
- A residual spectrum for each *component* in the method.

The software calculates the *cumulative residual spectrum* by subtracting the spectral information that is accounted for by all components in the calibrated method from the unknown sample spectrum. To generate each *component residual spectrum*, the software subtracts the spectral information for each component from the sample spectrum.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Residual Spectra command from the Diagnostics menu.

The diagnostic results are displayed in a *spectral window*.



If you ran the diagnostic on a CLS or PCR method, the software displays the sample spectrum followed by the *cumulative residual spectrum*. If a PLS method was active, the sample spectrum is displayed first followed by a residual spectrum for each component in the method. Each spectrum is placed in a separate *pane*. The selected spectrum is displayed in red.

Look for large features that resemble a spectral *peak* or a derivative peak in the residual spectra. These types of features represent portions of the spectral data that are not described by the calibrated method.

Use the Title box in the spectral window to display a list of the titles of the spectra that are displayed in the window. To select another spectrum, click its title in the list of titles or click the displayed spectrum.

You can use the View Finder and any of the commands in the View menu to change the limits of the spectral window or to rearrange the spectra in the window. You can also use the tools on the palette, such as the selection tool or the annotate tool, to interact with the displayed spectra.

Multiple Quantify

Use Multiple Quantify when you want to *quantify* two or more spectra using the current method and place the calculated results in a format that is accessible to other applications, such as a spreadsheet (Microsoft® Excel®) or text file. You can select one or more spectral data (*.SPA) files or a spectral group (*.SPG) file to be quantified or select an input file that lists the file names of the spectra to be quantified.

You can run Multiple Quantify on any TQ Analyst method. The method must be calibrated before Multiple Quantify can be used.

When you choose Multiple Quantify, the standard Windows Open dialog box appears listing spectral data files (*.SPA) in the SPECTRA directory.

Select the spectral data files you want to quantify. You may need to change directories or drives to locate them. To select more than one file, hold down the Control key and click the desired file names. To select a spectral group, set the Files of Type list box to Spectral Groups (*.SPG), choose the group file you want to analyze and then click Open.

If you want to quantify a series of spectral files that are listed in a text file, select the text file (*.TXT extension) that contains the list of file names. You can list all the files in the indicated directory by selecting All Files (*.*) from the Files of Type drop-down list box.

Note The input file must be formatted properly for use with Multiply Quantify. See the next section for more information. ▲

Click OK to begin the analysis. Depending on how the *method parameters* are set, you may be prompted to enter needed information, such as a pathlength value. Follow the instructions that appear on the screen. (If you are quantifying spectral files that are listed in an input file and the file includes any additional information that is required for the analysis, these prompts will not appear.)

When the analysis is finished, the results are displayed in an application window. If TQ Analyst locates the Microsoft® Excel® spreadsheet application on your computer, the data are displayed in an Excel application window. If Excel is not found, the data appear in a window for a text processing application, such as NotePad or Write. The results are formatted using the settings for the report parameters (see Report tab) in the current method.

When you are finished reviewing the results, close the results window by clicking the close button in the upper right corner of the window. The data will be saved in the file QUANTIFY.TXT (or QUANTIFY.XLS).

Notice The contents of the file QUANTIFY.TXT (or QUANTIFY.XLS) will be overwritten the next time you use Multiple Quantify. If you want to keep your *analysis results*, use the Save As command to save them with a new file name. ▲

Formatting an input file for Multiple Quantify

You can use the Multiple Quantify command in the Diagnostics menu to *quantify* a series of spectral files that are listed in a spreadsheet or text file. You can use any spreadsheet or text editor application to create the input file. If you use a spreadsheet application to create the input file, the file must be saved in text format with tab delimiters.

The input file must be formatted according to the sample and specifications provided below.

Example

file header information

Index	File name	Pathlength
1 {tab}	TQX_SBL1.SPA {tab}	1.0
2 {tab}	TQX_SBL2.SPA {tab}	1.0
3 {tab}	TQX_SBL2.SPA {tab}	1.0

You can include any number of lines in the file header information or use different column headings in the table. TQ Analyst starts reading the first line that begins with a "1". Any information that is placed at the top of the file will be ignored.

Make sure you use tabs to separate the entries in each column of the table.

The pathlength values are required only when the Pathlength Type parameter in the selected method is set to Known and the method is set up to prompt the operator to provide a pathlength value for each *sample* that is quantified (Pathlength Prompt *check box* is on). The pathlength values are ignored if the method doesn't require them. See the sections titled “Choosing a Pathlength Option” in the chapters on creating TQ Analyst methods for more information.

Note You may also use an External Validation input file with Multiple Quantify. Any additional information that is present in an External Validation input file will be ignored when the file is used with Multiple Quantify. See the section on “External Validation” in this chapter for more information. ▲

PRESS diagnostic

The PRESS diagnostic basically runs a cross validation but only on the calibration standards in a PLS method. It shows how the predicted residual error sum of squares (PRESS) value changes as the number of *factors* used to calibrate the *component* is increased. This information can help you choose the optimum number of factors to use for each component in a PLS method.

The PRESS diagnostic is only available for Partial Least Squares (PLS) quantitative methods. The method must be calibrated before the PRESS diagnostic can be used.

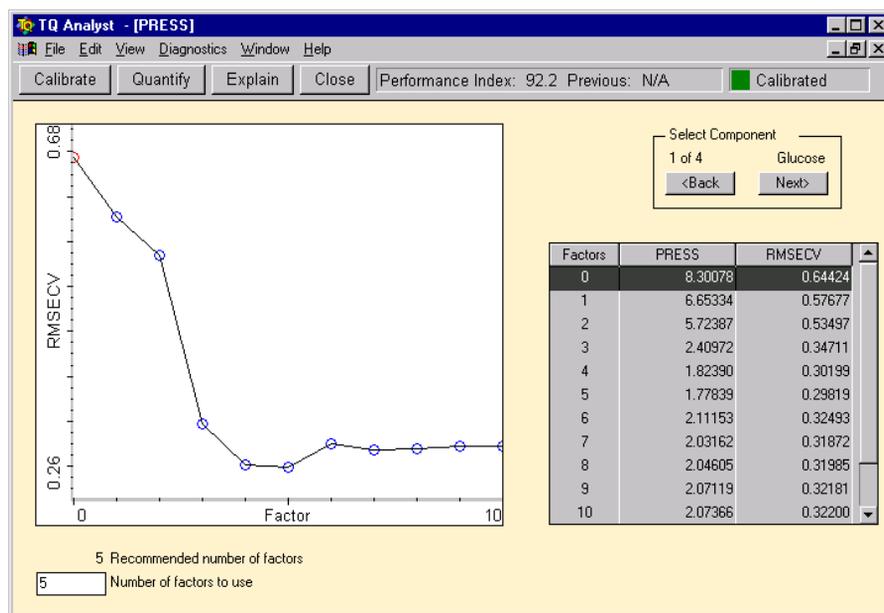
When a PLS method is calibrated, all of the relevant concentration information and spectral information in the *analysis region* or regions of the *calibration standards* is condensed into a set of factors. Each factor represents an independent source of variation in the data.

Factors are ranked by the amount of variance they describe. The first factor describes most of the variation in the calibration standards. Each additional factor describes most of the remaining variation. Therefore, the first factor contains most of the common information in the data. The rest of the factors contain information that is more specific, representing small variations in the data which are often important to the analysis.

The information provided by the PRESS diagnostic can help you choose the optimum number of factors to use for each component in a PLS method. For example, the *PRESS value* is an indicator of calibration error in a PLS method. Each time a factor that represents useful information is added to the *calibration model*, the error is reduced so the PRESS value decreases. At some point, the PRESS value will either reach a minimum, level off, or begin to increase. If you add factors after that point has been reached, the method's performance will not improve and could decrease if the calibration model becomes *overfit*.

Note Only the *standards* that have their usage set to calibration are used by the PRESS diagnostic. *Validation standards* are not used. The Usage parameter on the Standards tab specifies the usage of each standard. ▲

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the PRESS command from the Diagnostics menu. The diagnostic results are displayed in a task window.



The plot illustrates graphically how the PRESS value for the selected component changes as the software increases the number of factors used to calibrate the method. The plots starts at zero. The second point in the plot represents the first factor.

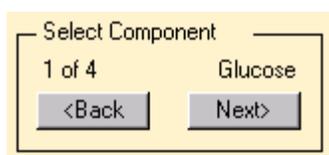
The table displayed in the lower right corner of the task window shows the numerical PRESS values for the selected component.

The recommended number of factors to use for the selected component is displayed below the plot. TQ Analyst automatically

selects the optimum number of factors to use the first time a PLS method is calibrated.

You can specify the number of factors to use for the selected component the next time the method is calibrated by entering a value in the Number of Factors to Use box.

To select the next or previous component in the method, click the Next or Back button in the Select Component group.



The name (or abbreviated name) and *index number* for the selected component are displayed above the buttons. When you select another component, the PRESS values for the new component are displayed in the table and plot.

If you're not sure what is the optimum number of factors to use for a component in your method, use the recommended value displayed in the PRESS diagnostic task window. If you want to use a different value, select the minimum number of factors that produce the lowest PRESS value. If there is no clear minimum in the PRESS plot for a component in your method, the Loading Spectra diagnostic routine may provide additional help in choosing the optimum number of factors.

Note If you want to limit the number of factors the software calculates for the components in a PLS method, see the Factors For Last Calibration table on the Other tab. ▲

Principal Component Scores diagnostic

The Principal Component Scores (PC Scores) diagnostic measures how accurately each *standard* in a PLS, PCR, or discriminant analysis method is represented by each *principal component* that was used to calibrate the method by calculating *score values*. A score value represents the multidimensional distance of a standard projected onto a principal component.

This diagnostic can only be run on a PLS, PCR, Discriminant Analysis or Distance Match method. The method must be calibrated or ready for calibration before the PC Scores diagnostic can be used.

