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Thermo Scientific
Chromeleon

HPLC Instrument Installation Qualification

Operating Instructions

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The information contained in this document is subject to change without notice.

This manual revision is backward compatible and replaces all prior manual revisions.

The descriptions in this manual revision refer to sequence templates version: 5.7 or later. Changes on sequence templates with a version later than 5.7 are described in the annex attached to the Release Notes. The annex is a complement to this manual. The version number of the sequence template is indicated in the name of the report template.

Contents

1 Using this Manual

1.1 About this Manual

1.1.1 Scope

The manual describes how the principle communication between Chromeleon™ and the connected modules is tested when Chromeleon is installed or upgraded. It is not intended to completely validate all communication functions.

The document is not intended to describe a procedure for Operational or Performance Qualification of the connected modules. However, Operational or Performance Qualification of the connected modules can be performed instead of Instrument Installation Qualification.

1.1.2 Overview

The following table provides a quick overview to the chapters in the manual.

Section	Content
2 Introduction	Describes the intention of the Installation Qualification, summarizes the supported devices and how to perform the qualification dependent on the installed system.
3 Requirements and Preparations	• Required parts • Preparation steps
4 Performing Automated HPLC IQ in Chromeleon	Describes how to prepare and perform the standard AutoQ™ checks. Since the procedures depend on the Chromeleon version, the procedures are described in two separate sections. Follow the instructions in this section in the given order. Descriptions for the Chromeleon version not used for the qualification can be skipped.
5 HPLC Systems – Test Design and Hints for Manual Test Execution	Describes the test design, expected measuring results, and how to test devices manually if no AutoQ test sequences are available for that device. Descriptions for modules that are not installed in the system can be skipped.
6 GC Systems	Describes how to test GC systems manually. Descriptions for modules that are not installed in the system can be skipped.
7 Appendix	Contains a filled in example form IQ_Report_Comm_Test.docx.

TIP We recommend that you read the manual thoroughly before starting Chromeleon Installation Qualification (AutoQ routines) in order to obtain full understanding of the procedure.

1.2 Conventions

This section describes the conventions that are used throughout this manual.

1.2.1 Terminology

The descriptions in all sections except section 4.2, page 35 use the terminology used in Chromeleon 6.80.

TIP In Chromeleon 7, the terminology differs from that in Chromeleon 6. For detailed information, refer to *Glossary – Chromeleon 7.x* included in the Documents folder of the Chromeleon 7 Installation.

All descriptions in this manual referring to Corona™ detectors equally apply to charged aerosol detectors.

1.2.2 Special Notices and Informational Notes

Special notices and informational notes in this manual appear different from the main flow of text. They appear in boxes and a note label identifies them. The label text appears in uppercase letters and in bold type.

NOTICE Highlights information necessary to prevent invalid test results and ensure a problem-free installation.

TIP Highlights information of general interest or helpful information that can make a task easier or optimize the performance of the equipment.

1.2.3 Typographical Conventions

These typographical conventions apply to the descriptions in this manual:

Notation	Description
Bold	Data input and output: • Input that you enter by the keyboard or that you select with the mouse • Buttons that you click on the screen • Commands that you enter by the keyboard • Names of, for example, dialog boxes, properties, and parameters • Paths: For brevity, long expressions and paths appear in the condensed form, for example: Click **Start > All Programs > Thermo Chromeleon 7 > Service Manager > Start Instrument Controller**.
Italics	• Particularly important words in the main flow of text • References to additional documentation
"text"	Messages that appear on the screen

1.2.4 Other Conventions

1.2.4.1 Viewpoint

If not otherwise stated, the expressions *left* and *right* in this manual always refer to the viewpoint of a person that is facing the device from the front.

1.2.4.2 Electronic Manual Version (PDF)

The electronic version (PDF) of the manual contains numerous links that you can click to go to other locations within the manual. These include:

- Table of contents entries
- Index entries
- Cross-references (in blue text), for example, to sections and figures

1.2.4.3 Part Numbers

For standards, solvents and columns, part numbers are Fisher catalog numbers.

2 Introduction

2.1 Installation Qualification

The HPLC instrument installation qualification is used to confirm that the principle communication between Chromeleon and the chromatography system corresponds to the expected behavior.

Communication is tested by evaluating the quality of the recorded detector signals. In Chromeleon, different parameters that can be checked are set for the modules. The data is checked for precision and accuracy; the limits for checking are quite wide. In addition, the test shall demonstrate that the "start of sample - injection - data acquisition - end of data acquisition - end of sample - start of the next sample" cycle runs as planned. The runs are short; they last few minutes only. The following basic functions will be tested:

- Module control in principle
- Check of the chromatography system including loading or running a sample sequence
- Qualitatively accurate data acquisition and evaluation
- Indirect test of database access

In addition, some functions and/or parameters are checked for each module by setting them on the control panel and comparing them to the values set on the module.

2.2 Supported Devices

The standard AutoQ checks are optimized for the following Thermo Scientific HPLC devices:

- Pumps
- Autosamplers
- Detectors
- Fraction collectors

For these devices, the qualification is performed automatically according to the descriptions in chapter 4, page 13 (for exceptions, see also section 2.3, page 13). Therefore, all manual checks described in chapter 5, page **Fehler! Textmarke nicht definiert.** and their documentation on the form IQ_Report_Comm_Test.docx can be omitted for the mentioned devices (Exception: For fraction collectors, see descriptions in chapter 5.12.2, page 63).

The standard AutoQ checks *not* are optimized for the following devices:

- Thermo Scientific HPLC column thermostats
- Third-party HPLC devices and systems
- Preparative HPLC devices and systems
- GC devices and systems

Depending on the device, either no checks are offered or warning or error messages can occur during batch ready check. If no checks are offered, the sequences and program files have to be created manually according to chapter 5, page **Fehler! Textmarke nicht definiert.**. If warning or error messages occur during batch ready check, the templates have to be adapted accordingly.

2.3 Known Restrictions

The standard AutoQ checks do not support systems with Vanquish mass spectrometry (MS) detector and fluorescence (FL) or Refractive Index (RI) detector. When using such a system, the AutoQ offers a check for the MS detector. To check the FL or RI detector, do the following:

1. In the **Chromeleon Server Configuration** program, remove the MS detector from your instrument.

2. Install any Vanquish UV detector in simulation mode.
3. Run the **Installation Qualification Wizard** for a second time.
4. Select only the specific detector test for the FL or RI detector.
5. When the message **The instrument queue contains sequences that are not finished. Should the qualification be continued and the instrument queue be cleared?** appears, select **No**.
6. Add the specific detector test sequence to the queue manually.
7. Add the MS detector to your instrument.
8. Delete the UV detector from your instrument.
9. Start the queue.

3 Requirements and Preparations

3.1 Software Requirement

Data acquisition must be performed using Chromeleon.

3.2 Analytical HPLC

This section lists preparations for specific device types. If a module is not listed, no specific preparation steps are required.

3.2.1 Dual Gradient Pumps

3.2.1.1 Preparations

Both pump units at one timebase – Tandem LC

1. When performing Instruments IQ for a timebase that includes a dual gradient pump, verify that the left pump unit (device name: **PumpLeft** or **LoadingPump**) is fluidically connected to the autosampler, column, and detector.

2. During the tests, only the left pump unit delivers the eluent. Set the flow rate for the right pump unit (device name: **PumpRight** or **MicroPump**) to 0°mL/min.

TIP For Vanquish Tandem LC systems, both valves are switched to position 1_2 to match with the default Tandem LC configuration for column A. Verify that the fluidic connections match with this valve configuration.

Pump units shared between two timebases – Dual LC

If the pump units of a dual gradient pump are shared between two different systems (i.e. timebases), no specific preparations are required.

3.2.2 Autosamplers

3.2.2.1 Materials Required

Autosampler Type	Material	Part Number
UltiMate 3000 WPS-3000(T)PL(RS)	Upgrade Kit for the 250 µL syringe	6820.0031
WPS-3000TBPL Analytical and WPS-3000T(B)FC Analytical	Standard configuration (sample loop volume: 50 µL)	-
UltiMate 3000 OAS-3600TXRS	Well plate with 96 wells	6820.4100

3.2.2.2 Preparations

Vanquish Dual Autosampler

Because the sampler units of a dual autosampler are shared between two different systems (i.e. timebases), no specific preparations are required.

WPS-3000(T)PL(RS)

Install the Upgrade Kit for the 250 µL syringe.
This kit also includes a sample loop (volume: 125 µL). If you want to use a different sample loop for the test, make sure that the sample loop has a minimum volume of 20 µL. Otherwise, the peak area deviation may exceed ± 5%.

WPS-3000TBPL Analytical and WPS-3000T(B)FC Analytical

1. Install the standard configuration (sample loop volume: 50 µL).
2. Enable the following autosampler model on the **Options** tab page in the Chromeleon Server Configuration:
   - WPS-3000TBPL Analytical: WPS-3000TBPL Analytical
   - WPS-3000T(B)FC Analytical: WPS-3000TFC/WPS-3000TBFC

UltiMate 3000 OAS-3600TXRS

Fill the requested well with at least 350 µL of the sample according to the position and sample given in the following detector sections applicable for your system.

3.2.3 Ultraviolet/Corona/Mass Spectrometry Detector

For HPLC systems with at least one UV detector, a Corona and/or MS detector, a mixture of uracil and caffeine dissolved in water is injected and separated in a column. For UV/Corona and/or MS tests, different standard concentrations are used. All other chromatographic conditions are identical.

