thermoscientific

Thermo Scientific Chromeleon

Operational Qualification/ Performance Qualification for HPLC Instruments

Operating Instructions

4828.3250A Revision 9.5 • January 2020

ThermoFisher SCIENTIFIC

Trademarks

PEEK is a trademark of Victrex PLC.

All other trademarks are property of Thermo Fisher Scientific and its subsidiaries.

This manual is provided "as is." Every effort has been made to supply complete and accurate information and all technical specifications have been developed with the utmost care. However, Thermo Fisher Scientific assumes no responsibility and cannot be held liable for any errors, omissions, damage, or loss that might result from any use of this manual or the information contained therein. We appreciate your help in eliminating any errors that may appear in this document.

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

Contents

1 Using this Manual

1.1 About this Manual

1.1.1 Chapters and Sections Overview

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

Section	Content
2 Introduction	Overview of the different qualification terms.
3 Standard Test Procedures	Contains all descriptions about how to perform the standard AutoQ™ checks. Follow the instructions in this section in the given order. Descriptions for modules that are not installed in the system can be skipped.
Subsections 3.1 to 3.5	• Required parts • All preparation steps • All system configuration steps
Subsections 3.6 to 3.8	Contains descriptions about the following tasks using Chromeleon™ 6.80: • Qualification template creation • Test execution • Test evaluation When using Chromeleon 7, skip these sections and use the descriptions in chapter 5.
Subsections 3.9 and 3.10	• Repeating individual checks • Known restrictions
4 Special Test Procedures for Individual Modules	Contains descriptions about how to perform special tests that require a different test setup than the standard AutoQ checks. Descriptions for modules that are not installed in the system can be skipped.
5 Chromeleon 7	Contains descriptions about the following tasks using Chromeleon 7: • Qualification template creation • Test execution • Test evaluation When using Chromeleon 6.80, skip these sections and use the descriptions in sections 3.6 to 3.8.
6 Appendix	Contains background information about supported instruments, overview of checks and limits, and about the test design. It also contains example instrument methods and reports.

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

1.1.2 Described Sequence Template Version

The descriptions in this manual refer to sequence templates version 9.5 or later. Changes on sequence templates with a version later than 9.5 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.

1.2 Conventions

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

1.2.1 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 damage to the instrument or invalid test results.

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

1.2.2 Naming Conventions

LightPipe Flow Cells

If not stated otherwise, the term LightPipe™ flow cell comprises both LightPipe flow cells, the 10 mm and the 60 mm path length versions.

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"	Text between quotation marks: Messages that appear on the screen
|	Separates options

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

2 Introduction

2.1 Background

The increasing number of standards and official regulations provide evidence that it is extremely important to monitor the used instruments and to make sure that they work as intended if you want to achieve reliable analytical results. To make the results transparent, quality management according to ISO 9000 and following monitors and documents the quality of the equipment at different times.

This is the purpose of the Operational Qualification (OQ) and Performance Qualification (PQ) procedures described in the sections below. This manual also lists modules that do not require any re-calibration.

2.2 Defining the Terms

The definitions given in the sections below are according to "The development and application of guidance on equipment qualification of analytical instruments" of P. Bedson and M. Sargent [Accred. Qual. Assur. (1996) 1: 265 - 274].

2.2.1 Operational Qualification

The purpose of Operational Qualification (OQ) is to prove and document that an analytical system functions according to its operating specification while the specific environmental conditions are taken into account.

In module specifications, suppliers must therefore define exactly the conditions that must be observed. As conditions vary, for example, varying ambient temperatures, higher limits must be used.

Usually, OQ is only performed directly after a new device has been installed.

2.2.2 Performance Qualification

The purpose of Performance Qualification (PQ) is to prove and document that an analytical system functions according to a specification that is suitable for the system's routine operation. As a system is subject to wear when being operated, it may happen that the supplier's specification no longer be met. This means that the procedures used for PQ are the same as those used for OQ, which simplifies the handling, but the tolerances for the PQ are less strict than those for the OQ. If required (for example, if stricter requirements apply for routine analysis), users can adapt the limits. However, the adapted limits must not be narrower than the OQ limits. PQ is usually performed after repair or after regular system service procedures have been performed.

2.2.3 System Suitability Test

The purpose of the System Suitability Test (SST) is to prove and document that the necessary limits are met for a specific measuring application. The specific conditions required for that application, e.g., solvents, column material, and temperature, must be taken into account. The check can be developed by the supplier on request. However, it is not part of the test procedures below.

Do not use SST limits that are more restrictive than the limits used for PQ.

3 Standard Test Procedures

3.1 Requirements and Preparations

3.1.1 Software Requirements

Qualification requires a Chromeleon™ version ≥ 6.80 SR11 or ≥ 7.1 SR1.

The following combinations of versions are supported for qualifying an Agilent HPLC system that is controlled by Agilent Instrument Control (ICF) and the Chromeleon "LC System" driver:

OQ/PQ Version	CM6 Version	CM7 Version	Agilent ICF version
8.0 – 8.4	6.80 SR11 – 6.80 SR11d	7.1 SR1 – 7.1 SR2 MUa	A.01.03
8.5 or later	6.80 SR12 or later	7.1 SR2 MUb or later	A.01.05 or later

3.1.2 Parts Required

If not mentioned otherwise, the materials in the table are needed for the qualification.

Material	Remarks	Part no.	Quantity
Performance Qualification Kit	Contains caffeine standards. For further details, see section 3.1.2.1 Performance Qualification Kit, page 17.	4832.5000A	1
Column Compartment PQ Kit	For details, see section 3.1.2.2 Column Compartment PQ Kit, page 18.	6732.0010	1
Temperature Sensor Type K	Needed for qualification of an ACC-3000(T) or ECD-3000RS column compartment.	6820.0010	1
Standards kits			
Standards kit	For details, see section 3.1.2.3, page 19.	3323.0010	1
Standards kit for RI detectors	Needed for qualifying an RI detector. For details, see section 3.1.2.3, page 19.	3325.0010	1

Material	Remarks	Part no.	Quantity
Solvents			
HPLC-grade methanol	Needed for testing the wavelength accuracy of a UV detector except the single-wavelength detectors (including the VWD-3400RS, VF-D40-A and VC-D40-A).	-	100 mL
HPLC-grade water	-	-	Approx. 1100 – 1700 mL
HPLC-grade water spiked with acetone Acetone concentration: General: 0.1% Vol (Exception: Systems with 60-mm-LightPipe flow cell: 0.02% Vol.)	Needed for qualification of gradient pumps.	-	Approx. 600 mL
Simulator cell			
Simulator cell (QualifierRS)	For qualifying an electrochemical detector with DC potentiostat module.	6070.4200	1 per DC potentiost at module to test
Simulator cell (PulseQualifierRS)	For qualifying an electrochemical detector with pulse potentiostat module.	6070.4300	1 per pulse potentiost at module to test
Supported UV detector with a flow cell with 10 mm light path	For qualifying the pump and autosampler in a HPLC system (except for the Thermo Scientific Vanquish™ systems (see section 3.5, page 42)): Both module types cannot be qualified using a different detector type (for example, a fluorescence detector) or UV detector with a flow cell with a differing light path (for example, 60 mm).	See *Operating Manual* for the detector	1
Upgrade Kit for a 250-µL syringe	For qualifying WPS-3000(T)PL and WPS-3000(T)PLRS autosamplers.	6820.0031	1
Waste Line	For qualifying a Vanquish dual gradient pump in a Tandem LC setup. This waste fluidic is part of the Vanquish Tandem LC Kit.	6083.2425	1

3.1.2.1 Performance Qualification Kit

The following required materials and parts are provided in the Performance Qualification kit (part no. 4832.5000A).

Part no.	Description	Quantity
709.8021	10 µL sample loop	1
754.ZU1M	SST connecting union 0.5 mm ID, 1/16 OD	2
2200.5502	Single-part, hand-tight fitting	2
3323.0010	Standards kit (caffeine and pyrene)	1
6000.0011	Finger-tight 33 mm fitting kit	1
5040.3000	Restriction tubing Viper™ SST(ID: 0.18 mm; length: 15 m)	1
2251.6001	PEEK tubing (ID: 0.25 mm)	2
The following parts are also provided in the kit. However, these parts are only required for the qualification of a Thermo Fisher Scientific Vanquish UHPLC Horizon System:		
22.61.5061	Viper connecting union	1
6041.5125	nanoViper capillary (ID: 50 µm; L: 950 mm)	1
2268.5014	Filter holder (10 µL Ti) and filter frit (2 µm)	1

TIP For a kit without sample loop, order part no. 4832.5010A.

3.1.2.2 *Column Compartment PQ Kit*

For qualifying a column compartment, a calibrated thermometer is required. The thermometer, and all other required equipment (temperature sensor mounting bracket, etc.) is provided in the Column Compartment PQ kit (part no. 6732.0010).

A replacement temperature sensor can be ordered (part no. 6705.0060). A separate temperature sensor mounting bracket for Vanquish column compartments VH-C10-A-02 and higher and / or VC-C10-A can be ordered (part no. 6732.0009).

TIP An additional flexible temperature sensor is required in addition to the Column Compartment PQ Kit when qualifying an ACC-3000(T) or ECD-3000RS column compartment. The temperature sensor is available as Temperature Sensor Type K for Thermometer P600/P700, part no. 6820.0010.

NOTICE When changing the temperature sensor, you may have to adapt the calibration values and sensor type setting of the thermometer. To do so, follow the *Operating Manual* shipped with the instrument. Otherwise, the thermometer may show the wrong temperature. For example, this is important when qualifying the ACC-3000(T), which is qualified using a type K temperature sensor. In addition, have a certified test center annually re-calibrate the thermometer and temperature sensor.

3.1.2.3 Standards Kits

General

The standards kit (part no. 3323.0010) contains the seven required caffeine and pyrene standards. 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 HPLC-grade methanol (see section 3.1.3.1, page 19). For single-wavelength detectors (including the VWD-3400RS, VF-D40-A and VC-D40-A) caffeine is used for the wavelength accuracy check, so you do not have to prepare (that is, dissolve) the pyrene standard.

RI Detectors

The kit with part no. 3325.0010 contains the five required standards with various concentrations for qualifying an RI detector.