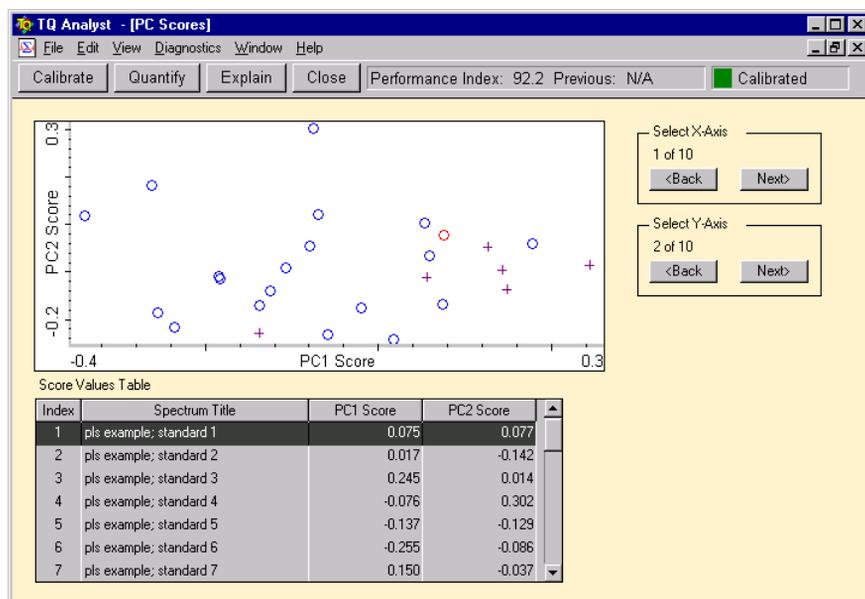
When a PLS, PCR, Discriminant Analysis or Distance Match method is calibrated, all of the relevant spectral information in the *analysis region* or regions of the calibration spectra is condensed into a set of principal components. Each principal component represents an independent source of spectral variation in the data.

The PC Scores diagnostic ranks the principal components according to the amount of variance they describe. The first principal component describes most of the variation in the calibration spectra. Each additional principal component describes most of the remaining variation. Therefore, the first principal component contains most of the common information in the data. The rest of the principal components contain information that is more specific, representing small variations in the data which are often important to the analysis.

The diagnostic results can help you determine whether the principal components that were calculated for the calibrated method accurately represent the spectral data for each standard in the method. They highlight any patterns or trends in your data, which may or may not be significant to your application. They can also help you identify standards that may be *outliers* or, if you are creating a discriminant analysis method, evaluate how the standards are assigned to the classes in the method.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Principal Component Scores command from the Diagnostics menu.

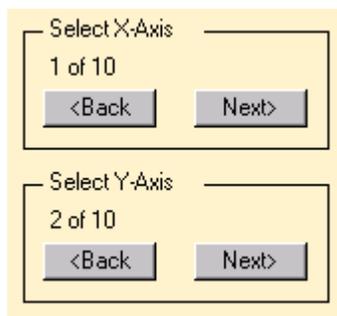
The diagnostic results are displayed in a task window.



The score values for principal component 1 and principal component 2 for each standard are displayed in a table at the bottom of the task window. These are the two most significant principal components in the method.

The plot shows graphically the "distance" between each standard and the two principal components that are selected for the X- and Y-axis of the plot.

Use the Back and Next buttons in the Select Y-axis and Select X-axis groups to select which principal component is assigned to each axis.



When you select a new principal component, the *index number* of the new principal component is displayed above the buttons.

If you are running this diagnostic on a quantitative method, data points in the plot that correspond with calibration standards are represented with a circle. A plus sign (+) is used to represent *validation standards* and a triangle is used for *correction standards*. The Usage parameter on the Standards tab defines how each standard is used in a method. For classification methods, the PC Scores diagnostic assigns each class a unique symbol (for up to seven classes).

Each score value in the table is linked to a data point in the plot. To see the data point that corresponds with a score value in the table, click anywhere in the corresponding row. The data point will be highlighted in the plot. To see the standard that is linked to a data point in the plot, click the data point. The title of the standard will be highlighted in the table.

If you are creating a PLS or PCR method, the data points in each PC Scores plot should be distributed randomly. The data points in the PC Scores plot for a Discriminant Analysis or Distance Match method should be clustered by class.

A data point that is isolated from the others indicates that the corresponding standard is different from the other standards in the method. This difference may be due to a characteristic of the standard

that is important to the analysis or it may be caused by an error or a problem with the spectrum.

If you find an outlier standard, check the data that was entered in the Standards table and display the spectrum for that standard. If the quality of the spectrum is poor, replace the spectrum with a new one. If the spectrum looks okay, consider the chemical makeup of the standard. It is up to you to decide whether an outlier standard should be included in the calibration or *validation set*.

Factor Loading diagnostic

The Factor Loading diagnostic shows the relationship between the spectral and concentration information that is described by each *factor* that is calculated for each *component* in a PLS method. This information can help you identify *standards* that may be *outliers* and decide whether or not to include them in your method.

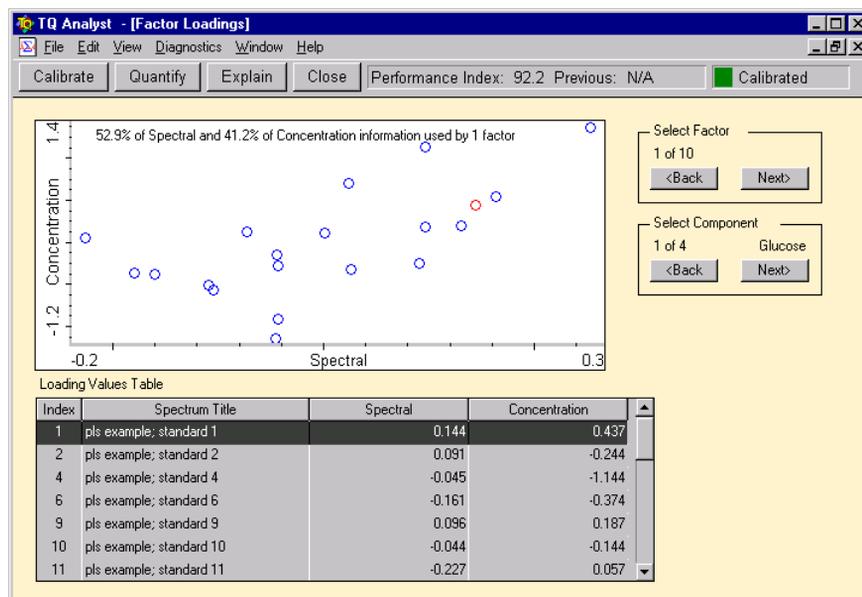
The Factor Loading diagnostic is only available for Partial Least Squares (PLS) quantitative methods. The method must be calibrated before the Factor Loading diagnostic can be used.

When you calibrate a PLS method, the software condenses all of the relevant concentration and spectral information in the *analysis region* or regions of the *calibration standards* into a set of factors. Each factor represents an independent source of variation in the data.

Factors are ranked by the amount of variance they describe. The first factor describes most of the variation in the calibration standards. Each additional factor describes most of the remaining variation. Therefore, the first factor contains most of the common information in the data. The rest of the factors contain information that is more specific, representing small variations in the data which are often important to the analysis.

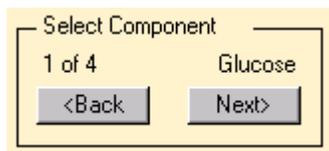
There is a set amount of variability in the spectral data for the *calibration standards*. There is also a set amount of variability in the concentration values of the standards. The Factor Loading diagnostic allows you to consider them separately for each standard in the method and evaluate how they contribute to the factors for each component. This information can help you identify standards that may be outliers and decide whether or not to include them in your method.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Factor Loading command from the Diagnostics menu. The diagnostic results are displayed in a task window.

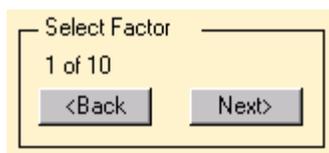


The *loading value* for the spectral contribution and the concentration contribution of each standard for the selected factor and the selected component are displayed in a table at the bottom of the task window. The plot shows graphically how the spectral loading values compare with the concentration loading values.

Use the Back and Next buttons in the Select Component group to select which component loading data you want to view. The *index number* and name (or abbreviated name) for the currently selected component are displayed above the control buttons.



Use the Back and Next buttons in the Select Factor group to select which factor loading data you want to view for the selected component. The number of the currently selected factor is displayed above the control buttons.



Each time you select a new component or factor, the software updates the spectral and concentration loading data in the table and plot.

Each standard in the table is linked to a data point in the plot. To see the data point that corresponds with a standard in the table, click anywhere in the corresponding row. The data point will be highlighted in the plot. To see the standard that is linked to a data point in the plot, click the data point. The title of the standard will be highlighted in the table.

For a given component and factor, the data points in an ideal factor loading plot will be distributed evenly along a line that is 45 degrees from both axes. This shows that the standards are contributing evenly to the factor and no standard is influencing the factor significantly more than the others. Data points that are close to this ideal line

represent standards that are well described for the corresponding factor. The higher the spectral and concentration *loading values* for a given standard, the more significant the standard is to the selected factor. A plot that shows some data points clustered together with an isolated point elsewhere may indicate an outlier.

Keep in mind, however, that the plots for each factor can be quite different. For example, the data points in the plot for the first factor are often distributed randomly because, although much of the variation is described, the information may not be highly correlated with concentration.

If most of the data points in a factor loading plot are clustered in the lower left corner of a plot (smaller spectral and concentration contribution) and one or more data points are clustered in the upper right corner of the plot (larger spectral and concentration contribution), the standards that have higher *loading values* have a significant influence on the factor and should be examined.

The difference in the loading value (spectral or concentration) may be due to a characteristic of the standard that is important to the analysis or it may be caused by an error or a problem with the spectrum. Check the data that was entered in the Standards table for the corresponding standards and display the spectrum of each standard. If the quality of the spectrum is poor, replace the spectrum with a new one. If the data look okay, consider the chemical makeup of each standard. It is up to you to decide whether or not an outlier standard should be included in the calibration or *validation set*.

Note If you want to limit the number of factors the software calculates for the components in a PLS method or change the number of factors used, see the Factors For Last Calibration table on the Other tab. ▲

Leverage diagnostic

The Leverage diagnostic provides the following information about the *calibration standards* in a PLS method.