3.2.3.1 Materials Required

Material	Description	Part Number
Standard	Standard mixture of uracil (50 µg/g) and caffeine (60 µg/g) dissolved in water For UV/Corona tests, use the standard undiluted. For MS detector tests, dilute the standard as described in the section Producing the Standard(s), page 18.	3321.0010A
Column	AcclaimTM 120, C18, 5 µm analytical (4.6 x 100 mm) or a similar column	059147

Material	Description	Part Number
Solvent	Methanol (HPLC-grade)	-
	Water (HPLC-grade)	-

3.2.3.2 Preparations

Preparations for the MS Detector

Notice Acceptable results can only be obtained, if the detector is properly calibrated before (positive and negative polarity). Before starting the installation qualification, verify that the MS detector is ready for operation according to the instrument operating manual.

Notice If a HESI / APCI dual source is used, ensure that the HESI probe is installed and the corona needle is in HESI position.

TIP Ensure that the used pump is set correctly in the **Associate pump info** tab of the detector in the Chromeleon 7 Instrument Configuration Manager. For dual gradient pumps apply the left pump unit in the **Associate pump info** tab (the flow rate of the right pump unit is set automatically to 0 mL/min).

Producing the Standard(s)

TIP If no suitable pipette for the stated volumes is available, you can adapt the dilution steps to your laboratory equipment.

For UV/Corona tests, use the standard stated above (part number 3321.0010A) to prepare an undiluted standard S1. For MS detector tests, prepare a diluted standard S2:

1. Pipette the entire standard (part number 3321.0010A) in a vial S1.
2. *For instruments with MS detector only*: Pipette 20 µL of S1 and 980 µL of water in a second vial, close the vial and shake it (sample solution (S2) – resulting concentration: uracil: 1 µg/g; caffeine: 1.2 µg/g).
3. Close vial S1.

Other Preparations

1. Prepare the eluent mixture for channel A(1): Water/methanol (60/40 Vol.%)
2. Put the sample(s) into the position as shown in the table below.

Autosampler Series	Sampler Position for UV/Corona Detector (S1)	Sampler Position for MS Detector (S2)
Vanquish	R:A1	R:A5
UltiMate	RA1	RA5
Any other autosampler	1	5

3. Connect the column between the autosampler and the (first) detector.
4. Optionally, place the column in a column thermostat.
5. Thoroughly rinse the HPLC system with eluent.

3.2.4 Fluorescence Detector

For HPLC systems including a fluorescence (FL) detector, pyrene dissolved in methanol is injected and separated in a column.

3.2.4.1 Materials Required

Material	Description	Part Number
Standard	Pyrene (solid) Due to legal shipping restrictions, the pyrene standard is shipped in solid form. Before you can use the standard, dissolve the solid pyrene in 1 mL of methanol as described in the following section.	3327.0010
Column	Acclaim 120, C18, 5 µm analytical (4.6 x 100 mm) or a similar column	059147
Eluent	Methanol (HPLC-grade)	-

3.2.4.2 Preparations

Dissolving Solid Pyrene

To dissolve the solid pyrene in 1 mL of methanol (HPLC-grade):

1. Unscrew the cap from the 1.5 mL vial labeled *3 µg pyrene*.
2. Add about 1 mL of methanol (HPLC grade), which is about half of the vial's volume.
3. Screw the cap onto the vial. Make sure that the cap seals tight.
4. Shake the vial for about 10 seconds to dissolve the solid pyrene.
5. Place the vial at the appropriate position in the autosampler.

Other Preparations

1. Prepare the eluent for channel A(1): 100% methanol
2. Put the sample into the position as shown in the table.

Autosampler Series	Sampler Position
Vanquish	R:A2
UltiMate	RA2
Any autosampler	2

3. Connect the column between the autosampler and the (first) detector.
4. Optionally, place the column in a column thermostat.
5. *If your HPLC system includes an FL detector but no UV or Corona detector:* Thoroughly rinse the HPLC system with eluent.
   If your system includes a UV or Corona detector, the eluent is changed and the HPLC system is rinsed automatically during qualification.

3.2.5 Electrochemical Detector

For HPLC systems including an electrochemical (EC) detector (for example, Thermo Scientific UltiMate 3000 ECD-3000RS), a catecholamine standard is injected and separated in a column.

3.2.5.1 Materials Required

TIP Several material may be available only via Fisher Scientific Shop.

Material	Description	Part Number	
Eluent	MOBILE PHASE TEST	70-3829	Both materials are also available together under the part number 70-7655
Standard	Catecholamine standard, consisting of: • Norepinephrine • Epinephrine • Dopamine Concentration (stock solution): 1mg/mL Prepare the standard is described in the following section.	45-0206	
Column	Acclaim 120, C18, 5 µm analytical (4.6 x 100 mm) or a similar column	059147	

3.2.5.2 *Preparations*

Producing the Standard

TIP If no suitable pipette for the stated volumes is available, you can adapt the dilution steps to your laboratory equipment.

Use the above stated stock solution (S1) with a concentration of 1 mg/mL and produce a sample solution (P1) with a final concentration of 100 ng/mL that is used for the qualification:

1. Pipette 5 µL of the stock solution S1 and 495 µL of the mobile phase in a vial, close the vial, and shake it (1st dilution – resulting concentration: 10,000 ng/mL = 10 µg/mL).
2. Pipette 5 µL of the 1st dilution and 495 µL of the mobile phase in a vial, close the vial and shake it (sample solution (P1) – resulting concentration: 100 ng/mL)

TIP At ambient temperature, the sample solution (P1) remains stable only for 12 hours.

Other Preparations

1. Prepare the eluent for channel A (1): 100% TEST mobile phase
2. Put the sample into the position as shown in the table.

Autosampler Series	Sampler Position
Vanquish	R:A3
UltiMate	RA3
Any other autosampler	3

3. Connect the column between the autosampler and the (first) detector.

4. Optionally, place the column in a column thermostat.

5. *If your HPLC system includes an EC detector but no other detector*:
   Thoroughly rinse the HPLC system with eluent.
   If your system includes any other detector, the eluent is changed and the HPLC system is rinsed automatically during qualification.

3.2.6 Refractive Index Detector

For HPLC systems inlcuding a refractive index (RI) detector, toluene dissolved in methanol is injected and separated in a column.

3.2.6.1 Materials Required

Material	Description	Part Number
Standard	Toluene dissolved in methanol (5 µL/mL) Producing the standard is described in the following section.	For toluene: T290-1
Column	Acclaim 120, C18, 5 µm analytical (4.6 x 100 mm) or a similar column	059147
Eluent	Methanol (HPLC-grade)	-

3.2.6.2 Preparations

Producing the Standard

TIP If no suitable pipette for the stated volumes is available, you can adapt the dilution steps to your laboratory equipment.

Use toluene and methanol and produce the standard with a final concentration of 5 µL/mL that is used for the qualification:

1. Pipette 5 µL toluene and 995 µL methanol in a vial
2. Close the vial.
3. Shake it.

Other Preparations

1. Prepare the eluent channel A (1): 100% methanol.
2. Put the sample into the position as shown in the table.
3. Connect the column between the autosampler and the (first) detector
4. Optionally, place the column in a column thermostat.

Autosampler Series	Sampler Position
Vanquish	R:A4
UltiMate	RA4
Any other autosampler	4

5. *If your HPLC system includes an RI detector but no UV or Corona detector*:
   Thoroughly rinse the flow cell of the RI detector with eluent.
   If your system includes a UV or Corona detector, the eluent is changed and the flow cell of the detector is rinsed automatically during qualification.

3.2.7 Evaporative Light Scattering Detector

TIP Evaporative light scattering (ELS) detectors are not covered by the standard AutoQ checks.

For HPLC systems including an ELS detector, glucose dissolved in water is injected.

3.2.7.1 Materials Required

- Glucose dissolved in water (0.125 mg/mL)
- Restriction capillary to connect the autosampler to the detector
- HPLC-grade water

3.2.7.2 Preparations

1. Prepare the eluent for channel A (1): 100% water.
2. Put the sample into any free autosampler position.
3. Connect the column between the autosampler and the (first) detector.
4. Optionally, place the column in a column thermostat.

3.2.8 Fraction Collector

For HPLC systems inlcuding a fraction collector, solvent is collected in a 1.5 mL standard vial.

3.2.8.1 Materials Required

Material	Description	Part Number
Empty vial	1.5 mL vial with pre-split cap	-
Column	Acclaim 120, C18, 5 µm analytical (4.6 x 100 mm) or a similar column or any restriction capillary (back pressure should be greater than 40 bar @ 0.6 mL/min flow rate)	059147
Eluent	Methanol (HPLC-grade) Water (HPLC-grade)	-

3.2.8.2 Preparations

Other Preparations

1. Ensure that the needle pusher position is in vial.
2. Select the 100 µm x 350 mm capillary in the Chromeleon Fraction Collector **ePanel** (**More Options** – **Delay Volume** – **Delay Capillary IDxL**).
3. Place the empty vial in the Fraction Collector at R:A1, Vanquish 54 Vial Rack.
4. Prepare the eluent mixture for channel A(1): As already used, e.g., water/methanol (60/40 Vol.%).
5. Connect the column or restriction capillary between the autosampler and the (first) detector.
6. Optionally, place the column in a column thermostat.
7. Thoroughly rinse the HPLC system with eluent.

3.2.9 System Equilibration

Use an appropriate control panel to equilibrate the chromatography system. If applicable for your system, consider following:

1. Turn on all modules in the system according to the operating instructions of the manufacturer.
2. Allow all modules to warm up.
3. Ensure that all modules are in operable state.
4. Purge the pump, system and the column sufficiently with the eluent to be used.
5. Apply the following basic chromatography conditions to the system and allow the system to equilibrate until the pressure, detector signal, and temperatures are stable:

- Flow rate: 0.6 mL/min
- Column thermostat temperature: 25 °C
- RI detector temperature: 35 °C

3.3 Preparative HPLC

3.3.1 Materials Required

3.3.1.1 Preparative HPLC Column

One of the customer's columns. As these may be quite different, no sample mixture is used for separation.