Substance	Concentration
Glycerin in water	5 mg/mL
Glycerin in water	10 mg/mL
Glycerin in water	15 mg/mL
Glycerin in water	25 mg/mL
Glycerin in water	35 mg/mL

3.1.2.4 Simulator Cells

For qualifying an electrochemical detector, you need one simulator cell (QualifierRS) for each DC potentiostat module that you want to test; simulator cells are available under part no. 6070.4200. For pulse potentiostat modules, you need a simulator cell (PulseQualifierRS) that is available under part no. 6070.4300.

TIP The simulator cells have a limited lifetime (for details, refer to the certificate shipped with the simulator cell).

3.1.3 Preparations

3.1.3.1 Pyrene Standard

Complete the following steps:

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 the vial 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.

TIP The pyrene standard is used for checking the wavelength accuracy of all UV detectors (except the UltiMate VWD-3400 and Vanquish VH-D40 detectors, and any other supported single-wavelength detectors. Concentration deviations of ±30% do not affect the test results.

3.1.3.2 Solvents

For the channels A - D, prepare the following solvents (if any channel supports several solvent lines, use the first on, e.g., A1 or B1):

Channel	Solvent	Quantity	Checks
A	Methanol (HPLC grade)	Approx. 100 mL	Wavelength accuracy of a UV detector (exception: single-wavelength detectors including the VWD-3400RS, VF-D40-A and VC-D40-A).
	Water (HPLC grade)	Approx. 600 – 1200 mL	All checks except the wavelength accuracy of a multi-wavelength and diode-array detectors.
B(1)	Water (HPLC grade) spiked with acetone Acetone concentration: • General: 0.1% Vol. • Systems with 60-mm-LightPipe flow cell: 0.02% Vol.	Approx. 300 mL	Gradient accuracy, gradient precision, and ripple.
C(1)	Water (HPLC grade)	Approx. 500 mL	
D(1)	Water (HPLC grade) spiked with acetone (for concentrations see B)	Approx. 300 mL	

(1) If channel is available

For the Thermo Scientific Accela autosampler, use solvent reservoir BT1 (bottle).

3.1.3.3 Vial Placement in the Autosampler

General

For single-wavelength and VWD-3400RS/VF D40-A detectors, sample position RA1 (or 1) is not used.

Sample Position – Sampler Variant			Substance	Concentration
Summit / UltiMate [1]	Vanquish	Any other		
RA1	R:A1	1	Pyrene in methanol	3 µg/mL
RA2	R:A2	2	Caffeine in water	10 µg/mL
RA3	R:A3	3	Caffeine in water	60 µg/mL
RA4	R:A4	4	Caffeine in water	140 µg/mL
RA5	R:A5	5	Caffeine in water	220 µg/mL
RA6	R:A6	6	Caffeine in water	300 µg/mL
RA7	R:A7	7	Caffeine in water	2000 µg/mL
RA8	R:A8	8	Water (solvent)	-

[1] Summit / UltiMate autosamplers: ASI-100(T), WPS-3000(T)SL / PL, WPS-3000TBPL Analytical, WPS-3000T(B)FC Analytical, WPS-3000(T)(X)RS, WPS-3000(T)PL RS, and ACC-3000(T)

Dual Gradient Pump with a WPS-3000T(B)FC Analytical autosampler

In order to qualify the flow precision of a dual gradient pump (DGP) in combination with a WPS-3000T(B)FC analytical autosampler, position the following additional standard in the carousel (concentration depends on the autosampler configuration) for the second pump (test sequence: **XQ_INJECTOR_FLOW_REPRO_DGP_LEFT**).

Sample Position	Substance	Concentration	WPS-3000T(B)FC Analytical – Configuration
RC1	Caffeine in water	140 µg/mL	Standard configuration (sample loop volume: 50 µL)
		60 µg/mL	"Large Volume" configuration (sample loop volume: 250 µL)

RI Detectors

The table below shows in which autosampler position the standards have to be placed.

Sample Position – Sampler Variant					Substance	Concentration
ASI 100	WPS-3000xx [1]	Vanquish[2]	Vanquish[3]	Any other sampler		
RA9	RB1	R:A9	R:B1	9	Glycerin in water	5 mg/mL
RA10	RB2	R:B1	R:B2	10	Glycerin in water	10 mg/mL
RA11	RB3	R:B2	R:B3	11	Glycerin in water	15 mg/mL
RA12	RB4	R:B3	R:B4	12	Glycerin in water	25 mg/mL
RA13	RB5	R:B4	R:B5	13	Glycerin in water	35 mg/mL

[1] xx: WPS-3000(T)SL / PL, WPS-3000TBPL Analytical, WPS-3000T(B)FC Analytical, WPS-3000(T)(X)RS, WPS-3000(T)PL RS, and ACC-3000(T).

[2] When using a vial holder with a capacity of 54 vials.

[3] When using a vial holder with a capacity of 40 vials.

3.2 Setting up the Flow Connections

The steps below describe the flow connections of the HPLC system. Perform *all* steps for each module in the system.

3.2.1 Autosampler – Detector

Parts required

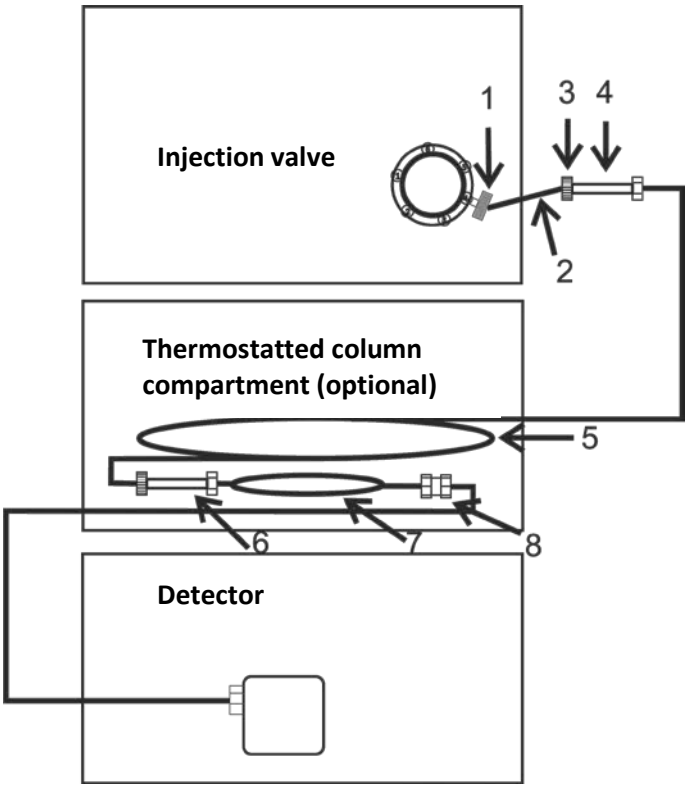

Figure 1: Restriction tubing installed between injection valve and detector

No.	Description	Part no.
Connection is not Viper-compatible		
1	Fitting, finger-tight, 33 mm	6000.0011
2	PEEK tubing, approximately 10 cm	2252.6001
3	Single-part fitting, finger-tight	2200.5502
4	Union	754.ZU1M
For all systems		
5	Restriction tubing	5040.3000

No.	Description	Part no.
Vanquish Horizon system only		
6	Viper union	2261.5061
7	High-pressure restriction tubing	6041.5125
Vanquish LightPipe flow cell only		
8	Filter frit	2268.5014

NOTICE Connections via 2, 3, and 4 are only allowed for non-Viper-compatible HPLC systems.

Follow these steps

1. Remove the column from the system.

TIP If the system includes several detectors that are connected in series, connect the restriction tubing to the detector that was connected to the column.

2. Connect the restriction tubing (no. 5 in Figure 1, page 23) directly to the injection valve (ViperTM fitting system) and the UV detector (Viper fitting system). Depending on the the HPLC system, additional components must be installed in the flow path:

   - *Non Viper-compatible injection valve connections only*:
     Connect one of the following device pairs with the PEEK tubing (no. 2 in Figure 1) and the union fittings (no. 4 in Figure 1) from the Performance Qualification kit:
     - Injection valve and restriction tubing (no. 5 in Figure 1)
     - Restriction tubing (no. 5 in Figure 1) and detector
   - *Vanquish Horizon System only*:
     For a qualification at a pressure of 850 bar, connect a high-pressure restriction tubing in series with a Viper union (no. 6 in Figure 1).
   - *Systems with Vanquish VH-D10-A Detector and LightPipe flow cell only*:
     To protect the flow cell, install a filter frit between the outlet of the restriction tubing (no. 5 or 7 in Figure 1) and the inlet capillary of the flow cell.

3. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying a dual gradient pump, proceed to section 3.2.2 Dual Gradient Pumps, page 25.
   - If qualifying a column compartment, proceed to section 3.3 Attaching Temperature Sensors to Column Compartments, page 29.
   - If none of the above applies, proceed to section 3.4 Configuring the System, page 33.

3.2.2 Dual Gradient Pumps

1. Depending on the pump configuration, observe one of the following:
   - *Pumps are shared between two different systems (e.g. Vanquish Dual LC system)*:
     If the pump units of a dual gradient pump are shared on two different systems, proceed as described in section 3.2.1, page 23. Qualification is comparable to a standard gradient pump (for example, UltiMate LPG-3400SD).
   - *Both pumps on the same system (e.g. Vanquish Tandem LC system)*:
     To qualify (within the same system) both pump units of a dual gradient pump with the same autosampler, restriction tubing, and detector, you have to use an external motorized switching valve, such as a valve in the Vanquish, UltiMate, or Summit TCC.

     Refer to the figures below for information on how to connect the fluidics of the entire system.
     - *Vanquish Tandem LC system:*

       The image below shows the fluid connection for testing the Vanquish dual gradient pump in Tandem LC operation using the lower valve (the valve is in position 1_2 for testing the right pump unit).