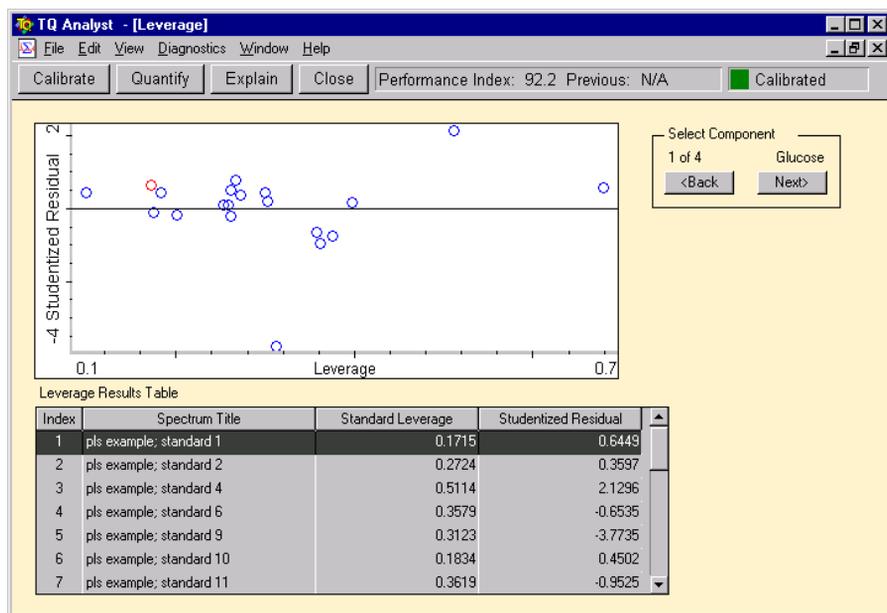
- How much influence each *standard* has on the *method model*.
- How accurately the *calibration model* describes each standard.

This information can help you identify standards that may be *outliers*.

The Leverage diagnostic is available only for Partial Least Squares (PLS) quantitative methods. The method must be calibrated before the Leverage diagnostic can be used.

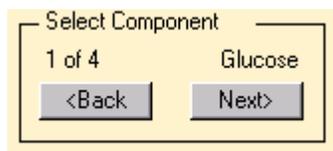
When you calibrate a PLS method, TQ Analyst saves the leverage and *residual values* for each *component* and each standard. The Leverage diagnostic shows the relationship between the leverage and residual values for each component and each standard in a PLS method. The residual values plotted by the Leverage diagnostic have been divided by their *standard error* to produce the “studentized” residual value. This places all standards on a similar scale, regardless of their *leverage values*, and makes it easier to identify standards that may be outliers.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Leverage command from the Diagnostics menu. The diagnostic results are displayed in a task window.



The numerical leverage and residual values for each standard are displayed in a table at the bottom of the task window. The plot shows graphically how the *leverage values* compare with the *studentized residual values* for the selected component.

Use the Back and Next buttons in the Select Component group to select which component leverage and residual data you want to view.



The *index number* and name (or abbreviated name) for the currently selected component are displayed above the control buttons. When you select a new component, the software updates the leverage and residual data in the table and plot.

Each standard in the table is linked to a data point in the plot. To see the data point that corresponds with a standard in the table, click anywhere in the corresponding row. The data point will be highlighted in the plot. To see the standard that is linked to a data point in the plot, click the data point. The title of the standard will be highlighted in the table.

The data points in the leverage plot should be distributed evenly throughout the plot. A data point that is isolated from the others indicates that the corresponding standard is different from the other standards in the method.

The center horizontal line represents zero residual values. If a standard has a high residual value (i.e., if it appears high above or below the zero residual line), the *analysis region* or regions of the spectrum for that standard contain features which are not modeled by the calibrated method.

If the *leverage value* for a standard is high, the standard is significant to the *calibration model* for the selected component. Standards that have high leverage values usually have low residual values because most of the spectral information in the *analysis region* or regions is included in the calibration model.

If you find a standard with a leverage value that is noticeably different from the leverage values for the other standards in the method, check the data that was entered in the Standards table and display the spectrum for that standard. If the quality of the spectrum is poor, replace the spectrum with a new one. If the data look okay, consider the chemical makeup of the standard. The difference in the leverage value may be due to a characteristic of the standard that is important to the analysis or it may be caused by an error or a problem with the spectrum. It is up to you to decide whether or not an outlier standard should be included in the calibration set.

Loading Spectra diagnostic

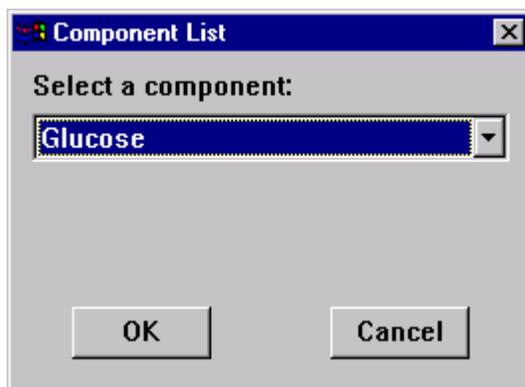
The Loading Spectra diagnostic displays a loading spectrum for each *factor* that is needed to describe 99.9% of the variation in the *standards* for a PLS method. A loading spectrum is the orthogonal spectrum that represents the amount of variability described at each X value for a given factor.

The software generates the loading spectra for each *component* in a separate operation. It creates at least one loading spectrum for each component (or a minimum of 10 loading spectra).

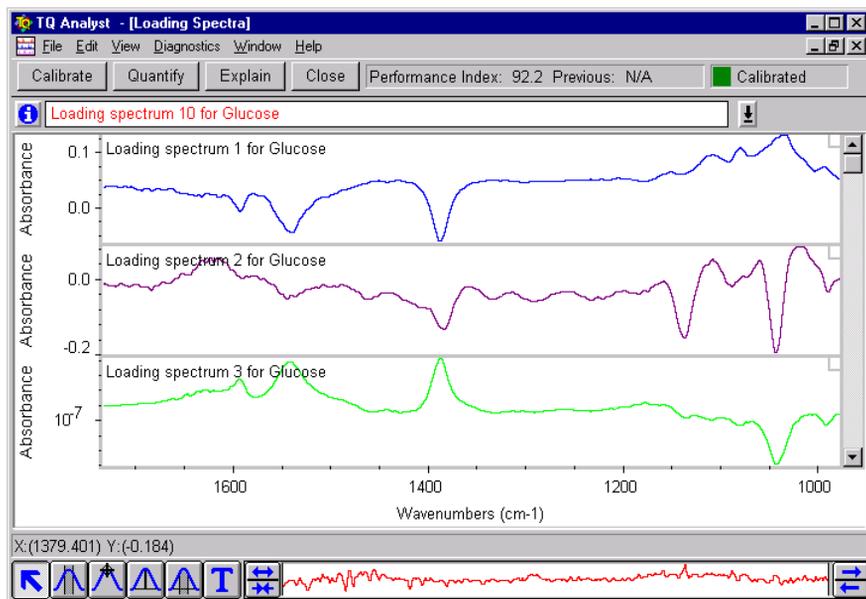
The loading spectra show how the spectral and concentration information in a *calibration set* is represented by the factors in a PLS method and how much of the total variance each factor describes. This information can help you determine if there are enough sources of variation in your data. You can also use this diagnostic to verify the number of factors required to describe the variation in a calibration set.

The Loading Spectra diagnostic is available only for Partial Least Squares (PLS) quantitative methods. The method must be calibrated before the Loading Spectra diagnostic can be used. The software uses the concentration data and all of the spectral data in the full spectral range of the calibration spectra to generate the loading spectra.

To start the diagnostic, select the method by clicking the *method window* or choosing the method name in the Window menu. Then choose the Factor Loading command from the Diagnostics menu. The following dialog box is displayed:



Select a component by clicking its name in the Select Component list box. The loading spectra for the component you selected are displayed in a *spectral window*. The selected loading spectrum is displayed in red.



Sources of variation in the calibration data will produce distinct features in the loading spectra. The features may look like a spectral *peak* or a *derivative peak*. A noisy or featureless loading spectrum indicates that the corresponding (and any subsequent) factor contributes little useful information to the calibration model.

Use the Title box in the spectral window to display a list of the titles of the loading spectra. To select another spectrum, click its title in the list of titles or click the displayed spectrum.

You can use the View Finder and any of the commands in the View menu to change the limits of the spectral window or to rearrange the spectra in the window. You can also use the tools on the palette, such as the selection tool or the annotate tool, to interact with the displayed spectra.

14 Troubleshooting Tips

The following comments may help you troubleshoot some common problems when creating or running a new method.

When creating or modifying a method:

- If a warning message about mismatched *standards* appears when you open additional standards, the parameter settings used to collect the new standard are significantly different from the settings that are saved with the current method. For optimum performance, all method standards should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the standard again using the proper settings for the collection parameters, edit the settings in the current method or ignore the warning and continue adding standards.
- If a software feature is unavailable (displayed in gray), it is either not appropriate for the selected analysis type or a required parameter (possibly located elsewhere) is not selected. Use the software's convenient Explain Help feature to get more information. Click the *Explain button* on the TQ Analyst *toolbar* to display the Explain help window. Then click the software feature you need information for. The help window updates to display information about the feature you clicked.

When using a method to analyze a sample:

- If you want to set the Collect and Bench parameters in Thermo Scientific OMNIC spectral analysis software and use the settings to collect a spectrum to be quantified, open the Quant Setup dialog box (choose Quant Setup from the OMNIC Analyze menu), select

the method and make sure the Collect and Bench *check boxes* in the Parameter group are off.

Note If the Collect and Bench *check boxes* in the Parameter group are on when you select a method, TQ Analyst will change the Collect and Bench parameter settings in your OMNIC software to match the settings that are specified in the selected method. ▲

- If a warning message about mismatched parameters appears when you select a spectrum to *quantify*, the parameter settings used to collect the *sample spectrum* are significantly different from the settings that are saved with the current method. For optimum performance, all standards and *samples* should be collected using the same settings for the collection parameters. You can deal with the warning three ways: collect the spectrum again using the proper settings for the collection parameters, edit the settings in the current method or ignore the warning and continue analyzing samples.
- If you turned on the Uncertainty Values *check box* (Report tab) for a PLS or PCR method but no *uncertainty values* appear in the *sample reports*, make sure at least one *validation standard* is specified in the method (see the Standards tab).