3.3.1.2 Standard for Reversed-Phase Chromatography

A pure uracil sample dissolved in methanol (or another quickly retarding, polar substance which is available for the customer in sufficient quantity).

3.3.1.3 Standard for Normal-Phase Chromatography

An appropriate dead time marker,for example, toluene.

If necessary, adapt the eluents and detector control parameters to the changed chromatographic conditions.

3.3.2 Tips for Concentrations

The modules of preparative HPLC systems are generally designed for higher concentrations. Therefore, adapt the concentration of the injected standard accordingly. The column has the greatest influence on defining a suitable concentration for the qualification.

The separation capacity of the column is proportional to its cross-section. This fact also determines the concentration to be used for the injection. The concentration used for testing the analytical system gives you a clue.

Example:

Parameter	Settings
Concentration of uracil for a column diameter of 4.6 mm	40 mg/L
Column diameter of the preparative column	30 mm
Cross-section of the column	$A = \pi d^2 / 4$
Cross-section of the analytical column	16.6 mm^2
Cross-section of the preparative column	706.8 mm^2

Parameter	Settings
Preparative concentration (= $Concentration_{Analytical}$ x $Cross\text{-}section_{Preparative}$ / $Cross\text{-}section_{Analytical}$)	40 mg/L x (706.8 / 16.6) = 40 mg/L x 42.6 ≈ 1600 mg/L

3.3.3 Tips for Flow Rate

Adapt the flow rate to the used components. The column has the greatest influence on defining a suitable flow rate, too.

The retention time is almost proportional to the column volume. This fact also determines the flow rate to be used. The flow rate used for testing the analytical system gives you a clue.

Example:

Parameter	Settings
Flow for a column of 4.6 x 100 mm	0.6 mL/min
Dimensions of the preparative column	30 x 250 mm (ID x l)
Column volume	$V = lD^2\pi/4$
Volume of the analytical column	1.66 mL
Volume of the preparative column	176.7 mL
Preparative flow (=$Flow_{Analytical}$ x $Volume_{Preparative}$ / $Volume_{Analytical}$)	0.6 mL/min x (176.7 / 1.66) = 0.6 mL/min x 106.4 ≈ 64 mL/min

3.4 GC Systems

The separation conditions for GC applications largely depend on the columns used. You have to prepare an appropriate test mixture and the separation method available. Make sure that the application completely separates the analyte from the solvent.

4 Performing Automated HPLC IQ in Chromeleon

4.1 Chromeleon 6

4.1.1 Installation Qualification Procedure

Chromeleon offers standard AutoQ checks that cover tests for HPLC pumps, autosamplers, and various detector types. The checks are optimized for Thermo Scientific modules and are described in chapter 5, page **Fehler! Textmarke nicht definiert.**. For column thermostats or other chromatographic systems, the standard AutoQ checks are not available or optimized. In this case, the seqences and program files have to be created manually according to chapter 5, page **Fehler! Textmarke nicht definiert.** or they have to be adapted accordingly.

A wizard guides you through the automated sequence creation. The sequences contain programs that contain all necessary commands. The programs are assigned automatically to the samples to process.

The sequences are added manually to the batch list. The batch is started manually after the Ready Check has been performed. During sample processing, you can observe the acquired date and whether all functions are performed. The audit trail lists all program commands including execution times, feedback from the injector, and name of the sample. After the sequence has been processed, the processed samples are evaluated highly automated with a report linked to the sequence templates.

The qualification for a system with pump, autosampler and one detector takes about 1.5 hours.

For more information about the test design, see chapter 5, page **Fehler! Textmarke nicht definiert.**.

4.1.2 Template Directory Structure on the Chromeleon CD

A data source named CM_CD is provided on the Chromeleon CD. The IQ directory in this data source has the following folder structure:

Folder Name	Description
DEMO	Including examples for HPLC and NANO_CAP_MIC_LC

Folder Name	Description
HPLC_Templates	Contains the sequences for Installation Qualification in an analytical LC configuration (HPLC / UHPLC or GC). Note that the program files included in this folder do not provide any specific commands that may be required for control of third-party modules. If, for this reason, any warnings or error messages occur during program execution, the templates have to be adapted accordingly.
NANO_CAP_MIC_LC_Templates	Contains the sequence for nano, cap, and micro LC systems.
Reports	When the sequences are copied from the CD, the report template is automatically copied to the corresponding directory. For information about the report structure, and how to adapt and sign reports, see section 4.1.6, page 33.

Chromeleon selects the appropriate template for the installed timebase automatically.

TIP All descriptions in this manual refer to the templates in the HPLC_Templates folder.

4.1.3 Creating Sequence Templates

To create the sequences required for your system, follow the steps:

1. Insert the Chromeleon CD. Or else, verify that you can access the **IQ** directory.
2. In the Chromeleon Browser, select **Qualification** > **Instruments IQ…**.
   A wizard guides you through the process of copying the sequences. Clicking **Next>** takes you to the next step in the procedure.
3. Select the computer and the timebase for which you want to perform Installation Qualification.
4. Select the source directory for the master sequences: Select **IQ**.
5. Select the directory where all sequence templates should be saved (default directory: Standard datasource\Timebase\IQ_Runs\HPLC_Templates <Date>)

TIP The standard datasource for a server is defined in the Server Configuration program. In this datasource, Chromeleon stores, for example, the daily audit trail. The standard datasource can be either a datasource or a directory in a datasource. In the directory of the standard datasource, Chromeleon automatically creates a subdirectory for each timebase.

6. Enter a unique folder name.

7. A number of sequences is displayed, matching the selected timebase and can contain the following sequences (maximum one per block):

Sequence / Order	Necessary Device Property / Additional Requirement
Block 1 **IQ_Pump(_xy)**	The **IQ_Pump(_xy)** sequence tests the flow rate for an analytical HPLC system.
IQ_Pump	**Pump.Flow** and UV/Corona detector
IQ_Pump_RI	**Pump.Flow** and RI detector, but no UV/Corona detector
IQ_Pump_FLD	**Pump.Flow** and FLD detector, but no UV/Corona/RI detector
IQ_Pump_ECD	**Pump.Flow** and ECD detector, but no UV/Corona/RI/FLD detector
Block 2 **IQ_Inject_Det(_xy)**	The **IQ_Inject_Det(_xy)** sequence tests the autosampler. Indirectly, this sequence also tests: • Corona detectors • RI detectors • Basic functions of Chromeleon
IQ_Inject_Det	**Inject** and UV/Corona detector if the UV detector does not include a LightPipe™ flow cell
IQ_Inject_Det_RI	**Inject** and RI detector, but no UV/Corona detector
IQ_Inject_Det_FLD	**Inject** and FLD detector, but no UV/Corona/RI detector
IQ_Inject_ECD	**Inject** and ECD detector, but no UV/Corona/RI/FLD detector
Block 3	
IQ_Inject_Det_CAD	**Inject** and Corona detector if the UV detector includes a LightPipe flow cell
Block 4	
IQ_UV_Det	This sequence tests a UV detector with variable wavelength. **Wavelength** <UV_VIS_1>(1)
Block 5	
IQ_Inject_Det_RI	**Inject** and RI and UV or Corona detector
Block 6	
IQ_FL_Det	This sequence tests a fluorescence detector. **Emission wavelength** <Emission_1>(1)
Block 7	
IQ_EC_Det	This sequence tests an electrochemical detector. **Potential** <ECDRS_1>(1)

(1) The device channel name (marked by angle brackets <>) can be selected freely and may deviate from the default value. The created sequences and their program files are adapted automatically to the used device and channel names.

8. Select the sequence(s) to be added.

TIP For systems with a non-Chromeleon controlled detector, it is sufficient to run the test sequences **IQ_Pump(_xy)** and **IQ_Inject_Det(_xy)**.

9. Click **Next**.
   The report is opened.
10. Make report adoptions as described in section 4.1.4, page 32.

4.1.4 Adapting the Report

4.1.4.1 General Information

When the sequences are copied from the CD, the report template is automatically copied to the corresponding directory and linked as preferred report to all test sequence templates. The report template consists of the following seven pages:

Page Name	Description
General	This page specifies the devices, test information (see section below) and limits. The limits are listed in column H, starting with line 20. You only have to change these limits if you do not want to use the limits recommended by Thermo Fisher Scientific Inc.
Results_Pump	These pages are used for the evaluation of the respective module.
Results_Inj_Det	
Results_UV	
Results_FL	
Results EC	
Audit Trail	

4.1.4.2 Adapting

TIP The instrument parameters are read automatically only if the sequences have already been processed. If you open a report without samples, the values are not read automatically.

Follow these steps

1. To disable the write protection of the report, click **Edit** > **Layout Mode**.
2. On **General** sheet, enter:
   a) Lot numbers
   b) Expiration dates
   c) Names of reviewer and operator

d) Serial numbers in lines O12-O18 (highlighted in light yellow):
Typically, the serial numbers of the instruments are automatically entered into the report. If a serial number field shows "n.a.": Delete the value in the respective field so that the existing **precond.xxx** entry in the Status Line is deleted and enter the serial number into this field.

e) Model types in lines L12-L18 (highlighted in light yellow):
Typically, the model types of the instruments are automatically entered into the report. If a model type field shows "n.a.": Delete the value in the respective field so that the existing **precond.xxx** entry in the Status Line is deleted and enter the model type into this field.