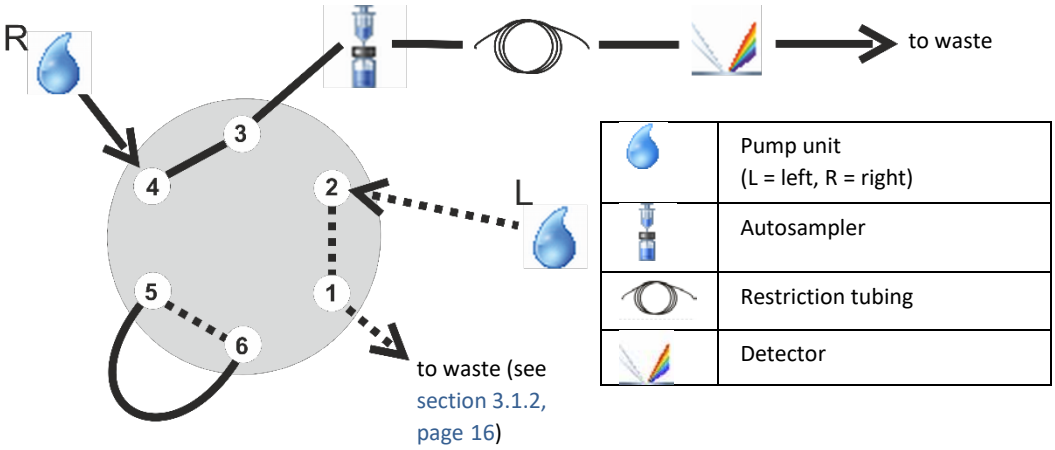

Figure 2: Fluid connection of lower valve in 1_2 position for testing the right pump unit

The image below shows the fluid connection for testing the Vanquish dual gradient pump in Tandem LC operation, using the lower valve (the valve is in position 6_1 for testing the left pump unit).

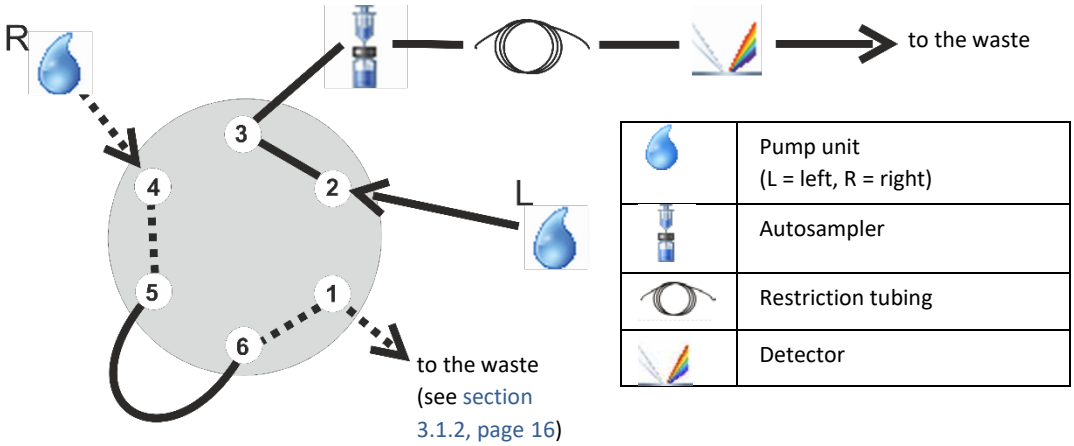

Figure 3: Fluid connection of lower valve in 6_1 position for testing the left pump unit

- *UltiMate / Summit:*
  - Case a): *6-port/2-position valve*

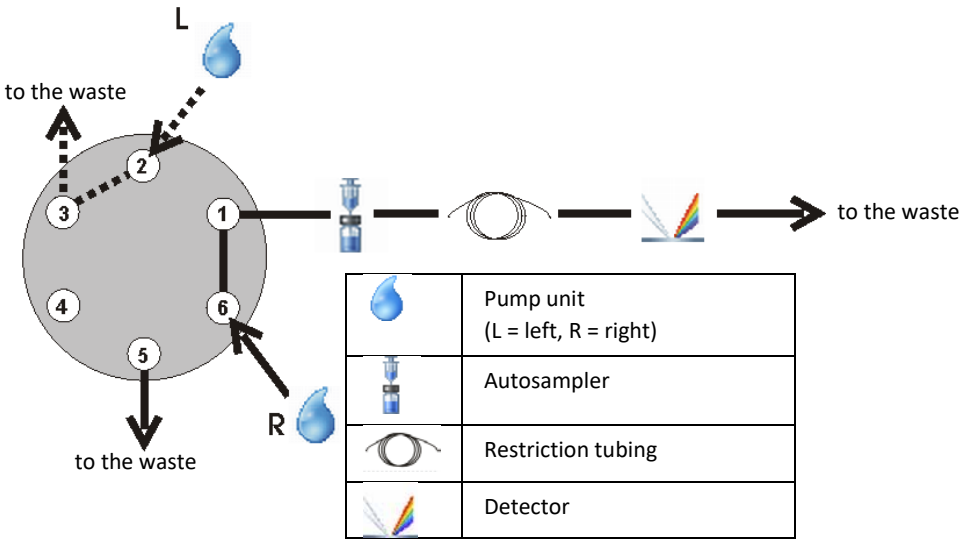

Figure 4: Fluid connection for testing the dual gradient pump, using a 6-port/2-position valve (the valve is in position A or 1, depending on the valve type)

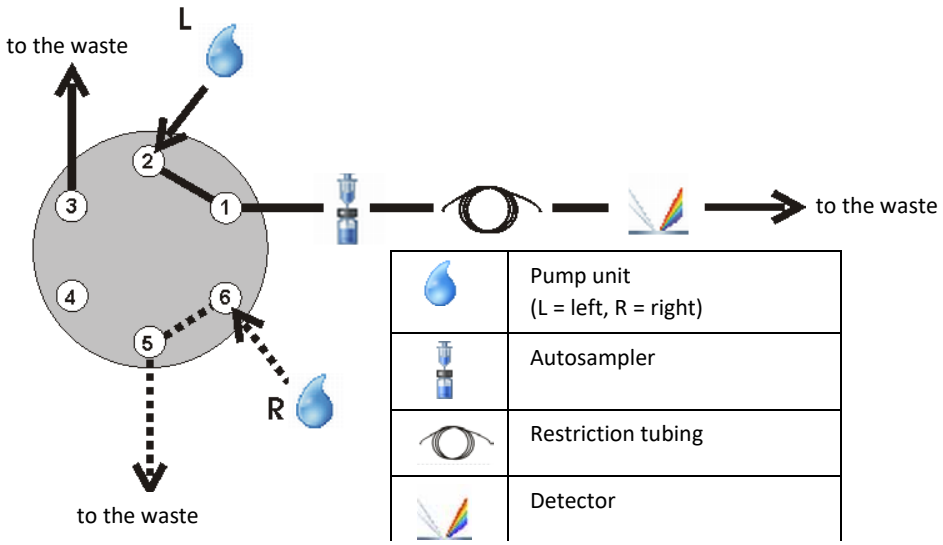

Figure 5: Fluid connection for testing the dual gradient pump, using a 6-port/2-position valve (the valve is in position B or 2, depending on the valve type)

- Case b): 10-port/2-position valve:

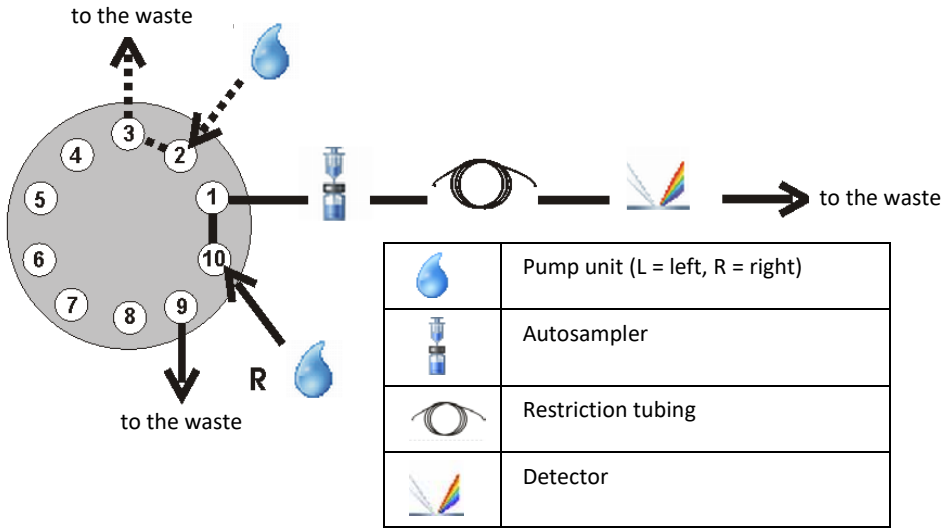

Figure 6: Fluid connection for testing the dual gradient pump, using a 10-port/2-position valve (the valve is in position A)

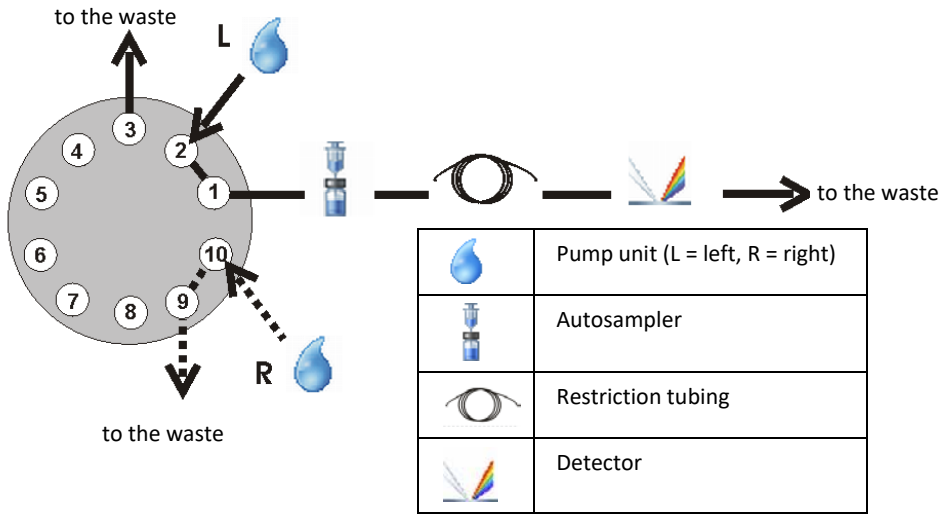

Figure 7: Fluid connection for testing the dual gradient pump, using a 10-port/2-position valve (the valve is in position B)

2. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying a column compartment, proceed to section 3.3 Attaching Temperature Sensors to Column Compartments, page 29.
   - If not qualifying a column compartment, proceed to section 3.4 Configuring the System, page 33.