Appendix A: Using the Keyboard

This chapter provides easy instructions on interacting with TQ Analyst software features using keyboard commands instead of a mouse. See your Windows documentation for complete information on using the keyboard to perform these and other standard Windows operations.

Tabbing through a window or dialog box

In a window or dialog box that contains several features, use the Tab key to move to the feature with which you want to interact. As you “tab” through a window or dialog box, the features become highlighted or marked with a dotted rectangle (or both) as they become active. Once a feature is active, you can use the appropriate keys on the keyboard to interact with it.

Selecting an item in a list

To select an item in a list, tab to the list and then use the up or down arrow key to move to the item.

Turning a check box on or off

To turn a check box on or off, tab to the check box and then press the space bar.

Selecting an option button

To select an option from a group of *option buttons*, tab to the option and then press the space bar.

Choosing a command button

To choose a command button (such as OK) after tabbing to it, press Enter.

Keyboard shortcuts

The next section describes how to use key combinations to open TQ Analyst menus or choose commands.

Selecting menus

Use the Alt key along with the underlined letter in a *menu name* on the screen to open the menu from the keyboard. For example, to open the File menu, hold down the Alt key and then type F.

Choosing commands

You can choose a menu command from the keyboard in two ways:

- Type the key combination. If there is a key combination for a command, the key combination will be displayed next to the command name in the menu. (The menu does not have to be selected in order to choose a command using its key combination.)
- If the menu that contains the command is selected, type the underlined letter in the command name on the screen or use the up and down arrow keys on the keyboard to select the command and then press the Enter key.

Appendix B: Comparison of Internal Reference and Peak Ratio Pathlength Types

This section describes two experiments which could be configured for either Internal Reference or Peak Ratio pathlength correction. The examples are included to demonstrate the difference between the Internal Reference and Peak Ratio pathlength options available in TQ Analyst and to help you understand when each of them should be used.

Case 1

This example is a contrived situation to show you a case where Peak Ratio does not work. In this example we measure a set of spectra of the same standard solution in several different pathlength cells.

Assume you are measuring a hypothetical analytical *peak height*, A , and a *pathlength peak height*, b , from each standard spectrum. Remember, there is always some error or *noise* associated with every *spectral measurement*. A small amount of error is included in the measurements as shown in the table on the next page.

The concentration, C , of each standard is the same, 1.00. For the purposes of this example, we assume that it has no error.

When setting up a *calibration curve*, concentration is generally plotted on the X-axis. This is the “known” value or the independent variable. The absorbance of the analytical measurement, A , is plotted on the Y-axis. This value depends on the concentration and is called the dependent variable. When an unknown *sample* is quantified, this

model is used in reverse; the independent variable, A, is measured and the corresponding dependent variable, C, is calculated.

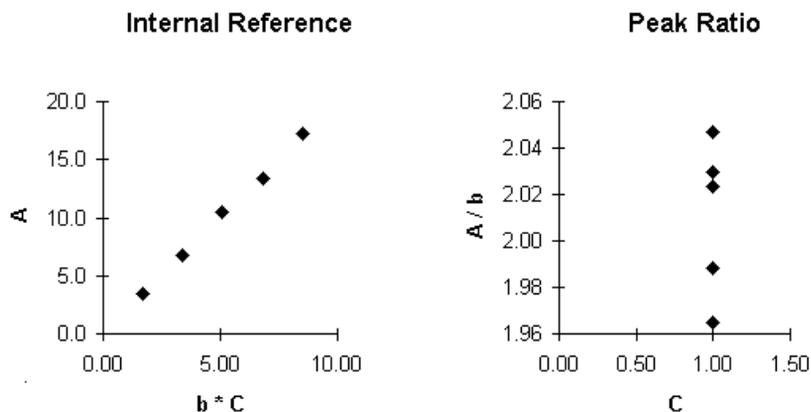
A calibrated method using Internal Reference, $A = b * C$, calculates the product of *pathlength* times concentration, $b * C$, for a measured value of the analytical *region*, A. A is the dependent variable and the product $b * C$ is the independent variable.

A method based on Peak Ratio, $A / b = C$, calculates just concentration, C. But this calculation is based on the pathlength corrected analytical measurement, A / b . The calibrated method cannot tell you anything about measurement error because this error is masked by ratioing the raw data before presenting it to the method.

The following data demonstrates this case. The pathlength times concentration column, $b * C$, shows the result calculated by a method using Internal Reference pathlength type. The absorbance over pathlength column, A / b , shows the pathlength corrected data that will be the dependent input into a method using the Peak Ratio pathlength type.

Std	A	b	C	$b * C$	A / b
1	3.45	1.70	1.00	1.70	2.03
2	6.76	3.40	1.00	3.40	1.99
3	10.44	5.10	1.00	5.10	2.05
4	13.36	6.80	1.00	6.80	1.96
5	17.20	8.50	1.00	8.50	2.02

If we plot this data you can easily see why the Peak Ratio pathlength type cannot be used. The calibration points will always form a vertical line. A concentration value of 1.00 will always result using this calibration, regardless of the actual spectral measurement.



For Internal Reference, the calibration points form a line which can be used as a calibration curve.

Case 2

This example is a more realistic situation where either Internal Reference or Peak Ratio could be used. However, it still demonstrates why Internal Reference is a better choice for pathlength type than Peak Ratio.

This example contains six hypothetical standards all at different concentrations and measured in different pathlength cells.

As with Case 1, assume you are measuring a hypothetical analytical peak height, A , and a pathlength peak height, b , from each standard spectrum. Remember, there is always some error or *noise* associated with every spectral measurement. These are the dependent variables.

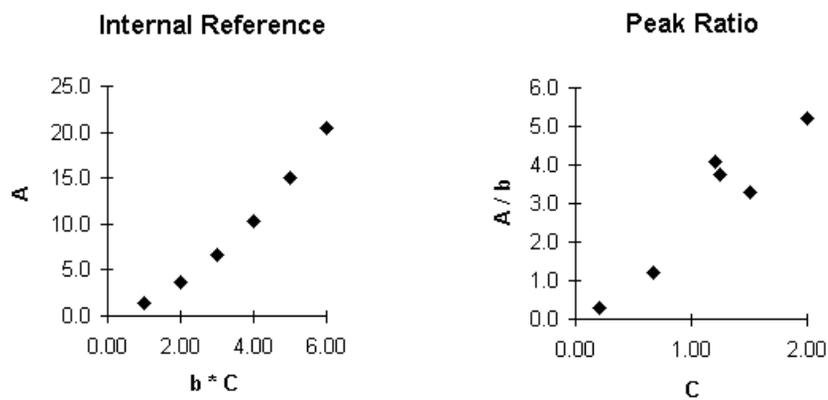
Again, for the purposes of this example, we assume that it has no error in the concentration values, C .

The pathlength times concentration column, $b * C$, shows the result calculated by a method using the Internal Reference pathlength type. A is the dependent variable; the product $b * C$ is the independent variable.

The absorbance over pathlength column, A / b , shows the pathlength corrected data that will be the dependent variable input into a method using Peak Ratio pathlength type. The independent variable is just concentration, C , in this case.

<u>Std</u>	<u>A</u>	<u>b</u>	<u>C</u>	<u>$b * C$</u>	<u>A / b</u>
1	1.4	5.0	0.20	1.00	0.28
2	3.6	3.0	0.67	2.00	1.20
3	6.6	2.0	1.50	3.00	3.30
4	10.4	2.0	2.00	4.00	5.20
5	15.0	4.0	1.25	5.00	3.75
6	20.4	5.0	1.20	6.00	4.08

If we plot the dependent vs. independent variables you can see how the data would be used to calculate a calibrated method or “working curve.”



The Internal Reference pathlength treatment does a better job showing the effect of measurement error in these standards. The slight non-linearity could easily be corrected using a second order *correction curve*.

Unlike Case 1, this data does allow you to construct a calibration curve from the Peak Ratio data. However, there is no trend to the errors or any way to smoothly compensate for them. Our only option here is to just fit the best straight line to the data.

Glossary

A

Absolute Maximum In Range region type A technique for measuring the intensity of a single point in a *spectral region*. Absolute Maximum In Range reports the maximum intensity value, either positive or negative, in the specified X-axis range.

absorbance A measure of how much of the incident radiation that is directed at a *sample* is absorbed by the sample. Absorbance is defined by the formula $A = \log_{10} (1/T)$, where T is the fractional *transmittance*.

absorbance peak A *region* of an *absorbance spectrum* where the *sample* absorbs radiation.

absorbance spectrum A *spectrum* that shows how the *absorptivity* of a *sample* varies with *frequency*, or *wavelength*.

absorptivity A property of a compound that describes how readily a fixed number of molecules absorb radiation at a specific X-axis location.

acceptance limits The highest and lowest measured or calculated values that are within the tolerance limits of a process control application.

acceptance limits check A sample checking feature that compares the measured or calculated values for the spectra you quantify with a method to the acceptance limits specified in the method. If a measured or calculated value is above the upper acceptance limit or below the lower acceptance limit, the sample is flagged and the specified action is taken.

action button

1) A feature on some method tabs that allows you to perform a task. For example, pressing the Edit Regions button on the Regions tab displays a task window for graphically editing analysis regions.

2) One of several features on the toolbar that allows you to perform a specific operation.

Action list box A drop down list box that allows you to select the action you want TQ Analyst to take if the corresponding sample checking feature is on and a spectrum you quantify with the current method fails the corresponding sample check.

active window The window that is affected by software operations.

analysis limits The upper and lower limits of the analysis range for a component.

analysis limits check A sample checking feature that compares the calculated values for the spectra you quantify with a method to the analysis limits specified in the method. If a component concentration value is above the upper analysis limit or below the lower analysis limit for that component, the sample is flagged and the specified action is taken.

analysis range The concentration range of a component that is described in a method model.

analysis region The portion or portions of the spectrum that will be used for the analysis. The analysis region can contain several bands.

analysis results The measured or calculated values that are displayed on the screen when you use a method to quantify an unknown sample spectrum.

Analysis Type parameter A parameter that defines the type of results a method will produce when you use it to analyze an unknown sample spectrum and the algorithm that will be used for the analysis.