3. If applicable, save the modifications and close the report.

4.1.5 Starting the Checks

TIP The program files included in the HPLC Templates folder do not provide any specific commands that may be required to control third-party modules. If, for this reason, any warnings or error messages occur during program execution, the templates have to be adapted accordingly.

1. If necessary, modify the created sequences (see sections 7.1 and 3.3).

TIP When an autosampler with variable injection volume is tested, the different volumes are not set in the sequence. The programs are different for each sample. They use the following command:
0.000 Inject Volume=20

2. Add the installed checks to the batch list. The order of the checks must match the order of the list given in step 7, section 4.1.3, page 30.

3. To start, click the **Start** button.
For troubleshooting deviating measuring conditions and results, refer to section 7.1, page 72.

4.1.6 Report Evaluation

TIP Before evaluating and signing the results, check that all peaks were detected properly. Otherwise, adapt the peak windows (within the quantification method) accordingly.

You can automatically evaluate the sequences, using the IQ_Report (Instruments_IQ_x_y) report template. If the measuring results are within the specified limits, the text in the

Results of Instrument… section of the respective **Results** page is **Test passed.** There are two possibilities to evaluate and sign the report:

- Paper based
- Electronic signature

TIP To be able to sign a test sequence electronically, the user has to be registered in the Chromeleon user database and has to have the necessary signature privileges. If, for example, a Field Service Engineer has to sign electronically, he has to be added to the Chromeleon user database or a registered substitute has to sign the report. For detailed information about the electronic signature process, see also the *Chromeleon Help*.

Paper based

In the Browser, select **File** > **Batch Report**.

By default, evaluation of the **IQ_Pump(_xy)** and **IQ_Inject_Det(_xy)** sequences is performed using the <UV_VIS_1> signal. If you want to evaluate a Corona detector, select the <CAD_1> channel in the dialog window of the **Batch Report…** function (see section 5.7.4, page 55). For all other sequences, it is not necessary to adapt the channel name.

Electronic signature

1. Select the sequence that you want to evaluate and sign electronically.
2. Click **File** > **Electronic Signature** > **Submit Results…**.
3. Select the channel to be used for evaluation:
   - UV detectors: <UV_VIS_1>
   - Corona detectors: <CAD_1>
4. To start signing the results, click **OK**.
5. Check the signed results and if the results are correct, click **OK**.
6. Enter your signature password and optionally a comment.
7. Click **OK**.
   The names and dates are automatically entered into the line **Submitter / Operator**.
8. Optionally, repeat all steps above for **Review Results…** and / or **Approve Results…**.

4.2 Chromeleon 7

4.2.1 Installation Qualification Procedure

Chromeleon offers standard AutoQ tests that cover tests for HPLC pumps, autosampler, and various detector types. They are optimized for Thermo Scientific modules and are described in chapter 5, page **Fehler! Textmarke nicht definiert.**. For column thermostats or other chromatographic systems, the standard AutoQ tests are not available or optimized. In this case, the seqences and instrument methods have to be created manually according to chapter 5, page **Fehler! Textmarke nicht definiert.** or they have to be adapted accordingly.

A wizard guides you through automated sequence creation. The sequences contain instrument methods that contains all necessary commands. The instrument methods are assigned automatically to the injections to process.

The sequences are added automatically to the queue. The queue is started manually after the Ready Check has been performed. During injection processing, you can observe whether all functions are performed and the data acquired. The audit trail lists all instrument method commands including execution times, feedback from the injector, and name of the injection. After the sequence has been processed, the processed sinjections are evaluated highly automated with a report linked to the sequence templates.

The qualification for a system with pump, autosampler and one detector takes about 1.5 hours.

For more information about the test design, see chapter 5, page **Fehler! Textmarke nicht definiert.**.

4.2.2 Creating Sequence Templates

1. From the Chromeleon 7 Console, select **Tools** > **Instrument Qualification….**
   A wizard guides you through the process of creating sequences.
2. To go to the next step in the procedure, click **Next>**.
3. Select the qualification type **Installation Qualification**.
4. Select an instrument for which you want to conduct the communication test (Installation Qualification).
5. Click **OK**.
   A number of test sequences is displayed, matching the instrument defined in this step and can contain the following sequences (maximum one per block):

Sequence / Order	Necessary Device Property / Additional Requirement
Block 1 **IQ_Pump(_xy)**	The **IQ_Pump(_xy)** sequence tests the flow rate for an analytical HPLC system.
IQ_Pump	**Pump.Flow** and UV/Corona/MS detector
IQ_Pump_RI	**Pump.Flow** and RI detector, but no UV/Corona/MS detector
IQ_Pump_FLD	**Pump.Flow** and FLD detector, but no UV/Corona/MS/RI detector
IQ_Pump_ECD	**Pump.Flow** and ECD detector, but no UV/Corona/MS/RI/FLD detector
Block 2 **IQ_Inject_Det(_xy)**	The **IQ_Inject_Det(_xy)** sequence tests the autosampler. Indirectly, this sequence also tests: • Corona detectors • MS detectors • RI detectors • Basic functions of Chromeleon
IQ_Inject_Det	**Inject** and UV/Corona if the UV detector does not include a LightPipe™ flow cell, but no MS detector
IQ_Inject_Det_MS	**Inject** and MS detector
IQ_Inject_Det_RI	**Inject** and RI detector, but no UV/Corona/MS detector
IQ_Inject_Det_FLD	**Inject** and FLD detector, but no UV/Corona/MS/RI detector
IQ_Inject_ECD	**Inject** and ECD detector, but no UV/Corona/MS/RI/FLD detector
Block 3	
IQ_Inject_Det_CAD	**Inject** and Corona detector if the UV detector includes a LightPipe flow cell
Block 4	
IQ_UV_Det	This sequence tests a UV detector with variable wavelength. **Wavelength** <UV_VIS_1>(1)
Block 5	
IQ_Inject_Det_RI	**Inject** and RI and UV or Corona detector
Block 6	
IQ_FL_Det	This sequence tests a fluorescence detector. **Emission wavelength** <Emission_1>(1)
Block 7	
IQ_EC_Det	This sequence tests an electrochemical detector. **Potential** <ECDRS_1>(1)
Block 8	
IQ_FC	This sequence tests a fraction collector.

(1) The device or channel name (marked by angle brackets <>) can be selected freely and may deviate from the default value. The created sequences and their instrument methods are adapted automatically to the used device and channel names.

6. Select the sequence(s) to be added.

TIP For systems with a non-Chromeleon controlled detector, it is sufficient to run the test sequences **IQ_Pump(_xy)** and **IQ_Inject_Det(_xy)**.

7. Select a directory where all qualification sequences are to be created and stored (default directory: ChromeleonLocal/Instrument Data/<Instrument Name>/Qualification/IQ <dd mmm yyyy>).

8. Click **OK**.
   All selected qualification sequences are automatically added to the instrument queue.

4.2.3 Starting the Qualification Tests

TIP The instrument method files included in the HPLC Templates folder do not provide any specific commands that may be required for control of third-party modules. If, for this reason, any warnings or error messages occur during instrument method execution, the templates have to be adapted accordingly.

1. If necessary, modify the created sequences (see sections 7.1 and 3.3).

TIP When an autosampler with variable injection volume is tested, the different volumes are not set in the injection list of the sequence. The instrument methods are different for each injection. They use the following command:
0.000 Inject Volume=20

2. To start the qualification sequences, click the **Start** button on the **Queue** tab of the ePanel.
   For troubleshooting deviating measuring conditions and results, refer to section 7.1, page 72.

4.2.4 Adapting the Report

4.2.4.1 Report Structure

The template consists of the following nine pages:

Page Name	Description
General	This page specifies the devices, test information (see section below) and limits. The limits are listed in column H, starting with line 23. You only have to change these limits if you do not want to use the limits recommended by Thermo Fisher Scientific Inc.
Results_Pump	These pages are used for the evaluation of the respective module. Remark: The result for the fraction collector has to be entered manually on **Results_FC** sheet. All other results are evaluated automatically.
Results_Inj_Det	
Results_UV	
Results_FL	
Results EC	
Results MS	
Results_FC	
Audit Trail	

4.2.4.2 Adapting

TIP The instrument parameters are read automatically only if the sequences have already been processed. If you open a report without samples, the values are not read automatically.

Follow these steps

1. Following cells should be unlocked by default. Otherwise, remove the protection of the **General** sheet via the **Home** ribbon and **Protection** group.

2. On **General** sheet, enter:
   a) Batch numbers

   b) Expiration dates

   c) Names of reviewer and operator

   d) Enter the serial numbers of the instruments into lines O12-O20 (highlighted in light yellow):
   Typically, they are automatically entered into the report. If a serial number or model type field shows "n.a.": Delete the value in the respective field so that the

existing **precond.xxx** entry in the Status Line is deleted and enter the serial number into this field.

e) Enter the model types of the instruments into lines L12-L20 (highlighted in light yellow):
Typically, they are automatically entered into the report. If a model type field shows "n.a.": Delete the value in the respective field so that the existing **precond.xxx** entry in the Status Line is deleted and enter the model type into this field.

TIP Notes regarding to the installation qualification can be documented on the individual module sheet of the report below the **Notes** section (field B78). If any remark is entered, remove the text **No remarks**.

3. If applicable, enable the protection of the **General** sheet.
4. If applicable, save the modifications and close the report.

4.2.5 Report Evaluation

TIP Before evaluating and signing the results, check that all peaks were detected properly. Otherwise, adapt the peak windows (within the processing method) accordingly.