3.3 Attaching Temperature Sensors to Column Compartments

When qualifying the column compartments, securely attach the temperature sensor of the thermometer to the heating block. When qualifying the following column compartments, refer to the following subsections for further details:

- Column compartment of the Accela autosampler
- Summit TCC-100 or UltiMate TCC-3x00(SD/RS)
- UltiMate ACC-3000(T) and ECD-3000RS
- Vanquish Column Compartments
- Shimadzu Column Compartments

3.3.1 Column Compartment of Accela Autosampler

1. Position the temperature sensor inside the oven near the internal temperature sensor.
2. Configure the system (see section 3.4, page 33).

3.3.2 Summit TCC-100 or UltiMate TCC-3x00(SD/RS)

1. Install the temperature sensor as shown in the figure below.

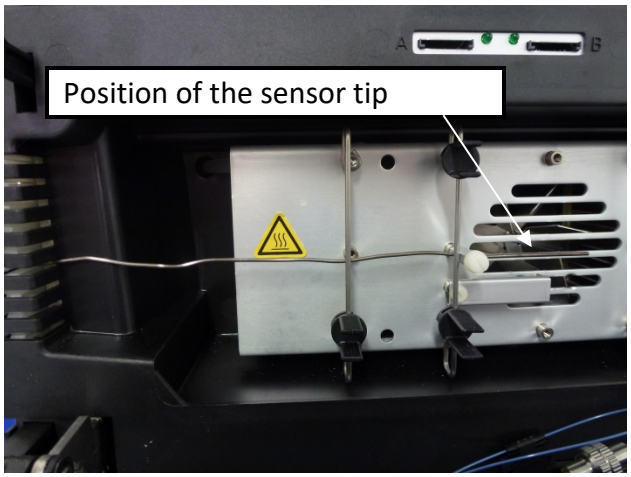

Figure 8: TCC-100/TCC-3x00(SD/RS) – Position of the temperature sensor

2. Configure the system (see section 3.4, page 33).

3.3.3 UltiMate ACC-3000(T) and ECD-3000RS

1. Install the temperature sensor as shown in the figure below.

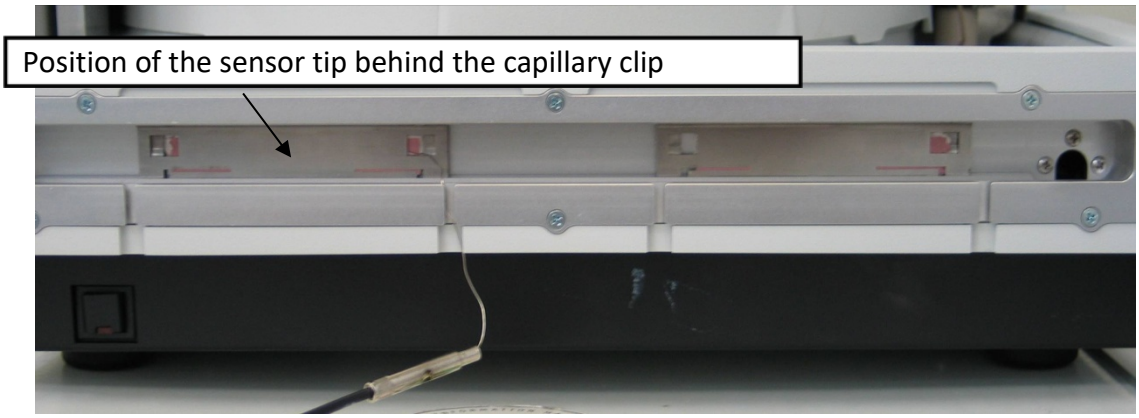

Figure 9: ACC-3000(T)/ECD-3000RS – Position of the temperature sensor

2. Use the type K temperature sensor (and not the sensor from the Column Compartment PQ Kit).
3. Install the temperature sensor behind the left capillary clip, from a vertical point of view in the center of the oven, and 2 cm away from the right edge of the heat-conductive pad.
4. Configure the system (see section 3.4, page 33).

3.3.4 Vanquish Column Compartments

NOTICE Be careful not to bend the measuring area of the temperature sensor when closing the door. This will damage the temperature sensor. Ensure that the entire active measuring area of the temperature sensor is inserted into the drilling hole.

1. Fix the temperature sensor as shown in Figure 10 for module VH-C10-A revision 01 and / or as shown in Figure 11 for VH-C10-A revision 02 and VC-C10-A.

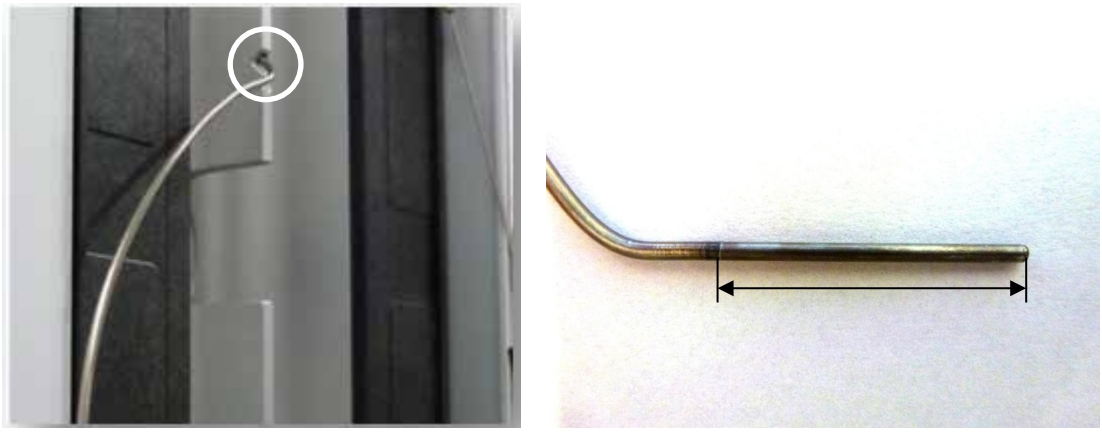

Figure 10: Left: VH-C10-A (revision 01) – Position of the temperature sensor; Right: measuring area of the temperature sensor

Figure 11: Left: VC-C10-A and VH-C10-A-02 (revision 02) – Position of the temperature sensor; Right: temperature sensor mounting bracket that has to be mounted manually in the compartment for qualification purposes

2. Configure the system (see section 3.4, page 33).

3.3.5 Shimadzu Column Compartments

1. Loosen a fastening screw.

2. Insert the sensor between the screw and the metal block, and carefully retighten the screw.

3. Configure the system (see section 3.4, page 33).

3.4 Configuring the System in Chromeleon

The steps below describe all configuration settings required for OQ and PQ in Chromeleon Server Configuration Program or on the instrument. Perform *all* steps *for each module* in the system. For all instruments that are not explicitly mentioned, the customer and/or default settings are used.

3.4.1 General Remarks

3.4.1.1 Device Names and Channel Names

You can use user-defined device names and channel names (as defined in the Chromeleon Server Configuration Program) for all devices except for those devices that are listed in the table below. All other device names may differ from the defaults. The PGM files in the sequences that were created as described above are automatically adapted to the used device and channel names.

Module	Name
Devices names of the pump's eluent channels	%A, (%B), (%C), (%D)
Device name of the external thermometer	Thermometer
Signal name of the external thermometer	TemperatureOVEN
Device name of the virtual channel	VirtualChannels_01

3.4.1.2 Systems with more than one Module of the Same Class

For systems with more than one module (A + B) of the same class (examples for module classes: pump, autosampler, column compartment, UV detector, FL detector, etc.), the principle procedure is as follows:

- Remove module B from the Server Configuration
- Run the OQ/PQ setups for the remaining module A.
- Perform the OQ/PQ tests.
- If you want to qualify a second module (B) of the same class, remove module (A) from the Server Configuration temporarily.
- Add module B.
- Run the OQ/PQ setup for module (B) to create the templates.

- Perform the OQ/PQ tests.

3.4.2 Autosamplers

3.4.2.1 WPS-3000(T)PL and WPS-3000(T)PLRS

For successful qualification of the WPS-3000(T)PL and WPS-3000(T)PLRS autosamplers, do as follows:

1. Make sure that the Upgrade Kit for a 250-µL syringe is installed.
2. Configure the detector (see section 3.4.4, page 36).

3.4.2.2 WPS-3000T(B)FC Analytical Autosampler

1. Activate the **WPS-3000TFC/WPS-3000TBFC** check box in the Server Configuration on the Options page of the autosampler: Supported Options (Volumes):

Option	Syringe volume	Buffer tubing size	Loop size
Standard	250 µL	500 µL	50 µL
Large Volume	250 µL	1000 µL	250 µL

2. Configure the detector (see section 3.4.4, page 36).

3.4.2.3 WPS-3000TBPL Analytical Autosampler

To ensure a successful qualification, do as follows:

1. Equip the WPS-3000TBPL analytical autosampler with the Standard or Large Volume configuration:

Option	Syringe volume	Buffer tubing size	Loop size
Standard	100 µL	500 µL	50 µL
Large Volume	250 µL	1000 µL	250 µL

2. Activate the **WPS-3000TBPL Analytical** check box in the Server Configuration on the Options page of the autosampler:

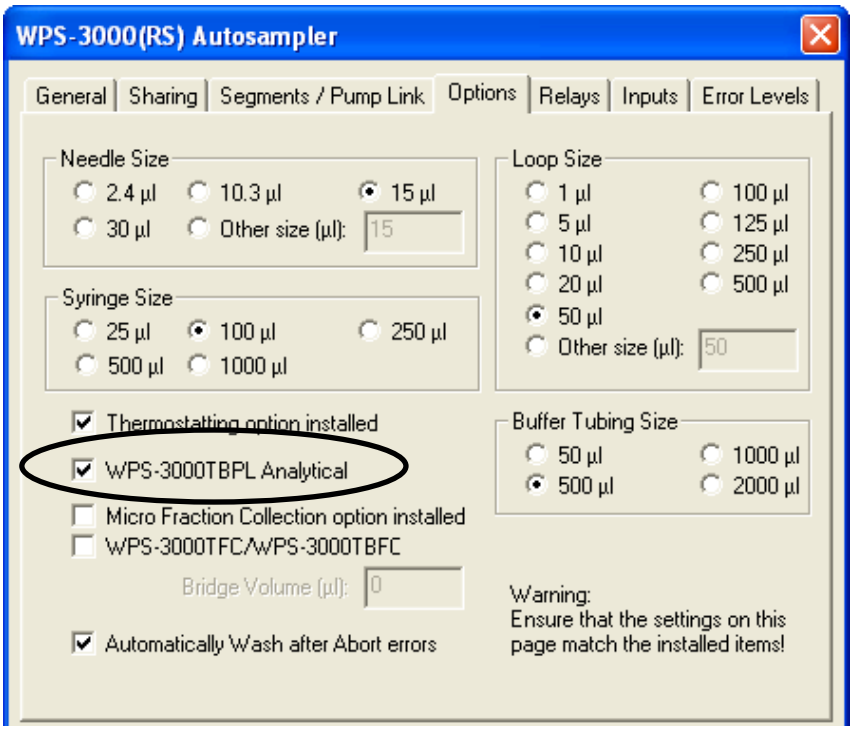

Figure 12: WPS-3000TB PL Analytical configuration

3. Configure the detector (see section 3.4.4, page 36).

3.4.2.4 UltiMate Autosamplers with User-Defined Sample Loop Volume

1. The sample loop volume must be at least 20 µL. In addition, you must set the sample loop volume in the Server Configuration program to a value predefined by Chromeleon (see table).