ANOVA An acronym for the standard Analysis of Variance statistical analysis. The ANOVA table is a standard format for reporting the degree of variation in a data set.

Area region type A technique for measuring the intensity of a spectral region. Area calculates the sum of the intensity values in the specified X-axis range. The trapezoidal rule is used to determine the area.

Average Height In Range region type A technique for measuring the intensity of a spectral region. Average Height In Range reports the average intensity value in the specified X-axis range.

Average In Range baseline option A technique for choosing baseline points in order to calculate a corrected measurement. Average In Range uses the average intensity value in the specified baseline range to define each baseline point.

B

background spectrum A single-beam spectrum obtained without a sample in place. A single-beam sample spectrum can be ratioed against the background spectrum to remove the effects of the background.

band A spectral feature that contains only one peak.

baseline The portion of an absorbance spectrum that is not part of the peaks. The baseline represents those regions where the sample absorbs little or no energy.

baseline corrected measurement The intensity of a peak or region measured from the spectral baseline. See corrected measurement.

Baseline Offset baseline type A technique for calculating a corrected measurement. Baseline Offset subtracts a constant from the specified measured peak or each point in the specified region. The Offset parameter defines the value of the constant.

baseline point A point through which the baseline of a spectral region will be drawn. The drawn baseline is subtracted from the intensity of the peak or region to produce a corrected measurement.

beamsplitter A device inside the interferometer that splits the infrared beam coming from the source into two beams of nearly equal energy. Usually one beam passes through the beamsplitter, is reflected from the interferometer's moving mirror and returns to the beamsplitter. The other beam is reflected from the beamsplitter and then is reflected from the interferometer's fixed mirror and returns to the beamsplitter. The recombined beam exits the interferometer, passes through the sample and travels to the detector.

Beer-Lambert-Bouguer law A mathematical law which states that absorbance increases in proportion to concentration: $A = a b c$

Where: A = absorbance
a = absorptivity (constant)
b = pathlength
c = concentration

C

Calibrate button A button on the toolbar that allows you to calibrate a method.

calibrate To analyze a set of standards in order to calculate a method model for predicting component concentrations in unknown samples.

calibration curve A method model that is based on two or more calibration standards which contain different amounts of the components to be measured in a quantitative analysis.

calibration model A mathematical relationship that describes how the spectral data for the calibration standards correlate with the concentration or classification data. See method model.

Calibration Results window The task window that appears after a quantitative, discriminant analysis, or Distance Match method is calibrated. The Calibration Results window shows the calibration tables and plots and the performance index for each component or class in the method.

calibration set The standards that are used to create the method model when a method is calibrated.

calibration spectrum The spectrum of a calibration standard.

calibration standard A standard that is used to create the method model during calibration. In TQ Analyst, calibration standards are also used to calculate a correction curve, if one is specified.

Chauvenet test A statistical test used to determine whether a standard is an outlier. If the deviation of a standard from the mean is so large that the probability of occurrence is less than 1/20, the Chauvenet test considers the standard an outlier.

check box A small box that can be selected to turn the adjacent parameter on and off. The parameter is on when a check mark appears in the box and off when the box is blank.

Christiansen effect A condition in which rapid changes in the refractive index of a substance in the vicinity of a sample peak leads to scattering which, combined with absorption of light, causes a shift in the sample peak's measured peak maximum.

class A group of calibration standards that have a common set of characteristics.

Classical Least Squares analysis type A quantitative analysis calibration technique based on the classical least squares algorithm.

classification analysis To find the calibration standard or class that most closely matches an unknown sample spectrum or verify that the sample spectrum is similar to the spectra in a specified class.

classification method A method that classifies an unknown sample spectrum by finding the calibration standard or class that most closely matches the sample spectrum or by verifying that the sample spectrum is similar to the spectra in a specified class.

classify To find the calibration standard or class that most closely matches an unknown sample or to verify that a sample spectrum is similar to the spectra in a specified class.

Close button A button on the TQ Analyst toolbar that allows you to close the active window.

Collection and Processing Information dialog

box A dialog box that contains information about the active spectrum. To display the collection and processing information for a displayed spectrum, select the spectrum and then double-click its title box.

command A word or phrase in a menu that you can choose in order to perform an action.

component A chemical compound contained in a sample mixture.

component residual spectrum The spectral information in an unknown sample spectrum that is not explained by a particular component. Each component residual spectrum is generated by removing the spectral information for a single component from the spectrum of the unknown sample. See residual spectrum and cumulative residual spectrum.

Computed Area region type A technique for measuring the intensity of a spectral region. Computed Area calculates the area of the spectral region that falls between the specified baseline points (or the specified baseline range).

concentration The amount of a component that is present in the standards used for calibration and validation or in an unknown sample mixture.

concentration unit The unit of measurement that is associated with the concentration values of a measured component.

concentration value The specified or reported concentration of a component in a standard or sample.

conformity spectrum A spectrum that shows point by point where deviations occur between two spectra.

Constant pathlength type A technique for handling pathlength differences in standards and unknown samples. Select the Constant pathlength option when the spectra of the calibration standards and the unknown samples are collected at the same pathlength. The pathlength value does not need to be known because it is not a factor in any calculations.

corrected concentration The concentration value multiplied by the specified correction coefficient.

corrected measurement The intensity of a peak or region measured from the spectral baseline. See baseline corrected measurement.

correction The process of calculating a linear or higher order polynomial which can be applied to the concentration values calculated by a calibrated method to improve the accuracy of the analysis.

correction coefficient The value for the variables c_0 , c_1 , c_2 , etc. that will be used to calculate each term in the polynomial equation for a correction curve. The general format for the equation is shown below:

$$X_c = c_0 + c_1X + c_2X^2 + c_3X^3 + c_4X^4 + c_5X^5$$

Where X is the calculated concentration of component X , X_c is the corrected concentration of component X , and c_i is the coefficient for each term in the equation (c_0 = constant, c_1 = first order, etc.).

correction curve A linear or higher order polynomial which can be applied to the concentration values calculated by a calibrated method to improve the accuracy of the analysis.

correction standard A standard that is used along with the calibration standards to calculate a correction curve. Correction standards are not used in calibration.

Corrections task window A task window that allows you to display the calculated versus actual plot for each component in a quantitative method and contains tools for defining a correction curve.

correlation coefficient A measure of the linear relationship between two variables. A value of "one" implies that there is a direct linear relationship between two variables. A value of "zero" implies that there is no relationship between the two variables.

Correlation search algorithm An algorithm for the Search Standards analysis that determines the correlation between the unknown sample spectrum and each calibration spectrum by calculating their correlation coefficients.

correlation spectrum A calculated spectrum that shows the correlation between the spectral information and component concentrations of a single standard. The correlation spectrum is produced by calculating the correlation coefficient at each X-axis value.

cross validation A technique for validating a quantitative method without using validation standards. A specified number of calibration standards are removed from the calibration set, then the method is calibrated and the new method model is used to quantify the standards that were removed. This process is repeated until all of the calibration standards have been quantified as validation standards.

cross validation standard A standard or group of standards that are used to validate a method model during cross validation. Cross validation standards are calibration standards that have been temporarily removed from the method model. The temporary model is then used to quantify the removed standard or standards.

cumulative residual spectrum The spectral information in an unknown sample spectrum that is not explained by the calibrated method that was used to quantify the spectrum. The cumulative residual spectrum is calculated by subtracting the spectral information that is accounted for by all components in the calibrated method from the unknown sample spectrum. See residual spectrum and component residual spectrum.

D

data collection parameter check A sample checking feature that compares the data collection parameter settings used to collect an unknown sample spectrum that is quantified with a method to the settings used to collect the calibration spectra. If the settings for certain key collection parameters don't match, the sample is flagged and the specified action is taken.

data collection parameters A set of parameters, such as resolution, that define the data collection process.

default setting The state each method parameter is set to when a new method is created.

derivative The rate of change of the Y value with respect to the X value. See first derivative.

detector A device inside the spectrometer or analyzer that produces an electrical signal in response to the intensity of the electromagnetic radiation (light) striking it.

detrending Removing a linear or quadratic curve from a spectral region in order to remove baseline variation.

diagnostic task windows Task windows that display diagnostic results and allow you to adjust related parameters.

dilution factor A mathematical function applied to the concentrations of standards and/or samples that are diluted by some amount. The concentration value of each diluted standard and sample should be divided by the specified dilution factor.

Discriminant Analysis analysis type A classification analysis technique. Discriminant Analysis applies the spectral information in the specified regions of an unknown sample spectrum to a stored method model in order to determine which class of standards is most similar to the unknown. A measurement of the Mahalanobis distance between the unknown sample and each reported class is also provided.

Distance Match analysis type A classification analysis technique. Distance Match calculates a residual spectrum for each class and measures its distance from the class average. The result is the percentage of frequencies that exceed the Distance Match threshold.

Distance Match threshold A parameter that defines the acceptable differences between the unknown sample and each class in a Distance Match analysis. The Distance Match threshold must be set in standard deviation units (typically 5).

distance value The numerical result from a Discriminant Analysis or Distance Match analysis. Discriminant analysis distance values are calculated using the Mahalanobis distance algorithm. The distance value from a Distance Match method represent the percentage of frequencies that exceed the distance match threshold.

The distance values from both of these analysis types tell you how well the sample matches the standards in each class that is reported. The smaller the distance value the better is the match.

distance value check A parameter that compares the distance values for the spectra you quantify with a discriminant analysis method to the threshold distance value specified in the method. If the distance value for the best matched class is above the threshold distance value, the sample is flagged and the specified action is taken.

Dixon test A statistical test used to determine whether a standard is an outlier. If the deviation of a standard from the mean is outside a 95% confidence threshold, the Dixon test considers the standard an outlier.

drop down list box A parameter that allows you to select an item from a list of available options.

E

Explain button An action button on the TQ Analyst toolbar that allows you to open the Explain help window.

Explain help window A window that provides help information on the selected feature in the active window. The active window can be a method window or a task window. Use the Explain button to open the Explain help window and the Close button to close it.

extension A period and three numbers or letters at the end of a file name. You can indicate the kind of data contained in a file by using an appropriate extension.

extraction factor A mathematical function applied to the concentrations of the standards and/or samples which are extracted by some amount. The concentration value of each extracted standard and sample should be multiplied by the specified extraction factor.