You can automatically evaluate the sequences, using the IQ_Report (Instruments_IQ_x_y) report template. If the measuring results are within the specified limits, the text in the **Results of Instrument…** section of the respective **Results** page is **Test passed.** There are two possibilities to evaluate and sign the report:

- Paper based
- Electronic signature

TIP To be able to sign a test sequence electronically, the user has to be registered in the Chromeleon user database and has to have the necessary signature privileges. If, for example, a Field Service Engineer has to sign electronically, he has to be added to the Chromeleon user database or a registered substitute has to sign the report. For detailed information about the electronic signature process, see also the *Chromeleon Help*.

Paper based

In the Chromeleon 7 Console (Data category), click **Print Report…** on the context menu. By default, evaluation of the **IQ_Pump** and **IQ_Inject_Det** sequences is performed using the <UV_VIS_1> signal. If you want to evaluate a Corona detector, select the <CAD_1> channel in the **Print** dialog of the **Print Report…** function (see section 5.7.4, page 55). If

you want to evaluate an MS detector, select the <MS Quantitation> channel in the **Print** dialog of the **Print Report…** function (see section 5.10.4, page 61). For all other sequences, it is not necessary to adapt the channel name.

Electronic signature

1. Select the sequence that you want to evaluate and sign electronically.
2. On the **Sequence Control** toolbar, click **Submit**.
3. Select the channel to be used for evaluation:
   - UV detectors: <UV_VIS_1>
   - Corona detectors: <CAD_1>
   - MS detectors: <MS Quantitation>
4. To create an electronic report, click **Finish**.
5. Enter your signature password and optionally a comment.
6. Click **OK**.
   The names and dates are automatically entered into the line **Submitter / Operator**.
7. Optionally, repeat all steps above for **Review Results…** and / or **Approve Results…**.

5 HPLC Systems – Test Design and Hints for Manual Test Execution

5.1 Introduction

This section describes the test design (basic measuring conditions, test procedure and the evaluation) for the different types of chromatography systems. The test design is implemented in the standard AutoQ checks and / or should be taken into account to perform the checks manually.

The sections *Basic Measuring Conditions* list the basic chromatographic parameters and their set values. For all other parameters, accept the values suggested by the wizard which ensures that the program includes all program steps required for the connected modules and that the syntax is correct. With the given basic measuring conditions, the expected measuring results should be archieved.

The test procedure lists the control parameters that are changed to test the functionality of the respective module.

5.2 Column Thermostats

TIP This test is not covered by the standard AutoQ checks. Perform the test manually and document the results on the form IQ_Report_Comm_test.docx.

5.2.1 Overview

Only those column thermostats are checked that are controlled by Chromeleon via the corresponding device driver. The following basic function is checked:

Setting the temperature

5.2.2 Procedure

1. Open a Chromeleon control panel.

1. Set three different temperatures for the column thermostat that are distributed over the operating range of the module.
2. If possible, verify the temperature settings on the thermostat display.
   The temperature parameters are logged in the audit trail.
3. On the IQ_Report_Comm_Test form, document (e.g. via screenshots) that the test has been performed.

5.3 Pumps

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.3.1 Overview

Only those pumps are checked that are controlled by Chromeleon via the corresponding device driver. The following basic functions are checked:

- Setting the flow rate
- Setting the pressure limits

5.3.2 Setting the Flow Rate

5.3.2.1 Procedure

A sequence is created that contains three samples.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Pump(_xy)** sequence, which contains the required samples.

If the IQ_Pump(_xy) sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

In both cases, the flow rate is varied as follows, based on the basic setting f_B (analytical example: 0.6 mL/min).

Portion of Basic Setting	Flow Rate (Analytical Example)
4/3	0.8 mL/min
f_B	0.6 mL/min
2/3	0.4 mL/min

5.3.2.2 Evaluation

For all three samples, the retention time t_r is evaluated for up to two peaks. The following three retention times will result:

- $t_{r(1)}$ for flow f_1 = 2/3 x f_B
- $t_{r(2)}$ for flow f_2 = f_B
- $t_{r(3)}$ for flow f_3 = 4/3 x f_B

The following applies:

$$f = V / t_r$$

$$t_r = m(1/f)$$

with:

Parameter	Description
f	Flow rate
V	Volume
t_r	Retention time
m	Slope

1. To calculate the slope m of this line, insert the three measured values in the two equations below:

$$m_1 = \frac{t_{r(1)} - t_{r(2)}}{1/f_1 - 1/f_2}$$

$$m_2 = \frac{t_{r(2)} - t_{r(3)}}{1/f_2 - 1/f_3}$$

2. Compare the m_1 and m_2 values.
   With a working pump, the deviation between these values must not exceed ± 10%.
3. If you use the flow rates mentioned above, use the equations below to calculate the slope m:

$$m_1 = 2 f_B (t_{r(1)} - t_{r(2)})$$

$$m_2 = 4 f_B (t_{r(2)} - t_{r(3)})$$

   Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).
4. When performing the evaluation using a Corona or an MS detector, set the signal (<CAD_1> or <MS Quantitation>) in the **Batch Report** dialog window (see section 5.7.4, page 55 or section 5.10.4, page 61).

5.3.3 Setting the Pressure Limits

1. On a Chromeleon control panel, set the pressure limits for the pump module.
2. If possible, verify the selected limits on the pump display.
   The pressure limits are logged in the audit trail.
3. Set the upper pressure limit to a value lower than the current system pressure.
   - Expectation 1: The pump is turned off automatically, and the corresponding action is logged in the audit trail.
   - Expectation 2: The pump flow is reduced automatically until the pressure is below the upper pressure limit. (Remark: The flow may be decreased to 0 mL/min if necessary).
4. Check whether the pump fulfils the expectations:
   - All Thermo Scientific pumps: Expectation 1 must be fulfilled
   - All other pumps: Either expectation 1 or 2 has to be fulfilled (as specified by the corresponding manufacturer).
5. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.4 Autosamplers

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.4.1 Autosamplers Controlled by Chromeleon

5.4.1.1 Overview

Only those autosamplers are checked that are controlled by Chromeleon via the corresponding device driver. The following basic functions are checked:

- Selecting the sample position
- Selecting the sample volume
- Feedback on injection

5.4.1.2 Procedure

Usually, the Chromeleon command used to select the sample position and the sample volume is a command combining the position and volume properties.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instrument Qualification Wizard copies the **IQ_Inject_Det(_xy)** sequence, which contains the required samples.

If the IQ_Inject_Det(_xy) sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

In both cases, the injection volume is varied, based on the basic settings.

Example for the variation of the injection volume (volumes may deviate depending on the autosampler to be qualified and the used detector type):

Portion of Basic Setting	Injection Volume (Analytical Example with UV Detector)
Basic setting	10 µL
1.5 times the basic setting	15 µL
2 times the basic setting	20 µL

5.4.1.3 Evaluation

1. If possible, verify the sample position and volume settings on the autosampler display. The injection parameters are logged in the audit trail.
2. Verify that the injection has been performed by checking the corresponding entry in the audit trail.
3. Evaluate the peak area of up to two peaks for all three samples.
   - The peak areas and the injection volumes must be proportional to each other.
   - For a working autosampler, the deviations from the setpoint must not exceed ± 5%. For qualification using a Corona or MS detector, the limit is ± 30%.

   Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).
4. When performing the evaluation using a Corona detector, set the signal (<CAD_1>) in the **Batch Report** dialog window (see section 5.7.4, page 55 or section 5.10.4, page 61).
5. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.4.2 Autosamplers Not Controlled by Chromeleon

5.4.2.1 Overview

If your HPLC system contains a manual injection valve or a non-controlled autosampler, Chromeleon waits for the feedback from the injector after the injection command has been performed. The following basic function is checked:

Injection feedback to Chromeleon

5.4.2.2 Procedure

A sequence is created that contains three samples and one program for all samples.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Inject_Det(_xy)** sequence, which contains the required samples.

If the IQ_Inject_Det(_xy) sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

The injection volume is identical for the three samples. Chromeleon starts sample processing. When the system receives the injection command, Hold mode is enabled. The system performs the injection, cancels the Hold mode, and then starts data acquisition. After having processed the sample, Chromeleon automatically starts the next sample and processes it in the same way.

5.4.2.3 Evaluation

1. Verify that the injection has been performed by checking the corresponding entry in the audit trail.
2. Evaluate the peak area of up to two peaks for each of the three samples.
   - For a working autosampler or injection valve, the peak area deviations of the three samples must not exceed ± 5%.
   - For qualification using a Corona or an MS detector, the limit is ± 30%.

   Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).
3. When performing the evaluation using a Corona detector, set the signal (<CAD_1>) in the **Batch Report** dialog window (see section 5.7.4, page 55 or section 5.10.4, page 61).
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.5 UV Detectors with Variable Wavelength

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.5.1 Overview

Only those detectors are checked that are controlled by Chromeleon via the corresponding device driver. The following basic functions are checked:

- Setting the wavelength
- Autozero command

5.5.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions:

Parameter	Setting
Eluent	Water/methanol (60/40 Vol.%)
Flow rate	0.600 mL/min
Program run time	8 minutes
Injection volume	10 µL
Sample position	Vanquish: R:A1 UltiMate: RA1 Any autosampler: 1
Wavelength of the UV detector	272 nm
Data collection rate for the UV signal	UltiMate: 5 Hz Accela: 10 Hz
Signal filter for the UV signal	1 s (ResponseTime – depends on the detector)
Data acquisition rate for the Corona signal	10 Hz
Column thermostat temperature (optional)	25 °C

Expected measuring results

The measuring conditions return a signal with two peaks for the UV detector. The peak height should be between 50 and 600 mAU.

If necessary, adapt the range settings or other signal-relevant parameters.

5.5.3 Setting the Wavelength

5.5.3.1 Procedure

A sequence is created that contains two samples.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_UV_Det** sequence, which contains the required samples.