Autosampler	User-defined sample loop volume	Sample loop volume setting
WPS-3000(T)PL / WPS-3000(T)PLRS	20 – 39 µL	20 µL
	40 – 99 µL	50 µL
	100 – 124 µL	100 µL
	> 125 µL	125 µL
WPS-3000(T)SL / WPS-3000(T)RS		
-	20 – 39 µL	20 µL
	40 – 129 µL	Micro
	> 130 µL	Analytical

Autosampler		User-defined sample loop volume	Sample loop volume setting
	With 250 µL injection volume kit	344 µL	344 µL
ACC-3000(T)		21 – 49 µL	20 µL
		51 – 199 µL	50 µL
		> 200 µL	200 µL

2. Configure the detector (see section 3.4.4, page 36).

3.4.3 Manual Injection Valve

1. Verify that the injection valve is fitted with a 10-µL sample loop.
2. Configure the detector (see section 3.4.4, page 36).

3.4.4 Detectors

3.4.4.1 UV Detector – General

1. On the Signals page for the UV detector in the Server Configuration program, ensure that the unit is **mAU**.
2. Ensure that the factor is **1.00**.

3.4.4.2 AD25 UV Detector

1. On the Signals page for the AD25 in the Server Configuration program, change the unit to **mAU** instead of AU.
2. Change the factor to **1000** instead of 1.00.
3. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If qualifying a column compartment, proceed to section 3.4.5 Column Compartment – General Instructions, page 38.
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If none of the above applies, prepare the system (see section 3.5, page 42).

3.4.4.3 RF2000 Fluorescence Detector

1. Before connecting the RF2000 fluorescence detector with Chromeleon, set the **ZWAVE** and **RATIO** parameter to **1** that you can only set on the module. It is not possible to set the value from Chromeleon:
   a) Disable the keyboard interlock by simultaneously pressing **Shift** and **CE**.
   b) Press **func** repeatedly until the ZWAVE command appears on the display.
   c) Press **1** on the number keypad.
   d) Confirm with **Enter**.
2. Enable remote operation to connect the module to Chromeleon:
   a) Press **func**, until the RS232 command appears on the display.
   b) Confirm with **Enter.**
   c) Press **func**.
   On the display, the reading is CONNECT.
   d) Confirm with **Enter**.
3. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If qualifying a column compartment, proceed to section 3.4.5 Column Compartment – General Instructions, page 38.
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If none of the above applies, prepare the system (see section 3.5, page 42).

3.4.4.4 ECD-3000RS EC Detector

TIP For qualification of the detector with up to eight channels simultaneously, HPLC OQ/PQ version 9.00 or higher is required. If any previous HPLC OQ/PQ version is used, the qualification of a module with more than four channels has to be performed in two steps with maximum four channels each.

1. Connect the simulator cells(s) to the potentiostats.
2. Click **Read Smart Cells** on the **Detector** page of the ECD-3000RS driver in the **Server Configuration** program and then, select the detected bays.
3. Check that the **DC Mode (nA)** or **Pulse Mode (nC)** option is selected under **Mode and Range**. Otherwise, no template for qualifying the detector will be offered.

4. Confirm your selection by clicking **OK**.
5. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If qualifying a column compartment, proceed to section 3.4.5 Column Compartment – General Instructions, page 38.
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If none of the above applies, prepare the system (see section 3.5, page 42).

3.4.5 Column Compartment – General Instructions

NOTICE - **Sensitive LightPipe flow cells**
If qualifying non-Vanquish column compartment in systems with Vanquish detector and LightPipe flow cell:
For the protection of the LightPipe flow cells, qualifying non-Vanquish column compartments in systems that include a Vanquish detector with an installed LightPipe flow cell as described in section 3.7.1 General Checks, page 3.7.1 is *not supported*. If you want to qualify a non-Vanquish column compartment, you have to perform the qualification as described in section 3.7.2, page 57.

The following three options require a modification of the Chromeleon Server Configuration. If this is not allowed due to customer restrictions, a manual qualification of the column compartment is recommended as described in section 3.7.2, page 57.

Option A: Using the Column Compartment PQ kit

- P500/600 thermometers:
  1. Connect the thermometer to a free COM port on the Chromeleon server PC.

TIP If a server PC without COM port is used, the Dostmann Thermometer P500/P600 can be connected using a USB-to-RS-232 adapter cable (part no. 6073.2000).

2. Install the Dostmann Thermometer P500/P600 driver (Chromeleon 7: P5xx/P6xx/P7xx Thermometer) in the **Chromeleon Server Configuration** program.
3. On the **General** page, select the COM port to which the thermometer is connected.
4. Install a virtual channel (device name: VirtualChannels_01; signal Name: TemperatureOVEN).

5. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying a Vanquish column compartment, proceed to section 3.4.6 Vanquish Column Compartment, page40.
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If none of the above applies, prepare the system (see section 3.5, page 42).

- P700 thermometer:
  Complete the following steps to connect the P700 thermometer with the Chromeleon server PC:

  1. Connect one end of the USB cable to the USB port of the thermometer.
  2. Connect the other end of the USB cable to a USB port on the Chromeleon server PC or to a USB port on another module that is connected to the Chromeleon server PC.
  3. If not done automatically, install the virtual COM port as follows: To install the virtual COM port, use the FTDI driver application provided on the Chromeleon installation medium in the **Drivers\USB Virtual COM Port** directory. As a result, the USB device will appear as an additional COM port in the **Windows Device Manager**. For installation details on the FTDI driver, refer to the installation instructions provided on the Chromeleon installation medium.
  4. Connect the thermometer to the virtual COM port on the Chromeleon Server PC.
  5. Stop and restart the **Chromeleon Instrument Controller**.
  6. Install a virtual channel (Device name: VirtualChannels_01; signal Name: TemperatureOVEN).
  7. Depending on your system configuration, proceed to one of the following steps:
     - If qualifying a Vanquish column compartment, proceed to section 3.4.6 Vanquish Column Compartment, page40.
     - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
     - If none of the above applies, prepare the system (see section 3.5, page 42).

Option B: Automatic data acquisition as analog signal

In the **Chromeleon Server Configuration** program, install the analog output of the external thermometer (Device: Integrator Driver) as an analog channel named **TemperatureOVEN**.

Option C: Manual data acquisition

TIP Option C (qualifying the column compartment with manual data acquisition) is not supported with Chromeleon 7 and for modules from Agilent, Shimadzu, and Waters modules. Thermo Fisher Scientific Vanquish modules are not supported, either.

1. In the **Chromeleon Server Configuration** program, install the **STH_manual** device. The driver is available under **Generic** on the **Manufacturers** list.
2. Verify that the driver is in **Demo Mode**.
3. Install a virtual channel (Device Name: VirtualChannels_01, Signal Name: TemperatureOVEN).
4. During the chromatographic run, enter the temperature indicated on the external thermometer on the **OQ_PQ_STH_manual** control panel.
5. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying a Vanquish column compartment, proceed to section 3.4.6 Vanquish Column Compartment, page40.
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If none of the above applies, prepare the system (see section 3.5, page 42).

3.4.6 Vanquish Column Compartment

1. Depending on the system or module components used, two different sequence templates with different temperature ranges are provided. The following table gives an overview of how the two available sequence templates are to be used:

VH-D10-A LightPipe flow cell installed	Post-column cooler configured	Sequence	Maximum temperature (OQ)
Yes	No	Column_Oven_LT (CM7: … Low Temp)	50 °C
No	Not relevant	Column_Oven (CM7: …High Temp)	> 50 °C
Yes	Yes	Column_Oven_LT (recommended) (CM7: … Low Temp)	50 °C
		Column_Oven (optional) (CM7: … High Temp)	> 50 °C

NOTICE Do *not* operate Vanquish LightPipe flow cells with temperatures over 50 °C. Before you start the qualification of the column compartment over the entire temperature range, make sure, that a post-column cooler is properly installed and connected.

TIP Disable the sequence **Column_Oven_LT** in the OQ/PQ Setup Wizard. To obtain the qualification template for the entire temperature range, enable the sequence **Column_Oven**.

2. Depending on your system configuration, proceed to one of the following steps:
   - If using Agilent Instrument Control Framework, proceed to section 3.4.7 Agilent Instrument Control Framework (ICF), page 41.
   - If not using Agilent Instrument Control Framework, prepare the system (see section 3.5, page 42).

3.4.7 Agilent Instrument Control Framework (ICF)

General

The OQ/PQ templates can only be used to qualify "pure" Agilent systems that include Agilent modules only.

Hint regarding the motor switching valve

The motor switching valve of the column oven is not required to qualify Agilent systems. However, if an Agilent system includes a column oven with motor switching valve, the created instrument methods will automatically set the valve position depending on the valve type (corresponds to the first position in the **Instrument Method Editor** window).

NOTICE For this case, make sure that the valve is not switched dry, to prevent damage to the valve.