F

F ratio The ratio of two variances. This ratio follows a well-defined distribution based on the number of degrees of freedom for both variances. The TQ Analyst feasibility wizard uses F ratios to determine if the variability in the method standards is significant.

factor A set of principal components that contain spectral and concentration information. Factors are used to describe the variation in a partial least squares analysis.

factorial design A technique for selecting the component concentrations of the standards in a quantitative analysis. A factorial design investigates the effects of a number of different variables simultaneously. A factorial design can be implemented using a full factorial design model or a fractional factorial design model.

feasibility standard A standard or sample used for the feasibility assessment.

feasibility assessment A test run on two representative samples to determine whether there is sufficient variability in the sample data that correlates with differences in sample composition to develop a successful quantitative method.

file A collection of spectral or method information, given a name and stored on a disk.

file name The name that identifies a file.

first derivative The rate of change of the Y value with respect to the X value. See derivative.

First Derivative In Range region type A technique for measuring a spectral region. First Derivative In Range measures an estimate for the first derivative at each data point in the specified region.

Fixed Location baseline option A technique for choosing baseline points in order to calculate a corrected measurement. Fixed Location uses the intensity value at the specified baseline location to define each baseline point.

Fixed Location Height region type A technique for measuring the intensity of a single point in a spectral region. Fixed Location Height finds the nearest data point to the specified X-axis location and uses that point and the two points on either side to find the interpolated maximum. Then, it measures the intensity at that location.

fixed mirror The mirror in the interferometer of a Fourier-Transform Infrared (FT-IR) spectrometer that reflects the infrared beam back to the beamsplitter.

Force Through Zero parameter A parameter that forces a correction curve to pass through the origin at zero concentration.

fractional factorial design model A technique for selecting the component concentrations of the standards in a quantitative method. The fractional factorial design model selects a well-organized portion of the total number of combinations found in the full factorial design model.

frequency The number of light wave cycles that occur per unit time or space. In TQ Analyst, frequency is expressed in wavenumbers (cm^{-1}).

full factorial design model A technique for selecting the component concentrations of the standards in a quantitative method. A full factorial design model uses all combinations of the variables that can be formed. Since this often requires a large number of standards, a fractional factorial design model is typically used.

full spectrum check A sample checking feature that compares each unknown sample spectrum that is quantified with a method to the calibration spectra. The comparison is made over the full spectral range. The result of the comparison is a fit value. If a spectrum fit value falls below the threshold fit value, the sample is flagged and the specified action is taken.

full spectrum fit value The numerical result from the full spectrum check or the measurement region spectrum check. The fit value tells you how well the sample matches the calibration standards. The fit values range from 0 to 100. A fit value of 100 indicates a perfect match.

full width half height (FWHH) See Peak Width (At Half Maximum) region type.

H

hidden spectrum A spectrum that has been removed from the screen by using the Hide Spectrum command in the View menu. The spectrum remains open and can be displayed again by using the Show Spectrum command.

I

index number Numbers used throughout TQ Analyst software to indicate which component, standard, analysis region, etc., out of the total number defined in a method is being examined.

infrared (IR) The region of the electromagnetic spectrum extending from approximately $12,800\text{ cm}^{-1}$ to 30 cm^{-1} .

infrared beam The infrared beam of light emitted by the source in an infrared spectrometer. The beam travels from the source to the detector.

intercept The distance from the origin of coordinates along a coordinate axis to the point at which a line or curve intersects the axis.

interferogram The signal produced by the constructive and destructive addition of light when two infrared beams in the interferometer of a Fourier-Transform Infrared (FT-IR) spectrometer are recombined.

interferometer A device that splits and then recombines the infrared beam in a Fourier-Transform Infrared (FT-IR) spectrometer. The output signal is an interferogram.

Internal Reference pathlength type A technique for handling pathlength differences in standards and unknown samples. Select the Internal Reference pathlength option when you want to specify a peak that will be used to compensate for differences in sample pathlength.

K

Known pathlength type A technique for handling pathlength differences in standards and unknown samples. Select the Known pathlength option when the pathlength values of the calibration standards and the unknown samples vary but can be measured by a technique that is not based on infrared absorbance. The pathlength values must be specified by the user and are used in the calibration and analysis of unknown samples.

L

least squares algorithm A statistical quantitative analysis technique. The least squares algorithm determines the most probable value of a quantity from a set of measurements by choosing the value which minimizes the sum of the squares of the deviations of those measurements.

leverage value The distance between a calibration standard and the center of a method model. The leverage values tell you how much the standard is influencing the model. The leverage values range between 0 and 1, where 1 indicates the highest leverage.

linear correction The mathematical equation of a straight line ($Y = m (x + b)$) that can be used to apply a multiplicative factor or offset value to a measured intensity value. For example, $\text{Conc} = m (A + b)$, where A = measured absorbance, m = the multiplicative factor, and b = the offset value.

Linear Removed baseline type A technique for calculating a corrected measurement. Linear Removed calculates a linear least squares fit over the specified region. Then it subtracts the value of this line at each data point in the region from the spectrum intensity at the same data point. The resulting region has all of its linear information removed, leaving only the higher order part of the region.

loading value The distance of a calibration standard in the spectral or concentration direction from the selected factor. If the spectral or concentration loading value for a given standard is large (positive or negative), the standard is significant to the factor.

Location At 1% (2%, 5%, 10%) of Peak Maximum region type A technique for finding where the intensity in a spectral region is reduced to 1%, 2%, 5%, or 10% of its maximum height.

locked pane A stacked pane into which a spectrum cannot be moved. Locking a pane that contains a spectrum allows you to scroll spectra on the screen while maintaining the position of the spectrum in the locked pane. An X appears in the pane lock of a pane when the pane is locked.

M

Mahalanobis distance An algorithm for calculating the distance of a sample from the mean of a set of standards.

match The result of a Search Standards, Discriminant Analysis, Distance Match, or QC Compare Search analysis. The match is the spectrum (Search Standards) or class (Discriminant Analysis, Distance Match, QC Compare Search) that is most similar to the unknown sample spectrum.

Match Type parameter A parameter that defines the scale for the match values calculated from a Similarity Match analysis. If Match Type is set to "Find Similarities," the match values will be scaled from 0 to 100 where 100 is a perfect match. If Match Type is set to "Find Residual Differences," the match values will be scaled from 100 to 0 where 0 is a perfect match.

match value The numerical result from a Similarity Match, Search Standards, Distance Match, or QC Compare Search analysis.

match value check A sample checking feature that compares the match values for the spectra you quantify with a method to the threshold match value specified in the method. If a match value does not meet the threshold match value, the sample is flagged and the specified action is taken.

maxi-min strategy A strategy for selecting standards from a large pool of possible standards. The basic steps are outlined below:

1. Find the standard that is closest to the mean for all standards and add it to the calibration set.
2. Add the standard that is farthest away from the mean.
3. For each remaining standard in the pool, calculate its distance to each standard already in the calibration set. Find the minimum of these distances and assign it to the remaining standard.

4. Find the remaining standard with the maximum distance assigned to it and add it to the calibration set.

5. Repeat steps 3 and 4 until the requested number of standards are chosen.

Maximum Height In Range region type A technique for measuring the intensity of a single point in a spectral region. Maximum Height in Range reports the maximum intensity value in the specified X-axis range.

Maximum In Range baseline option A technique for choosing baseline points in order to calculate a corrected measurement. Maximum In Range uses the maximum intensity value in the specified baseline range to define each baseline point.

mean spectrum A calculated spectrum that shows the average of the spectral features that are present in a group of spectra.

measurement warnings Sample checking features which can be used to monitor the unknown sample spectra that are quantified with a method for possible problems. Use the Report tab to turn the measurement warnings on and off and to specify how they will operate.

Measurement Only analysis type A TQ Analyst analysis type that allows you to measure the heights or areas of peaks in a spectrum, take the ratio of two measured peaks, measure the noise in a given region, measure the width at half maximum of the largest peak in a region, locate the largest peak in a region, or find where a peak is reduced to 1%, 2%, 5%, or 10% of its maximum height.

measurement region spectrum check A sample checking feature that compares each unknown sample spectrum that is quantified with a method to the method's calibration spectra. Only the specified analysis regions are compared. The result of the comparison is called a fit value. If a spectrum fit value falls below the threshold fit value, the sample is flagged and the specified action is taken.

measurement unit The unit of measurement that is associated with a measured peak or region.

menu A list of commands that you can choose to carry out an action or see information.

menu bar The horizontal list of menu names near the top of the TQ Analyst window.

menu name The name of a menu that appears in the menu bar. You can see the commands available in a menu by selecting the menu name.

method A set of parameters and spectra that can be used to create a method model.

method file name The standard Windows file name you enter when you save a TQ Analyst method on a disk (*.QNT extension).

method model A mathematical relationship that describes how the spectral data for the calibration standards correlate with the concentration or classification data. See calibration model.

method parameter A parameter that specifies how a method will work. There are several distinct types of method parameters, including text boxes, readouts, option buttons, check boxes, and drop down list boxes.

method title A one-line description of a TQ Analyst method. The method title is displayed in the Open Method dialog box when the method file name is selected. The title can also be displayed or printed with the analysis results.

method window A window that contains parameters and functions for creating a TQ Analyst method.

micrometer An X-axis unit typically used for infrared wavelength. One micrometer equals 1×10^{-6} meter.

Minimum Height In Range region type A technique for measuring the intensity of a single point in a spectral region. Minimum Height In Range reports the minimum intensity value in the specified X-axis range.

Minimum In Range baseline option A technique for choosing baseline points in order to calculate a corrected measurement. Minimum In Range uses the minimum intensity value in the specified baseline range to define each baseline point.

mixture spectrum A spectrum of a sample that contains two or more components.

moving mirror The mirror in the interferometer of a Fourier-Transform Infrared (FT-IR) spectrometer that reflects the infrared beam back to the beamsplitter while moving toward and away from the beamsplitter in a repeating cycle.

multiple correlation spectrum A calculated spectrum that shows the correlation between the spectral information and the concentrations of all of the components in all of the standards. The multiple correlation spectrum is produced by calculating the correlation coefficient at each X-axis value over all of the standards.

multiplicative factor A numerical value that can be applied to a measured intensity value according to the following equation:

$$\text{<Corrected value>} = \text{factor} \times \text{<measured intensity>}$$

Multiplicative Signal Correction pathlength type A technique for handling pathlength differences in standards and unknown samples. Select the Multiplicative Signal Correction pathlength option when you want to use a mathematical function to compensate for differences in sample pathlength and there is a multiplicative contribution to the spectral signal that correlates with sample pathlength.