If the IQ_UV_Det sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

In both cases, the wavelength is varied as follows, based on the basic settings:

Parameter	Setting
Wavelength 1 (basic)	272 nm
Wavelength 2	290 nm

If you use a substance other than caffeine, adapt the settings accordingly. Select two wavelengths for which you expect clearly distinct signal heights.

5.5.3.2 Evaluation

Signal evaluation depends on the standard used:

- If caffeine has been used, the signal height of the caffeine peak is evaluated for both samples. The signal height ratio is as follows:

  $$Ratio = \frac{Signal\ Height(272nm)}{Signal\ Height(290nm)}$$

  For a working detector, the ratio must be between 1.5 and 10.0. Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).

- If a substance other than caffeine has been used, it is only possible to perform qualitative signal evaluation.

Follow these steps

1. If possible, verify the wavelength setting on the detector display.
   The wavelength parameters are logged in the audit trail.
2. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.5.4 Autozero Command

1. Execute injection from a Chromeleon control panel.
2. Select the **Autozero** command on the control panel while a substance elutes.
3. On the Chromeleon signal plot, verify that the signal changes as expected.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.6 FL Detectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.6.1 Overview

Only those FL detectors are checked that are controlled by Chromeleon via the corresponding device driver. The following basic functions are checked:

- Setting the excitation and emission wavelength
- Autozero command

5.6.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions:

Parameter	Setting
Eluent	100% methanol
Flow rate	0.600 mL/min
Program run time	8 minutes
Injection volume	10 µL
Sample position	Vanquish: R:A2 UltiMate: RA2 Any autosampler: 2
Excitation wavelength	335 nm
Emission wavelength	390 nm

Parameter	Setting
Data acquisition rate for the signal	5 Hz
Step for the fluorescence signal	0.2 s
Column thermostat temperature (optional)	25 °C

Expected measuring results – Summit RF Series

The conditions return a signal with one peak (pyrene) for the FL detector of the Summit RF series for which the peak height should be between 50 and 700 mV.

If necessary, adapt the range settings or other signal-relevant parameters.

Expected measuring results – UltiMate and Vanquish Series

For the FL detector of the UltiMate and Vanquish series, you receive a signal with one peak (pyrene) with an expected peak height between 2 million and 30 million counts.

If necessary, adapt the range settings or other signal-relevant parameters.

5.6.3 Setting the Excitation and Emission Wavelength

5.6.3.1 Procedure

For verification of the two parameters, a sequence with four samples is created.

If your system includes an FL detector and a UV and/or a Corona detector, you will be asked during processing of sample 1 to change the eluent and to flush the system with methanol. Follow the instructions provided by Chromeleon.

If your system includes only an FL or RI detector, this step is skipped automatically.

If you use the sequence templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_FL_Det** sequence, which contains the required samples.

The excitation (λEx) and emission (λEm) wavelength is varied as follows, based on the basic settings:

- Sample 1: Change eluent, if necessary [water / methanol (60/40 Vol. %) to methanol (100 Vol. %)]
- Sample 2 (basic setting): λ_{Ex} = 335 nm / λ_{Em} = 390 nm

- Sample 3 (variance (λ_{Ex}): λ_{Ex} = 350 nm / λ_{Em} = 390 nm
- Sample 4 (variance (λ_{Em}): λ_{Ex} = 335 nm / λ_{Em} = 430 nm

If the IQ_FL_Det sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself. For the excitation (λEx) and emission (λEm) wavelength variations, see above.

If a substance other than pyrene has been used, you may have to adapt the settings accordingly. Select two excitation and emission wavelengths for which you expect clearly distinct signal heights.

5.6.3.2 *Evaluation*

Signal evaluation depends on the standard used:

- If pyrene was used, the signal height of the pyrene peak will be evaluated for all samples. The signal height ratio [Ratio(λ_{Ex}) or Ratio(λ_{Em})] is as follows:

$$Ratio(\lambda_{\mathrm{Ex}}) = \frac{Signal\ \ height(\lambda_{\mathrm{Ex}} = 335\ \mathrm{nm} / \lambda_{Em} = 390\ \mathrm{nm})}{Signal\ \ height(\lambda_{\mathrm{Ex}} = 350\ \mathrm{nm} / \lambda_{Em} = 390\ \mathrm{nm})}$$

$$Ratio(\lambda_{\mathrm{Em}}) = \frac{Signal\ \ height(\lambda_{\mathrm{Ex}} = 335\ \mathrm{nm} / \lambda_{Em} = 390\ \mathrm{nm})}{Signal\ \ height(\lambda_{\mathrm{Ex}} = 335\ \mathrm{nm} / \lambda_{Em} = 430\ \mathrm{nm})}$$

  For a working detector, both ratios must be larger than 3. Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).
- If a substance other than pyrene has been used, it is only possible to perform qualitative signal evaluation.

Follow these steps

1. If possible, verify the excitation and emission wavelength setting on the detector display.
   The wavelength parameters are logged in the audit trail.
2. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.6.4 Autozero Command

1. Execute injection from a Chromeleon control panel.
2. Select the **Autozero** command on the control panel while a substance elutes.
3. On the Chromeleon signal plot, verify that the signal changes as expected.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.7 Corona Detectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.7.1 Overview

Only those Corona detectors are checked that are controlled by Chromeleon via the corresponding device driver. The following basic functions are checked:

Change of the peak area depending on the substance concentration.

TIP A system that includes only a Corona detector as detector device can also be used to qualify a pump (see section 5.3, page 44) or autosampler (see section 5.4, page 46).

5.7.2 Basic Measuring Conditions / Expected Measuring Results

Use the measuring conditions listed in section 5.5, page 49.

Expected measuring results

For the Corona detector you will also receive a signal with two peaks. Only the second peak (caffeine), however, will be evaluated. The height of the second peak should be between 1 and 50 pA.

5.7.3 Procedure

The sequence **IQ_Inject_Det** is used for evaluation of a Corona detector with one exception: If the HPLC system includes a Vanquish VH-D10-A UV detector with a LightPipe™ flow cell, the sequence **IQ_Inject_Det_CAD** is used.

If you use the sequence templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Inject_Det(_CAD)** sequence, which contains the required samples.

If the IQ_Inject_Det(_CAD) sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

5.7.4 Evaluation

Perform the evaluation as described in section 5.4.1.3, page 47.

NOTICE Only the caffeine peak is used for evaluation. If you use the sequence **IQ_Inject_Det** and you perform the evaluation using the **Batch Report…** file command, make sure that the Corona detector channel (default channel name: <CAD_1>) is selected in the Batch Report dialog window in the **With selected channel** box. If the sequence **IQ_Inject_Det_CAD** is used, the last step is not necessary.

5.8 Electrochemical Detectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.8.1 Overview

Only those electrochemical detectors are checked that are controlled by Chromeleon via the corresponding device driver. In direct current mode, the following basic functions are checked:

- Setting the potential (using channel 1)
- Functional test of channel 2 (if available)
- Autozero command

5.8.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions to check the coulometric cells (6011RS, 6020RS) and amperometric cells (6041RS) with glassy carbon working (GC) or boron-doped diamond (BDD) electrode:

Parameter	Setting
Eluent	100% TEST mobile phase
Flow rate	0.600 mL/min
Program run time	12 minutes
Injection volume	10 µL

Parameter	Setting
Vial position	Vanquish: R:A3 UltiMate: RA3 Any autosampler: 3
Potential channel 1	
Coulometric cells (6011RS, 6020RS) and amperometric cells (6041RS) with glassy carbon working (GC) electrode	+400 mV
Amperometric cells (6041RS) with boron-doped diamond (BDD) working electrode	+800 mV
Potential channel 2 (if available)	+400 mV
Data acquisition rate	10 Hz
Filter constant	5 sec
Gain range (optional)	500 nA
Column thermostat temperature (optional)	35 °C

When qualifying electrochemical cells that have two channels (for example, coulometric cell 6011RS for UltiMate 3000 ECD-3000RS), check both channels. To do so, perform the test described in section 5.8.3, page 57.

NOTICE After changing the cell conditions (potential), equilibrate the cell at least for 5 minutes.

Expected measuring results – EC Detector with Coulometric Cell (6011RS, 6020RS)

The basic measuring conditions return a signal with three peaks (norepinephrine, epinephrine and dopamine) for which the peak heights should be between 30 and 500 nA.

Only applicable for the 6011RS cell: When testing channel 1, the peaks are typically seen on channel 1 and not channel 2. When testing channel 2, the peaks are typically seen on channel 2 and not on channel 1.

If necessary, adapt the range settings or other signal-relevant parameters.

Expected measuring results – EC Detector with Amperometric Cell (6041RS)

Independent of the used working electrode, the basic measuring conditions return a signal with three peaks (norepinephrine, epinephrine and dopamine) for which the peak heights should be between 0.5 and 30 nA.

If necessary, adapt the range settings or other signal-relevant parameters.

5.8.3 Setting the Potential / Functional Test

5.8.3.1 *Procedure*

A sequence with two samples is created for this test.

If you are using the template sequences shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard creates a copy of the sequence **IQ_EC_Det**, which includes the required samples.

If the sequence IQ_EC_Det is not offered or if you are not using the templates, you must create the sequence by yourself.

In both cases, the potential is varied as follows, starting with the basic setting:

Cell	Potential 1 (Basic Setting)	Potential 2
Coulometric cell (6011RS, 6020RS) / amperometric cell (6041RS) with GC electrode		
channel 1	400 mV	-175 mV
channel 2, if available	400 mV	400 mV
Amperometric cell (6041RS) with BDD electrode	800 mV	-175 mV

If you are using a different standard than the catecholamine standard for this test, you may have to adapt the settings. In this case, select two potentials where you expect a noticeable difference regarding signal areas.