3.5 Preparing HPLC Systems

To prepare the HPLC system for OQ or PQ, follow the steps below. Perform *all* steps for the modules in your system, observing the correct order.

3.5.1 Vanquish Solvent Monitor

1. To avoid potential queue ready check errors, disable at least solvent A(1) channel of the Vanquish Solvent Monitor via the specific CM ePanel. After finishing the OQ or PQ, enable all previously disabled channels again.

2. Proceed to the steps in section 3.5.2 Pump, page 42.

3.5.2 Pump

Follow the steps in this section depending on the system configuration:

1. To qualify a Corona™ detector, follow the instructions in section 4.4, page 71.
2. To qualify a mass spectrometry detector, follow the instructions in section 4.5, page 73.
3. For systems with RI detector: Follow the steps described in section 3.5.4.4, page 44.
4. Purge channel A(1) as follows:
   - *Systems with single-wavelength detectors and UltiMate VWD-3400RS / Vanquish VF-D40-A, VC-D40-A detectors, or if the wavelength accuracy is not checked*: Use water to purge channel A(1).
   - *Systems with multi-wavelength detectors and photodiode-array detectors*: Use methanol to purge channel A(1).
5. Purge all other available channels of the pump sufficiently with the solvents listed in section 3.1.3.2, page 20.

NOTICE Rinsing the HPLC system with a Corona detector included in the flow path can damage the Corona detector. To avoid damage, separate the fluidics of the Corona detector from the HPLC system before rinsing the HPLC system.

6. If a Corona detector is included in the flow path, disconnect the fluidics of the Corona detector from the HPLC system.
7. If a mass spectrometry detector is included in the flow path, disconnect the fluidics of the mass spectrometry detector from the HPLC system.

8. Rinse the entire HPLC system with the solvent used in channel A(1).
9. Proceed to the steps in section 3.5.3 Autosampler, page 43.

3.5.3 Autosampler

TIP Although methanol is used as solvent for the first OQ and PQ check, rinse the autosampler with water, as water is the solvent for all successive checks. Automatically rinsing the system after the wavelength accuracy check ensures that the fluid system is sufficiently prepared.

1. Rinse the autosampler thoroughly with water by injecting 250 µL of water at least five times. If the allowed maximum injection volume of the autosampler is smaller, inject five times the largest possible volume.
2. Make sure that the fluid components are free of air bubbles.
3. Proceed to the steps in section 3.5.4 Detectors, page 43.

3.5.4 Detectors

3.5.4.1 UV Detector

1. Turn on the UV detector lamp at least six hours before you start the check.
2. When using a detector with additional VIS lamp, turn off the VIS lamp.
3. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If not qualifying another detector, check the fluidics (see section 3.5.5, page 44).

3.5.4.2 Fluorescence Detector

1. Prepare the detector:
   - *Detectors with a continuously burning lamp (for example, Summit RF2000)*:
     Turn on the detector lamp approximately 30 minutes before you start the check.
   - *Detectors with a flash lamp (for example, UltiMate FLD-3x00)*:
     Make sure that the detector is sufficiently equilibrated (for example, the flow cell temperature).
2. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If not qualifying another detector, check the fluidics (see section 3.5.5, page 44).

3.5.4.3 Electrochemical Detector

TIP Even when qualifying only the detector (but not the pump and autosampler), make sure that there is sufficient eluent (water) in the pump's eluent supply and a restriction capillary is connected.

1. Separate the detector and the flow cell from the HPLC system fluidics.
2. Connect a simulator cell (QualifierRS for DC Mode or PulseQualifierRS for Pulse Mode) to all potentiostats that you want to qualify.
3. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If not qualifying another detector, check the fluidics (see section 3.5.5, page 44).

3.5.4.4 RI Detector

1. Turn on the module at least one hour before you start the check.
2. Ensure that no air bubble is trapped in the flow cell. If there are air bubbles, rinse the flow cell with methanol or isopropanol.
3. Rinse sample and reference part of the flow cell via the **Purge** button at a flow rate of 1.0 mL/min (mobile phase: water).
4. *All detectors except the UltiMate VWD-3x00 / Vanquish VF-D40-A, VC-D40-A detectors and all single wavelength detectors*: If you check the wavelength accuracy of the UV detector using methanol, disconnect the fluid components of the RI detector from the HPLC system.
5. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If not qualifying another detector, check the fluidics (see section 3.5.5, page 44).

3.5.4.5 Evaporative Light Scattering Detector

1. Turn on the detector lamp approximately 30 minutes before you start the check.
2. Depending on your system configuration, proceed to one of the following steps:
   - If qualifying another detector, proceed to the corresponding section below.
   - If not qualifying another detector, check the fluidics (see section 3.5.5, page 44).

3.5.5 Fluidics

On the injection valve or autosampler, do the following:

1. Verify that there are no pressure fluctuations when the valve switches from Load / Bypass to Inject and vice versa.
   Pressure fluctuations indicate system leakage or contamination.
2. Eliminate any leaks and contamination before you start the check.
3. Prepare Chromeleon for the checks (see section 3.6, page 46).

3.6 Preparing the Test Templates in Chromeleon

3.6.1 Template Directory Structure on the Chromeleon CD

The **PQ_OQ** directory on the Chromeleon CD has the following subdirectories (see figure below):

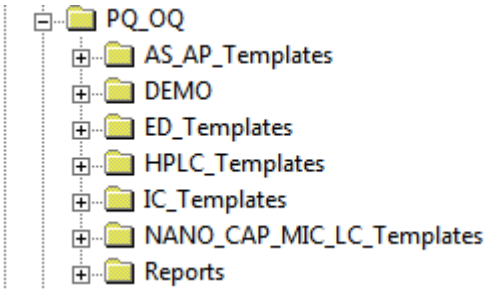

Figure 1: PQ_OQ directory structure on the Chromeleon CD

When creating the sequence templates, the wizard provides only those sequences that match the timebase:

System Configuration	Provided Directory
IC and BioLC systems	IC_TEMPLATES
Systems with an electrochemical detector	ED_TEMPLATES
Nano, cap, and micro systems	NANO_CAP_MIC_LC_TEMPLATES
HPLC systems	HPLC_TEMPLATES

The **HPLC_TEMPLATES** directory contains all master sequences required for OQ or PQ of a common HPLC configuration. This directory has a **SPECIAL HPLC TEMPLATES** subdirectory for special checks (see chapter 4, page 64).

TIP The sequences in the **HPLC_TEMPLATES** directory do not support qualification of systems that include an UltiMate FLM-3x00 Flow Manager or an NCS system or NCP pump. Sequences for qualifying these systems are available in the **NANO_CAP_LC_TEMPLATES** directory.

3.6.2 General Procedure Description

NOTE These OQ/PQ operating instructions refer only to the sequences of the **HPLC_TEMPLATES** directory.

Running Operational Qualification or Performance Qualification in Chromeleon comprises two basic steps:

1. *Only if the system has been installed or if the system configuration was changed*:
   To create configuration-specific system master templates, select **Qualification** > **PQ Setup…|OQ Setup…**. See (a) in Figure 2 and section 3.6.3, page 48.
   A wizard generates sequence templates from the master sequences of the Chromeleon CD, providing only sequences that match the timebase. In addition, the wizard adapts the programs automatically to the devices installed in the timebase. This step has to be performed every time a system has been installed or a system configuration has been changed.
2. Adapt the report and method. See section 3.6.4, page 52.
3. *Only if PQ Setup…|OQ Setup…has been performed and the system configuration was not changed:*
   To perform the checks, select **Qualification** > **Instruments PQ…|Instruments OQ…** . See (b) in Figure 2 and section 3.7, page 54.
   For each check performed on the same system configuration, a separate copy of the configuration-specific system master template is created. OQ/PQ is then performed with the sequences of the copied templates.

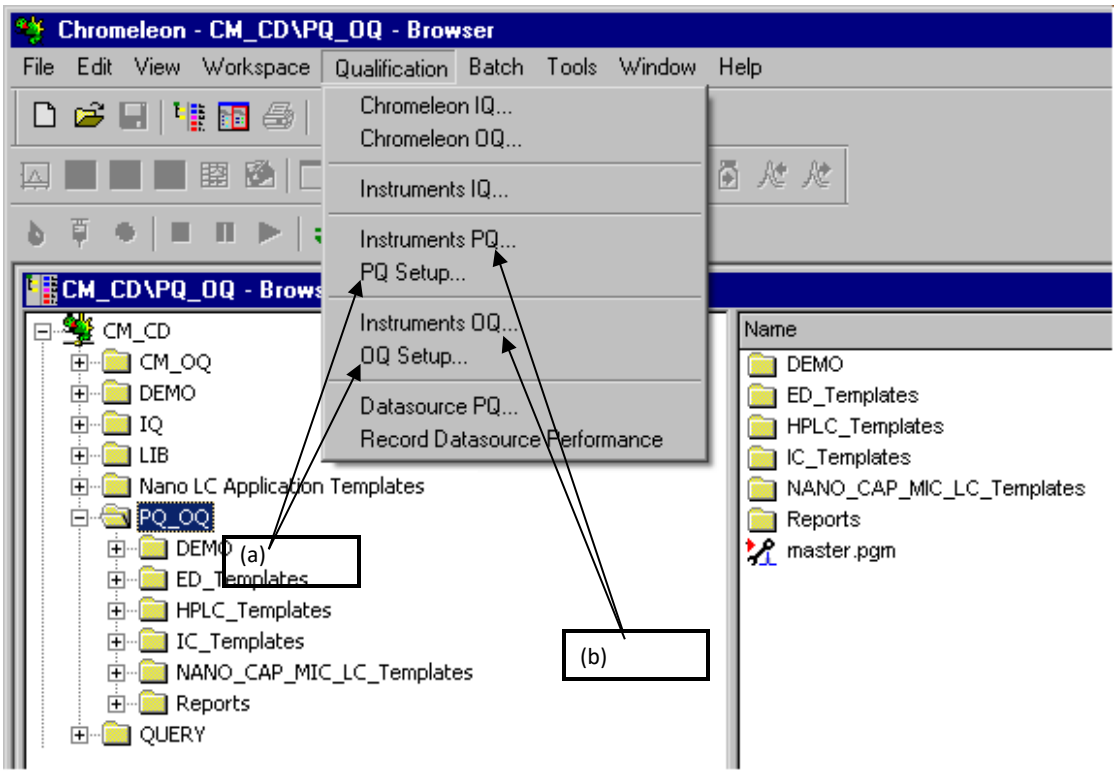

Figure 2: Performing OQ/PQ: (a) Step 1: OQ/PQ Setup; (b) Step 2: Instruments OQ/PQ