N

noise Random signals produced by a number of components in a spectrometer, including the detector and signal-processing electronics.

non-linear A measured response, y , that does not vary in proportion to some other quantity, x . ($y \propto ax + b$). In a quantitative analysis, non linear usually means that spectral absorbance, y , does not vary in proportion to concentration, x (i.e., the component does not obey the Beer-Lambert-Bouguer Law).

0

offset value

1) A numerical value that can be applied to a measured intensity value according to the following equation:

$$\text{<Corrected value>} = \text{<measured intensity>} + \text{offset}$$

2) A constant that is subtracted from each data point in a region to determine the baseline corrected measurement. See baseline offset.

OMNIC* Thermo Scientific comprehensive software package for general FT-IR applications.

One Point baseline type A technique for estimating the baseline of a spectral region. A one-point baseline is a horizontal line that passes through the spectrum at the specified baseline point. The software subtracts the drawn baseline from the intensity of the peak or region to produce a corrected measurement.

Open Method dialog box A dialog box that allows you to open a TQ Analyst or other compatible analytical method that is stored on a disk.

optical bench The component of a spectrometer or analyzer that contains the optics, generally including the source, interferometer, and detector.

option button A small circle in a dialog box or window that you can click to select one option from a group of options.

orthogonal spectrum A spectrum that represents an independent source of variation in a data set. In method types such as PLS and PCR, mixture spectra are reduced to a smaller set of orthogonal spectra which, through linear combination, can be used to approximate the original mixture spectrum.

orthogonal vectors Directions in multidimensional space that are at right angles to one another. For example, the X- and Y-axes in a plot are orthogonal vectors in two dimensional space. In a quantitative analysis, orthogonal vectors are spectra that represent independent sources of variation in a data set.

outlier A standard that is significantly different from the other standards in a calibration set as determined by the diagnostic plots, the Dixon test, or the Chauvenet test. The difference may be due to valid differences in the composition of the standard or to an error in preparing the standard or entering its associated data.

overfit model A calibration model that is very specific to the calibration standards. An overfit model can quantify the calibration standards with great accuracy but cannot produce the same level of accuracy when quantifying a validation standard or sample.

P

pane A rectangular area of a spectral window in which a spectrum can be displayed.

parameter A setting within the software that affects how the active method analyzes an unknown sample spectrum or reports the analysis results.

partial least squares algorithm A statistical quantitative analysis technique. The partial least squares (PLS) algorithm examines the specified region or regions of the calibration spectra to determine which areas are varying statistically as a function of component concentration. The PLS calibration model is developed in one operation using spectral and concentration information from the standards. Intensity and wavelength information are used in the analysis.

Partial Least Squares analysis type A quantitative analysis technique based on the partial least squares algorithm.

pass/fail indicator A sample checking feature that uses the threshold match value to determine whether a "pass" or "fail" message will be displayed or printed next to the match values from a Similarity Match, Distance Match, Search Standards, or QC Compare Search analysis.

pathlength The distance that a beam of electromagnetic energy travels within a sample. When analyzing samples that are in the solid state, "sample pathlength" is often referred to as "sample thickness."

pathlength peak A spectral peak that can be used to compensate for differences in pathlength between the standards and the unknown samples. The measured intensity of the pathlength peak must vary only with pathlength; it cannot include absorptions from other components or impurities in the sample mixture. This can be a peak from a weighed amount of a component that is added to the calibration standards and unknown samples or it can be a peak that is due to the sample matrix.

Pathlength Type parameter A parameter that defines how the method will account for differences in pathlength between the standards and the unknown samples.

peak A region of a spectrum where the sample absorbs radiation.

peak height The intensity (Y value) of a spectrum at a given X value.

Peak Location (Interpolated) region type A technique for finding the exact location of a peak in a spectral region. Peak Location (Interpolated) reports the location of the largest peak in the specified region.

Peak Ratio pathlength type A technique for handling pathlength differences in standards and unknown samples. Select the Peak Ratio pathlength option when you want to specify a reference peak that will be used to compensate for differences in sample pathlength. All of the data points in the analysis region or regions of the calibration spectra are divided by the reference peak value. The pathlength correction is applied before the method is calibrated.

Peak Width (At Half Maximum) region type A technique for consistently measuring the width of a peak in a spectral region. Peak Width (At Half Max) reports the width at half maximum for the largest peak in the specified region. This type of measurement is also referred to as FWHH (full width at half height).

peak-to-peak noise A measurement of signal noise in which the lowest noise peak is subtracted from the highest noise peak.

Peak-To-Peak Noise region type A technique for measuring the noise in a spectral region. Peak-to-Peak Noise reports the calculated peak-to-peak noise value for the specified region.

performance index A measure of how accurately a calibrated method can quantify or classify the validation standards. TQ Analyst provides two algorithms for calculating the performance index for quantitative methods: % Difference (default) and RMSE. The average distance ratio algorithm is used to calculate the performance index for discriminant analysis methods.

Predict pathlength type A technique for handling pathlength difference in standards and samples. Select the Predict Pathlength option when you want to use the pathlength information in the calibrated method to predict the pathlength of each sample that is analyzed with the method. The predicted pathlength value is used to calculate component concentrations in the unknown samples.

Predicted Residual Error Sum of Squares See PRESS value.

PRESS value An acronym for Predicted Residual Error Sum of Squares. The PRESS value for a given factor in a partial least squares analysis is the sum of the (residual concentration error)² over the standards that are removed for each cross validation iteration.

principal component One or more orthogonal vectors that describe the spectral or concentration variation in a principal component regression or partial least squares analysis, or the spectral variation in a discriminant analysis. The first principal component describes most of the variation in the calibration standards. Each additional principal component describes most of the remaining variation.

principal component regression algorithm A statistical quantitative analysis technique. The principal component regression (PCR) algorithm examines the specified region or regions of the calibration spectra to determine which areas are varying statistically as a function of component concentration. The PCR method model is generated in two steps: step 1 uses the spectral data from the calibration standards to determine spectral variation; step 2 correlates spectral variation with concentration. The intensities of all data points in the specified region or regions are used in the analysis.

Principal Component Regression analysis type
A quantitative analysis technique based on the principal component regression algorithm.

principal component spectrum The orthogonal spectrum that represents the amount of variation described by a principal component.

pure component spectrum A calculated spectrum that represents the spectral information that correlates with the concentration information for a given component in all of the standards.

Q

QC Compare search analysis type A classification analysis technique. QC Compare search compares the spectral information in the specified region or regions of an unknown sample spectrum with the spectra of the calibration standards to determine which class of standards is most similar to the unknown. The result of this comparison is the single best match from each reported class.

Quadratic Removed baseline type A technique for calculating a corrected measurement. Quadratic Removed calculates a 2nd order polynomial over the specified region. Then it subtracts the value of the curve at each data point in the region from the spectrum intensity at the same data point. The resulting region has all of its (quadratic) curvature removed, leaving only the higher order part of the region.

qualitative analysis To identify the composition of a sample mixture.

quantify To use a calibrated method to analyze an unknown sample.

Quantify button A button on the TQ Analyst toolbar that allows you to quantify an unknown sample spectrum using the active method. The method must be calibrated before it can be used to quantify samples.

Quantify dialog box A dialog box that displays the analysis results after an unknown sample spectrum is quantified.

quantitative analysis To measure the concentrations of one or more components in a sample mixture.

R

random design A method for selecting the component concentrations of the standards that will be used to calibrate a quantitative method. The random design technique applies the maxi-min strategy to a large set of potential standards to select which standards will be used to create the method model. The random design allows you to easily add standards later.

readout A field used to display information or values provided by TQ Analyst, such as the version number of a method. Readout fields have a colored background, which indicates that their contents cannot be changed.

region A portion of a spectrum that lies between two X-axis locations. See spectral region.

Region Selection task window A task window that allows you to display the spectrum of one or more standards and contains tools for editing the analysis regions used in a method.

residual concentration error The calculated concentration of a component in a standard minus the actual concentration of the component in that standard.

residual spectrum The spectral information in an unknown sample spectrum that is not explained by the calibrated method that was used to quantify the spectrum. Only the spectral information in the specified analysis region or regions are used to calculate the residual spectrum or spectra. See cumulative residual spectrum and component residual spectrum.

residual value A value that indicates how well a spectrum is described by a method model. The residual value is calculated from the amount of the spectrum that is not described by the model divided by the overall size of the spectrum. A small residual value indicates a better fit.

resolution A measure of how well closely spaced peaks in a spectrum are differentiated. The higher the resolution, the more separated two closely-spaced peaks will appear. Increasing the resolution requires that the distance traveled by the moving mirror in the interferometer be increased.

RESULT™ Thermo Scientific spectral analysis software for process applications.

result warnings Sample checking features which can be used to monitor the analysis results for possible problems. Use the Report tab to turn the result warnings on and off and to specify how they will operate.

RMS (Root Mean Square) noise A statistical measurement of random instrument noise.

RMS Noise region type A technique for measuring noise in a spectral region. RMS Noise reports the RMS noise value for the specified region.

RMSE An acronym for Root Mean Square Error. In TQ Analyst, RMSE is one of the algorithms available for calculating the performance index for quantitative methods. The closer the RMSE value is to zero, the better the method will perform.

sample A compound being analyzed.

sample checking To monitor the unknown sample spectra that are analyzed with a method or the analysis results for possible problems. See analysis limits check, acceptance limits check, full spectrum check and measurement region spectrum check.

sample matrix A compound in which the components in a sample mixture are dispersed or dissolved.

sample mixture A sample that contains two or more components.

sample report All of the information that is displayed on the screen after using a TQ Analyst method to quantify an unknown sample spectrum, including the analysis results.

sample spectrum The spectrum of an unknown material being analyzed.

sampling technique The technique used to prepare and collect the spectrum of a sample or standard. Examples include transmission and diffuse reflection.