5.8.3.2 *Evaluation*

Signal evaluation depends on the standard used:

- If the catecholamine standard was used, the signal areas of the three peaks are evaluated for both samples. The signal area ratio is calculated for all three peaks.

Channel Number	Formula
1	$Ratio_{Min\_Channel1} = \frac{Signal\ area(Potential1)}{Signal\ area(Potential2)}$
2	$Ratio_{Min\_Channel2} = \frac{Signal\ area(Potential2\_Channel2)}{Signal\ area(Potential2\_Channel1)}$

The smallest ratio of each channel must be greater than 10 for a fully functional detector. The test is evaluated in Chromeleon with the IQ_Report created for this test (see chapter 4, page 28).

- If a different standard than the catecholamine standard is used, the signals can only be evaluated qualitatively.

Follow these steps

1. Check the detector display, if applicable, to find out whether the selected potential is set correctly.
   The Audit Trail logs the detection parameters.
2. Use the IQ_Report_Comm_Test.docx form to document this test.

5.8.4 Autozero command

1. Execute injection from a Chromeleon control panel.
2. Select the **Autozero** command on the control panel while a substance elutes.
3. On the Chromeleon signal plot, verify that the signal changes as expected.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.9 RI Detectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.9.1 Overview

Only those RI detectors are checked that are controlled by Chromeleon via the corresponding device driver. The following basic function is checked:

Peak area change depending on the substance concentration.

5.9.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions:

Parameter	Setting
Eluent	100% methanol
Flow rate	0.600 mL/min
Program run time	8 minutes
Injection volume	10 µL
RI detector temperature	35 °C
Polarity	Plus
Baseline shift	0
Data collection rate	5 Hz
Rise time	2 s
Column thermostat temperature (optional)	35 °C

Expected measuring results

These conditions return a signal with one peak (toluene) for the RI detector for which the peak height should be between 50 and 500 µRIU.

If necessary, adapt the range settings or other signal-relevant parameters.

5.9.3 Procedure

For checking an RI detector, the sequence **IQ_Inject_Det_RI** is used. In contrast to the sequence **IQ_Inject_Det**, the sequence **IQ_Inject_Det_RI** contains two additional samples to change the eluent and to rinse the RI detector.

If your system contains an RI and an UV and/or Corona detector, you will be prompted to change the eluent and to switch to methanol during sample 1. Follow the instructions in Chromeleon.

If your system only contains an RI detector, the mentioned step and rinsing the RI detector will be skipped.

If the sequence templates provided by Chromeleon are used (see chapter 4, page 28), the Instruments_IQ Wizard creates a copy of this sequence that contains all necessary samples.

If the sequence IQ_Inject_Det_RI is not offered or if the sequence templates are not used, you have to create the sequence yourself.

5.9.4 Evaluation

Perform the evaluation as described in section 5.4.1.3, page 47.

5.10 MS Detectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

Notice Acceptable results can only be obtained if the detector is properly calibrated before (positive and negative polarity). Before starting the installation qualification, verify that the MS detector is ready for operation according to the instrument operating manual.

5.10.1 Overview

Only the following basic function of Vanquish MS detectors is checked:

Peak area depending on the polarity and mass.

TIP A system that includes only an MS detector as detector device can also be used to qualify a pump (see section 5.3, page 44) or an autosampler (see section 5.4, page 46).

5.10.2 Basic Measuring Conditions / Expected Measuring Results

Use the measuring conditions listed in section 5.5, page 49. In addition, use the following MS settings:

Parameter	Setting
Source	HESI
Source settings	Easy, default settings depend on the set flow rate

Parameter			Setting
Method type			Basic
Scans			
	Uracil		
		Mass	111.0 amu
		Polarity	Negative
		Source CID voltage	20 eV
	Caffeine		
		Mass	195.1 amu
		Polarity	Positive
		Source CID voltage	20 eV
Acquisition rate			
	Min. baseline peak width		3.0 s
	Desired scans per peak		6

Expected measuring results

For the MS detector, you will receive a signal with two peaks. The area of the first peak (uracil) should be above 500 counts*min and the area of the second peak (caffeine) should be above 20000 counts*min (injection volume: 6 µL).

5.10.3 Procedure

The sequence **IQ_Inject_Det_MS** is used for evaluation of an MS detector.

If you use the sequence templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Inject_Det_MS** sequence, which contains the required samples.

If the IQ_Inject_Det_MS sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

The polarity and the mass are varied, as described in the MS scan settings (see section 5.10.2, page 60).

5.10.4 Evaluation

Perform the evaluation as described in section 5.4.1.3, page 47.

5.11 ELS and other Detectors

TIP ELS and other detectors are not covered by the standard AutoQ checks. Perform the test manually and document the results on the form IQ_Report_Comm_test.docx.

5.11.1 Overview

Only those detectors are checked that are controlled by Chromeleon via the corresponding device driver.

If the detector is the only module of the HPLC system, follow the steps described in section 5.4.2, page 48 to check the basic functions of Chromeleon (see section 2.1, page 12).

5.11.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions for an ELS detector:

Parameter	Setting
Eluent	100% water
Flow rate	0.500 mL/min
Program run time	3 minutes
Injection volume	20 µL
Nebulizer temperature	50 °C
Evaporator temperature	90 °C
Gas flow rate	1.6 SLM at 4.1 bar
Step for the ELS signal	0.2 seconds

Expected measuring results – ELS detector

These conditions return a signal with one peak (glucose) for the ELS detector for which the signal height should be between 500 and 1000 mV*s.

If necessary, adapt the range settings or other signal-relevant parameters.

5.11.3 Procedure

1. Open a Chromeleon control panel.
2. Set a control parameter for the detector module.

Typical control parameter influencing the signal is the evaporator temperature (for evaporative light scattering detectors).

3. If possible, verify the selected parameter setting on the detector display.
   The parameters are logged in the audit trail.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

5.12 Fraction Collectors

TIP These tests and their documentation on the form IQ_Report_Comm_test.docx can be omitted when the automatic instrument installation qualification is performed - see also section 1.1.2, page 8.

5.12.1 Overview

Only those fraction collectors are checked that are controlled by Chromeleon via the corresponding device driver. The following basic function is checked:

Time controlled solvent collection in a 1.5 mL standard vial.

5.12.2 Basic Measuring Conditions / Expected Measuring Results

Use the following basic measuring conditions:

Parameter	Setting
Eluent	100% solvent A (as already used, e.g., water/methanol (60/40 Vol.%) or 100% methanol)
Flow rate	0.600 mL/min
Program run time	1 minute
Injection type	Blank
Fraction collection	Collect by time (Collection period: 60 s; start time: 0.1 min, end time: 0.9 min)
Collection time frame	Start time: 0.1 min, end time: 0.9 min, flush: No
Thermostat temperature	Off
Wash mode	NoWash
Rinse mode	NoRinse
Collect path mode	Horizontal
Needle positioning mode	InVial

Expected measuring results

These conditions return a vial (R:A1) filled with collected solvent for which the volume should be between 0.1 and 0.9 mL. Document the result in the IQ report on sheet **Results_FC** – results table.

TIP If available, the volume can also be measured with a precision pipette (1 mL). The expected volume should be between 0.4 and 0.6 mL (the limits can be adapted on the **General** page of the report – see chapter 4.2.4.1, page 38).

If necessary, adapt relevant parameters.

5.12.3 Procedure

For checking a fraction collector, the sequence **IQ_FC** is used.

If the sequence templates provided by Chromeleon are used (see chapter 4, page 28), the Instruments_IQ Wizard creates a copy of this sequence that contains all necessary samples.

If the sequence **IQ_FC** *is not offered or if the sequence templates are not used*, you have to create the sequence yourself.

6 GC Systems

6.1 Checking all Systems

TIP Dependent on the system, these tests are not necessarily covered by the standard AutoQ checks. If the Chromeleon does not offer any sequence templates, perform the test manually and document the results on the form IQ_Report_Comm_test.docx.

The separation conditions for GC applications largely depend on the used columns. The user must make the appropriate test mixture and the separation method available. Make sure that the application fulfills the conditions described in section 3.4, page 27.

NOTE The used columns and detectors influence the operating range of the GC systems. That is why the user must provide information about the operating range. Adapt the parameters as necessary.

6.1.1 Procedure

In each test run, as many modules as possible are tested. This is to optimize the test effort for GC systems.

1. Create a sequence that contains three samples for each combination to be tested.
2. Create a program and an evaluation method that are both used for all three samples.
3. Create a separate sequence for each detector that is controlled by Chromeleon.
4. Create a separate sequence for each of the configured inlet valves (see also section 6.4, page 67).
5. Open a Chromeleon control panel and set some parameters on the GC.
6. If possible, verify the settings on the display of the GC module.

6.1.2 Evaluation

1. Evaluate the peak area of up to two peaks for each of the three samples.
   The peak area deviations of the three samples must not exceed ± 5%. Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).
2. On the IQ_Report_Comm_Test form, document that the test has been performed.

6.2 GC Column Thermostats

Only those column thermostats are checked that are controlled by Chromeleon via the respective driver.

1. In addition to the check described in section 6.1, page 66, set three different temperature values for the column thermostat (observe the operating temperature range of the column used).
2. If possible, verify the selected temperatures on the display of the GC.
   The temperature parameters are logged in the audit trail.
3. Check each column thermostat of the GC system.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

6.3 GC Detectors

Only those GC detectors are checked that are controlled by Chromeleon via the respective driver.