3.6.3 Creating the System Master Templates

To install the sequences required for your system, follow the steps below. In case you are using different types of flow cells for the qualification of a system that includes a Vanquish VH-D10-A detector, you have to create sequence templates for each type of flow cell:

1. Insert the Chromeleon CD or verify that you can access the **PQ_OQ** directory.

2. In the Browser, click **Qualification** > **OQ Setup|PQ Setup**.

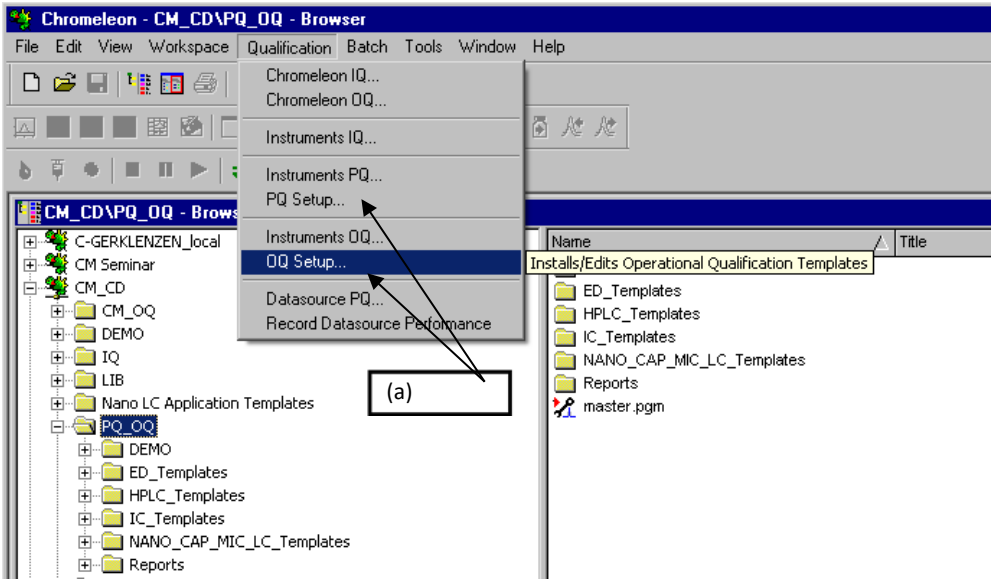

Figure 13: (a) Selecting OQ or PQ setup

A wizard guides you through copying of the sequences.

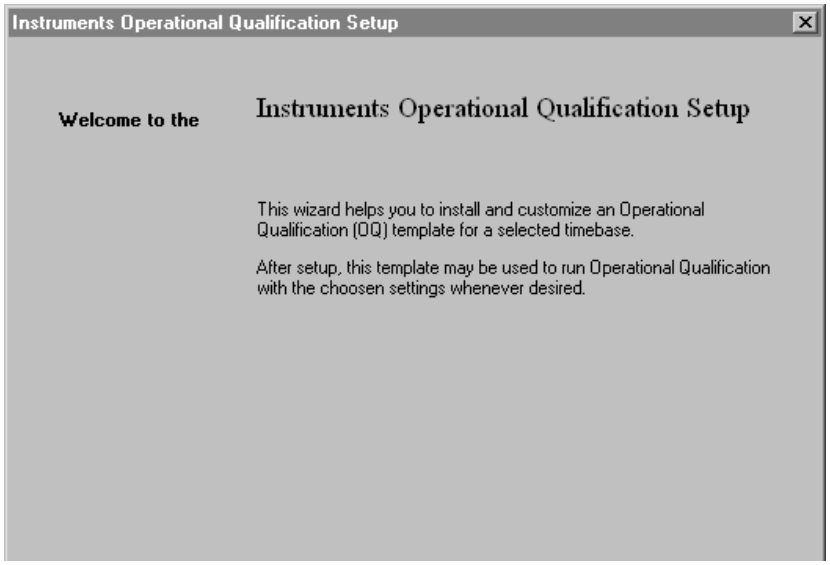

Figure 14: OQ/PQ setup wizard welcome page

3. Click **Next >** to go to the next step.

4. Select the timebase for which you want to perform OQ or PQ and enter the name of the computer on which the timebase is installed.

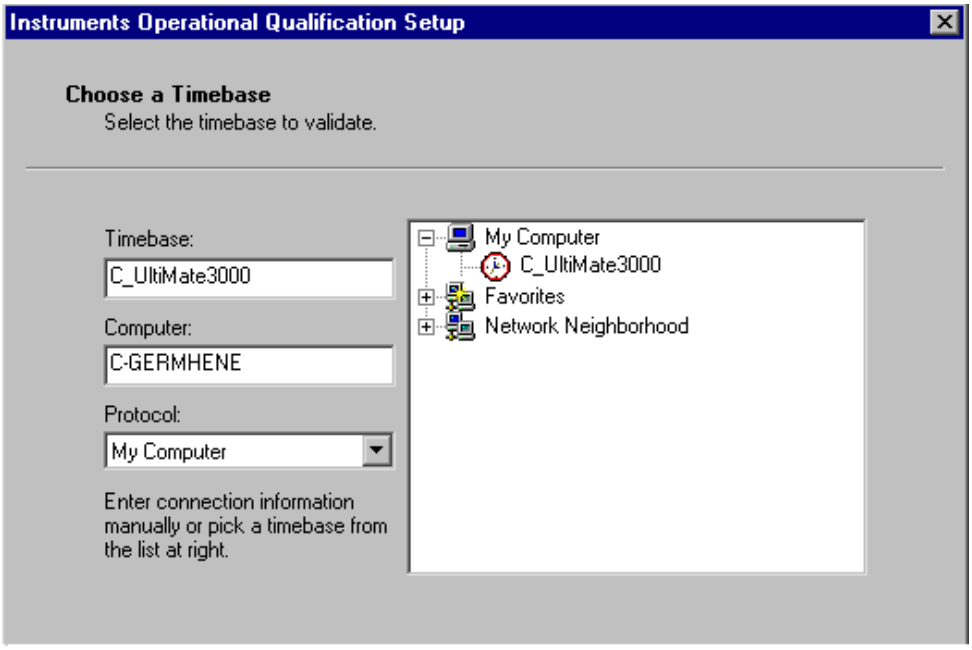

Figure 15: Selecting a timebase

5. Select **PQ_OQ** as the source directory of the master sequences.

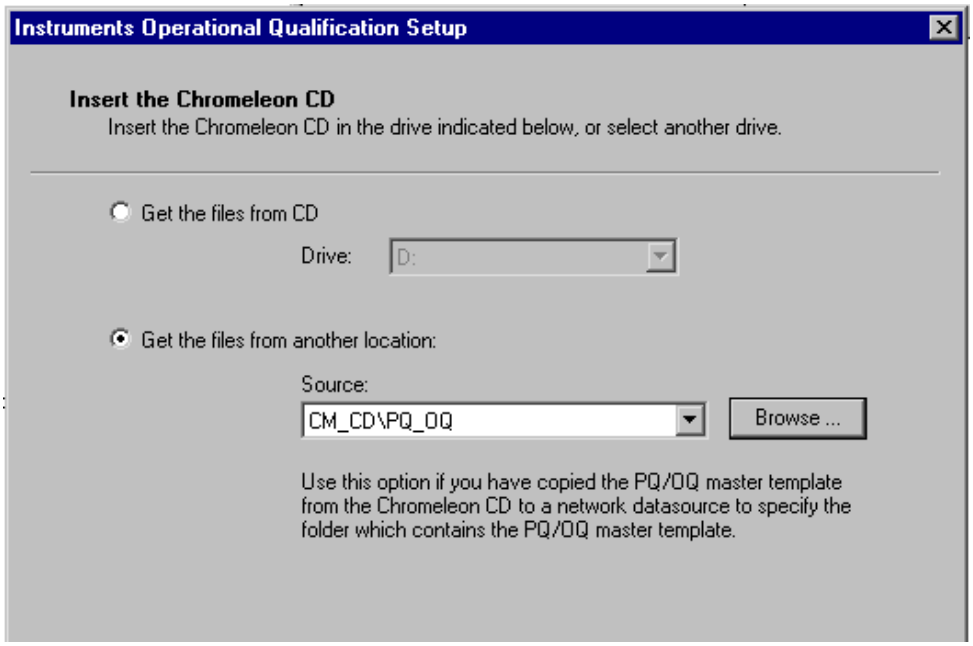

Figure 16: Selecting the source directory

6. Select a unique name under which the sequence directory containing all sequence templates for this instrument is saved.

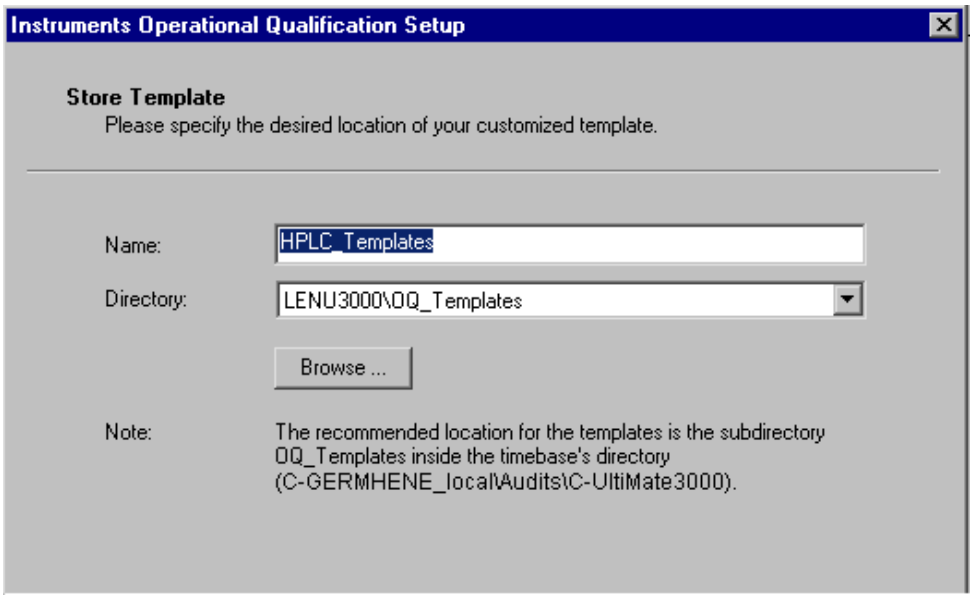

Figure 17: Selecting the storage location

7. Click **OK**.
   A list of checks / sequences is displayed. The list is adapted to the instrument configuration of the selected timebase as defined in the Chromeleon Server Configuration program.