S

score value A measurement of the multidimensional distance of a standard projected onto a principal component. The score value shows how much of the variation in the spectrum of the standard is described by the principal component.

scroll bar A bar running along the right side or bottom of a list box or window that is used to scroll text or stacked spectra into view. Items can be scrolled by clicking one of the scroll arrows at either end of the scroll bar or by dragging the scroll box along the scroll bar.

search algorithm The algorithm used to compare the unknown sample spectrum with each calibration spectrum in a Search Standards analysis.

Search Standards analysis type A classification analysis technique. Search Standards compares the specified region or regions of an unknown sample spectrum with each calibration spectrum and finds the standards that most closely match the unknown. The index number and title of each reported standard are given as well as a match value between 0 and 100.

Search Type parameter A parameter that allows you to select the search algorithm that will be used for the Search Standards analysis.

second derivative The rate of change of the first derivative with respect to the X value.

Second Derivative In Range region type A technique for measuring a spectral region. Second Derivative in Range measures an estimate for the second derivative at each data point in the specified region.

shoulder A small hump or variation in curvature on the side of a peak. A shoulder may indicate the presence of a small peak that is obscured by the larger peak.

signal-to-noise ratio (SNR) The ratio of the intensity of a signal to the intensity of the noise that accompanies it.

SIMCA An acronym for Soft Independent Modeling of Class Analogy. SIMCA is a classification technique that generates an independent model for each class. Each model is based on its own set of principal components. When the method is used to quantify an unknown sample spectrum, the software compares the sample spectrum with each model and applies a statistical analysis to the pooled results. For details see Wold, S. Pattern Recognition 1976, 8, 127-139.

Similarity Match analysis type A classification analysis technique. Similarity Match compares the spectral information in the specified region or regions of an unknown sample spectrum with that of a known set of standard spectra to determine how closely the sample matches the standards. The result of this comparison is called a match value.

Simple Beer's Law analysis type A quantitative analysis technique based on the Beer-Lambert-Bouguer Law. The calibration model is the best fit straight line in an absorbance measurement versus concentration plot. A separate calibration curve is created for each component.

single-beam spectrum A spectrum (data in the frequency domain) obtained by Fourier transforming an interferogram (data in the time domain). A single-beam spectrum shows the response at all frequencies in the spectral range. This response is influenced by the beamsplitter, relative source output, detector, and other optical components in the instrument. A sample single-beam spectrum can be ratioed against a background single-beam spectrum to produce a sample spectrum with the background information removed.

slope The rate at which an ordinate of a point of a line on a coordinate plane changes with respect to a change in the abscissa (i.e., the rise divided by the run).

source A component inside the optical bench of an infrared spectrometer or analyzer that emits the infrared radiation that travels to the detector.

spectral measurement A peak height, peak area, or region of a spectrum measured from a null reference point. An absorbance spectrum, for example, would be measured from zero absorbance units.

spectral measurement method A TQ Analyst analysis technique that measures spectral features and reports the measured values. You can set up a spectral measurement method that measures peak heights or peak areas in a sample spectrum, calculates the ratio of two measured peaks, measures random noise or peak width, or finds peak locations.

spectral region A portion of a spectrum between two X-axis locations.

spectral window A window used to display a spectrum or spectra. A spectral window contains one or more panes, each of which can contain one spectrum.

spectrum A graphical representation of the intensity of the radiation reaching the detector at each X-axis location measured. The intensity at a given X-axis location is determined by the characteristics of the sample, if one is present.

spectrum collection date and time The date and time a spectrum was collected and saved on a disk. The collection date and time is stored with the spectrum during sample collection. You can view a spectrum's collection date and time by displaying the spectrum in a spectral window, selecting the spectrum, and then double-clicking the title box.

spectrum file name The standard Windows file name you enter when you save a spectrum in a file on a disk (*.SPA extension).

Spectrum Range region type A technique for measuring a spectral region. Spectrum Range measures the intensities of all the data points in the specified region rather than a single value.

spectrum title A description of a spectrum that is saved with the spectrum.

spectrum warnings Sample checking features which can be used to monitor the unknown sample spectra that are quantified with a method for possible problems. Use the Report tab to turn the spectrum warnings on and off and to specify how they will operate.

Squared Derivative search algorithm An algorithm for the Search Standards analysis. The squared derivative difference is defined as the sum of the squares of the differences between the first derivative of the sample spectrum and the first derivatives of the calibration spectra in the specified regions, where the sums are taken over all data points. The sample and calibration spectra are placed on a common Y-axis scale before the squared derivative differences are calculated.

Squared Difference search algorithm An algorithm for the Search Standards analysis. The squared difference is defined as the sum of the squares of the differences between the unknown sample spectrum and each calibration spectrum in the specified regions, where the sums are taken over all data points. The sample and calibration spectra are placed on a common Y-axis scale before the squared differences are calculated.

stacked spectra Spectra with panes that are displayed in a column on the screen.

standard Known samples that model the behavior of the unknown samples that will be analyzed with the method. For quantitative analysis, standards are samples which have known concentrations of each component the method will be used to analyze. For classification analyses, standards are samples that have the characteristic you want to track.

standard deviation A measure of sample distribution about the mean or average. For a normal distribution, the probability that a measured value lies within one standard deviation, σ , of the mean is approximately 67%; within 2σ is approximately 95%. Standard deviation is the square root of variance.

$$\sigma = \left(\frac{\sum_i (y_i - y_{ave})^2}{n - 1} \right)^{1/2}$$

The standard deviation on variance values indicates the spread or scatter in a set of data.

standard error check A sample checking feature that compares the analysis results for the spectra you quantify with a method to the standard error limit specified in the method. If the standard error value associated with a calculated component result is above the standard error limit, the sample is flagged and the specified action is taken.

standard error limit A parameter that defines the maximum standard error value allowed in order to pass the standard error check.

standard error value The relative error of a measurement expressed as a percentage of the actual values.

Standard Normal Variate pathlength type A technique for handling pathlength difference in standards and samples. The Standard Normal Variate (SNV) pathlength setting scales the spectral data in order to compensate for differences in sample pathlength. This allows you to analyze samples with different pathlengths when it is difficult or impossible to obtain an independent measure of sample pathlength. The spectra of the standards and any unknown samples the method is used to analyze are all scaled independently.

standards library A collection of spectra of known materials used for calibration, validation, or correction. A method standards library is contained in two files which have the same base name as the method (method filename.LBD and method filename.LBT).

Stepwise Multiple Linear Regression analysis type A quantitative analysis technique based on the multiple linear regression algorithm. Stepwise Multiple Linear Regression (SMLR) expresses concentration as a function of the absorbance at multiple frequencies. When the SMLR algorithm is used, the software selects the analysis regions. Each component is calibrated independently.

studentized residual value The residual value of a spectrum after it has been scaled by the leverage value.

T

tab A software feature that looks like a file card and contains a group of method parameters. Each file card has a tab that is always visible at the top of the active method window.

task window A special window that appears when you choose certain commands or action buttons. A task window contains the features needed to use the command or complete the action. Examples of task windows include the Region Selection task window, the Corrections task window, and the diagnostic task windows.

text box A parameter that allows you to enter information that you want to save with a method, such as the method title. Text boxes have a white background, which indicates that their contents can be changed.

threshold distance value A parameter that defines the maximum distance value allowed to pass the distance value check.

threshold F ratio An internal parameter that defines the minimum F ratio required to pass the feasibility assessment. The threshold F ratio is a fixed value and cannot be changed. If the calculated F ratio is greater than or equal to the threshold F ratio, we can conclude that the variability in the feasibility standards is significant at a 95% confidence level and the method is feasible.

threshold fit value A parameter that defines the minimum fit value required to pass the full spectrum check or the measurement region spectrum check.

threshold match value A parameter that defines the minimum match value required to pass the match value check or trigger the pass/fail indicator.

toolbar A long narrow strip at the top of the TQ Analyst window that contains action buttons.

TQ Analyst window The window that contains the TQ Analyst menu bar and toolbar. The main portion of the TQ Analyst window may be used to display method windows, spectral windows and task windows.

transmission spectrum A spectrum that shows how the transmittance of a sample varies with frequency or wavelength.

transmittance (%) The fraction of the radiation that remains after a beam of electromagnetic radiation passes through a sample. Percent transmittance is defined by the formula $T = (P/P_0) * 100$, where P is the radiation that passes through the sample and P_0 is the radiation when no sample is present.

Two Point baseline type A technique for estimating the baseline of a spectral region. A two-point baseline is a line that passes through the spectrum at two specified baseline points. The software subtracts the drawn baseline from the intensity of the peak or region to produce a corrected measurement.

U

uncertainty limit The highest uncertainty value allowed in order to pass the uncertainty value check.

uncertainty value An empirically derived value that indicates the relative, estimated precision of a quantitative result.

uncertainty value check A parameter that allows you to monitor the uncertainty values that are reported when a method is used to quantify an unknown sample spectrum. If the uncertainty value check is on and a reported uncertainty value is above the uncertainty limit, the specified action is taken.

uncorrected measurement A peak height, peak area, or region of a spectrum measured from a null reference point. An absorbance spectrum, for example, would be measured from zero absorbance units.

unknown sample spectrum The spectrum of a sample of unknown composition.

Usage parameter A parameter that defines how each standard will be used in a TQ Analyst method.

V

validation Using a calibrated method to quantify validation standards. Because validation standards are not used to create the method model, validation can be used to verify that a method is capable of quantifying unknown sample spectra with the desired accuracy.

validation set The standards that are used to validate the method model after a method is calibrated.

validation spectrum The spectrum of a validation standard.

validation standard A standard that is used to evaluate the performance of a calibrated method. The results from the validation standards are also used to calculate the performance index.

variance spectrum A calculated spectrum that shows the spectral variance in the standards. The variance spectrum is produced by calculating the square root of the spectral variance at each X value over all of the standards.

W

wavelength The distance between corresponding points in consecutive light waves. Wavelength is measured in micrometers and is often used as the X-axis unit of an infrared spectrum.

wavenumber The number of waves per centimeter, expressed as cm^{-1} . Wavenumber is the inverse of wavelength and is often used as the X-axis unit of an infrared spectrum.

window A rectangular area on the screen that can contain method tabs, spectral data, data plots or software features. Examples include method windows, spectral windows, and task windows.

working curve See calibration curve.

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