1. In addition to the check described in section 6.1, page 66, open a control panel and set three different temperature values for the detector heating.
2. If possible, verify the selected temperatures on the display of the GC.
   The temperature parameters are logged in the audit trail.
3. Check each detector of the GC system.
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

6.4 Temperature Settings on the Sample Inlet Valve

To check the temperature settings:

1. Open a control panel.
2. Set three different temperature values for the sample inlet valve.
3. If possible, verify the selected temperature settings on the display of the GC.
   The temperature parameters are logged in the audit trail.
4. Check each sample inlet valve of the GC system.
5. On the IQ_Report_Comm_Test form, document that the test has been performed.

6.5 GC Sample Injection

For all injection types, the feedback on injection is checked. This manual distinguishes between controlled autosamplers (see section 6.5.1, page 68) and non-controlled (see section 6.5.2, page 69) autosamplers.

6.5.1 Controlled GC Autosamplers

6.5.1.1 Overview

Only those GC autosamplers are checked that are controlled by Chromeleon via the respective device driver. The following basic functions are checked:

- Selecting the sample position
- Selecting the sample volume
- Feedback on injection

6.5.1.2 Procedure

Usually, the Chromeleon command to select the sample position and the sample volume is a command combining the position and volume properties. A sequence is created that contains three samples.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Inject_Det** sequence, which contains the required samples.

If you do not use the sequence templates, you have to create the sequence yourself.

In both cases, the injection volume is varied as follows, based on the basic settings (see section 6.1, page 66):

- Basic setting (Example: 1 µL)
- 2 times the basic setting (Example: 2 µL)
- 4 times the basic setting (Example: 4 µL)

The three samples are injected from different positions.

6.5.1.3 *Evaluation*

1. If possible, verify the sample position and volume settings on the autosampler display. The injection parameters are logged in the audit trail.
2. Verify that the injection has been made by verifying the corresponding entry in the audit trail.
3. Evaluate the peak area of up to two peaks for all three samples.
   - The peak areas and injection volumes must be proportional to each other.
   - With a working autosampler, the deviations from the setpoint must not exceed ± 5%.

   Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28)
4. On the IQ_Report_Comm_Test form, document that the test has been performed.

6.5.2 Non-Controlled Autosamplers

6.5.2.1 *Overview*

If your GC system contains a manual injection valve or a non-controlled autosampler, Chromeleon waits for the feedback from the injector after the injection command has been performed. The following basic function is checked:

Injection feedback to Chromeleon

6.5.2.2 Procedure

A sequence is created that contains three samples and one program for all samples.

If you use the templates shipped with Chromeleon (see chapter 4, page 28), the Instruments_IQ Wizard copies the **IQ_Inject_Det** sequence, which contains the required samples.

If the IQ_Inject_Det sequence is not offered or if you do not use the sequence templates, you have to create the sequence yourself.

The injection volume is identical for the three samples. Chromeleon starts sample processing. When the system receives the injection command, Hold mode is enabled. The system performs the injection, cancels the Hold mode, and then starts data acquisition. After having processed the sample, Chromeleon automatically starts the next sample and processes it in the same way.

6.5.2.3 Evaluation

1. Verify that the injection has been performed by checking the corresponding entry in the audit trail.
2. Evaluate the peak area of up to two peaks for each of the three samples.
   For a working autosampler, the peak area deviations of the three samples must not exceed ± 5%.

   Chromeleon evaluates the test based on the IQ_Report (see chapter 4, page 28).

7 Appendix

7.1 Troubleshooting

The table below contains tips for deviating measuring conditions and results.

Problem	Cause	Remedial Action
Peak flattening and/or different injection volumes	Using a higher injection volume may result in that you leave the linear range of the detector or overload the column. As a result, the peak flattens.	Dilute the used standard by the factor by which the injection volume was increased.
	Another reason for peak flattening may be an unfavorable ratio between the absorption path length and the flow cell volume.	Increase the flow rate.
Peaks are too low or too high	The signal parameters of detectors whose signals are transmitted to Chromeleon as analog signals very often determine the gain factor at the module's analog output.	Optimize these parameters if the signals are too low or too high. Verify that the gain factor is set correctly in the Server Configuration program. If the signals are too high, you may as well dilute the used standard. If you still do not achieve the signal heights described above, refer to the tips for preparative HPLC systems in section 3.3, page 26.
Peaks do not appear or bad separation	Large dead volumes in the system may result in that the two substances do not elute during the runtime of the program. Composition of eluent Water/methanol (60/40 Vol.%) is not correct.	Extend the runtime. A runtime of 20 minutes should be sufficient for the two peaks to appear. If the signals still do not appear, replace the column by a capillary coupling. This is to exclude the column as source of trouble. Besides, refer to the tips in the table cell above when the signals are too low. Check and / or exchange the eluent.

7.2 IQ_Report_Comm_Test_x_y.docx form

For a filled-in IQ_Report_Comm_Test_5.7.docx form (with an example of an Agilent instrument), see the attached pages below.

A template is available as electronic document.

TIP On the first page, enter the instrument details, test case(s) and test result(s). On the attachment sheet, enter the sceenshots.

Installation Qualification: Communication Test Report

Description of the Tested System

Name of CM Server:	CSFKST		
Name of Timebase:	HP 1100		
Installed Instruments:			
Name of Instrument:	**Supplier:**	**Model:**	**Serial Number:**
Pump	Agilent	G1312A (Binary Pump)	123456
Sampler	Agilent	G1313A	123456
ColumnThermostat	Agilent	G1316A	123456
UV	Agilent	G1314A VWL	123456

Test Description

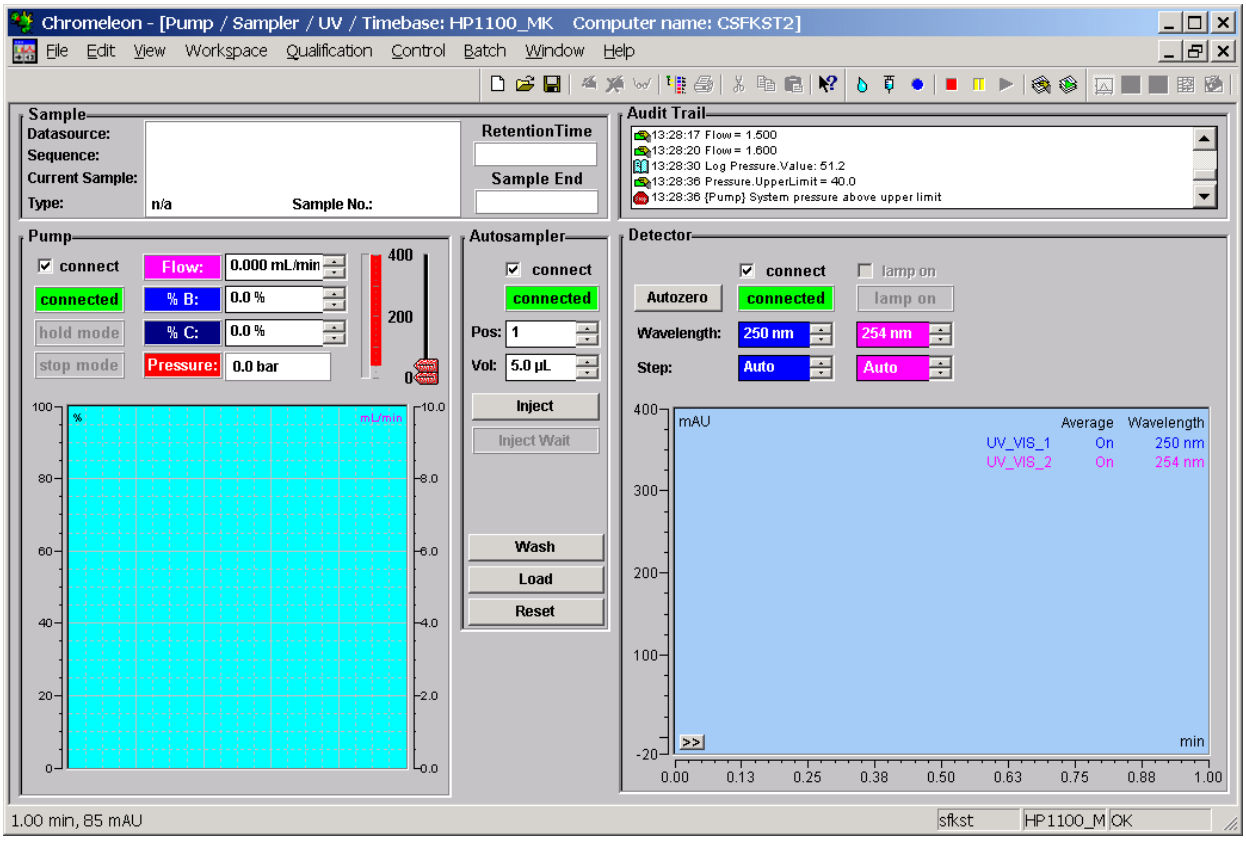
Tested Instrument:	Pump		
Tested Functionality:	Pressure Limits		
Test Case:	System pressure 50 bar. Set upper pressure limit to 40 bar.		
Test Result:	New settings are logged in the Audit Trail. Pump stopped flow. Audittrail: [] 13:28:30 Log Pressure.Value: 51.2 [] 13:28:36 Pressure.UpperLimit = 40.0 [Abort] 13:28:36 {Pump} System pressure above upper limit		
X Test Passed		☐ **Test Failed**	**X Attachment(s)**

Service Engineer	2013-02-20	*Customer*
Operator	Date of Performance	Reviewer

Installation Qualification: Communication Test Report - Attachment

Description of Tested System

Name of CM Server:	CSFKST
Name of Timebase:	HP 1100
Tested Instrument:	Pump
Tested Functionality:	Pressure Limits



Service Engineer	2013-02-20	*Customer*
Operator	Date of Performance	Reviewer