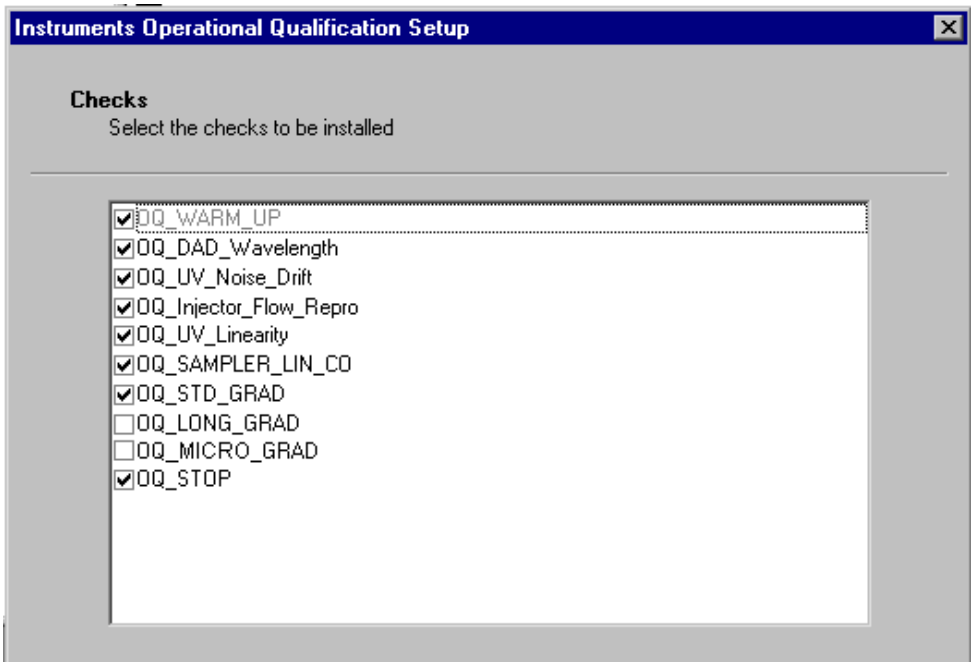

Figure 18: List of checks for the timebase

When Chromeleon cannot automatically determine the mixing chamber volume, you can select the sequences as required. This applies to the Summit P680, UltiMate, and Vanquish Flex pumps. For details, see section 6.3.1.2, page 137. In all other cases, the selection is read-only. Select the sequences required for the checks that you want to perform (see chapter 6, page 92). The selected sequences are automatically copied to the corresponding data source. When installation is complete, the report opens on the Specification page.

TIP If you use the TSP UV1000 UV detector, sequences are only offered if the UV lamp is installed in the detector.

8. Adapt the report and method (see section 3.6.4, page 52).

3.6.4 Adapting the Report and Method

1. To disable the write protection of the report, click **Edit** > **Layout Mode**.
2. Enter:
   a) Batch numbers
   b) Expiration dates
   c) Actual concentration of the standards
   d) Names of customer and tester
   e) Name of the item that is used to generate the backpressure
      Default: capillary (L: 15 m; ID: 0.18 mm)
      For all supported modules, the device name and the limits recommended by Thermo Fisher Scientific Inc. are entered automatically in the report when the report is opened after the "Warmup" sample has finished. The information is not yet entered at the time the sequence is copied.

NOTICE Do not fill in the report for the supported devices (see below). The limits can be found in lines 231 and following; you must change the limits only if you want to use other limits than the limits recommended by Thermo Fisher Scientific Inc.

   f) When using Summit detectors UVD 170S, UVD 340S, UVD 170U, or UVD 340U with non-analytical flow cells, enter the specifications listed in section 4.2 on page 65 and section 6.2.7 on page 127 manually in the report. Automatic detection of the flow cell is not supported.

g) Typically, the serial number is entered automatically. If this is not the case, enter the serial number in column K from line 201 on (the fields have a yellow background). To delete the value in the related box, on the Edit menu, click Clear Values. This removes the Chromeleon variable from the cell and clears the audit.xxx entry for the cell on the status bar.

h) For third-party devices, enter the manufacturer's name in column D from line 201 on.

i) Typically, the model number is entered automatically. If this is not the case, adapt or enter the model name in column H (cells with a yellow background), deleting the existing audit.xxx entry as before.

j) From line 231 on, enter the limits in the column with the related model name.

3. To enable the write protection, click **Edit** > **Layout Mode**.
4. To save the report, click **Workspace** > **Save Report Definition**.
5. To check the linearity of the UV detector, adapt the amounts in the QNT file of the sequence to the actual amounts of the used standards.
6. Start the checks (see section 3.7.1, page 54).

3.7 Performing the Checks

This section contains all descriptions about how to perform the general checks. Chapter 4, page 64 contains descriptions about how to perform special checks that require a different test setup than the standard checks.

3.7.1 General Checks

NOTICE - Sensitive LightPipe flow cells
If qualifying non-Vanquish column compartment in systems with Vanquish detector and LightPipe flow cell:
For the protection of the LightPipe flow cells, qualifying non-Vanquish column compartments in systems that include a Vanquish detector with an installed LightPipe flow cell as described in this section is *not supported*. If you want to qualify a non-Vanquish column compartment, you have to perform the qualification as described in section 3.7.2, page 57.

To create a copy of the template (see section 3.6.3, page 48), do the following:

1. In the Browser, click **Qualification** > **Instruments OQ…** or **Instruments PQ…**.
   A wizard guides you through copying of the sequences.
2. To go to the next step, click **Next**.
3. Select the timebase for which you want to perform OQ or PQ.
4. Enter the name of the computer on which the timebase is installed.
5. Select the source directory of the template to be used.

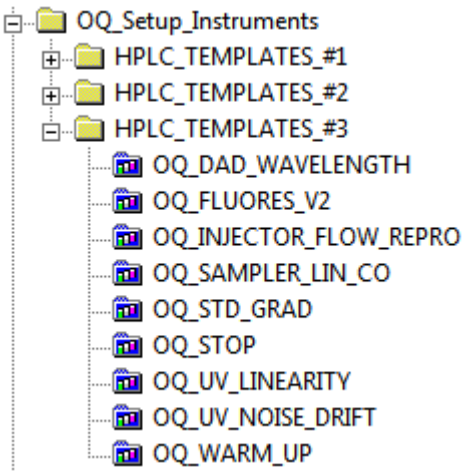

Figure 19: Selecting the source directory of the template to be used

Each directory in the **OQ_Setup_Instruments** folder contains a series of module-specific template sequences (see section 3.6.3, page 48), as shown for the **HPLC_TEMPLATES_#3** directory.

6. To select the OQ_Setup_Instruments directory, click **Browse**.
   A list of directories with the instrument-specific sequence templates is shown:

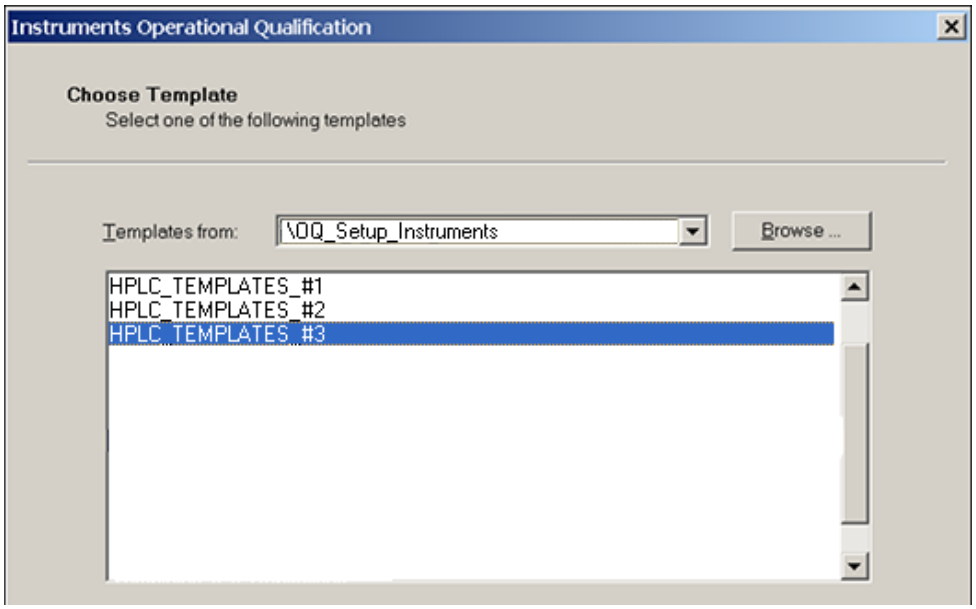

Figure 20: Selecting a template directory

7. Enter a unique name for saving the copy (default: template name + date).
   A list of all sequences of the corresponding template is displayed.
8. Click to select the sequences required for the checks (see chapter 6, page 92).
   A copy is created. After the sequences have been copied, the batch list of the corresponding timebase is automatically opened.
9. Start the batch to run the sequences.
   The batch list contains the checks in the following order:

Order	Checks	Sequence
1.	Fluid preparation of the system	Warm up
2.	Temperature accuracy of the column compartment for manual data acquisition	Column Oven
3.	Wavelength accuracy of the UV detector	Wavelength
4.	Baseline noise, and drift, of the UV detector	UV Noise Drift
5.	Precision of injection volume and flow	• Injector Flow Repro • Injector Flow Repro_DGP_Left (optional)
6.	Linearity of the UV detector	UV Linearity

Order	Checks	Sequence
7.	Linearity of the injection volume	Sampler Lin CO
8.	Carry-over by the autosampler	Sampler Lin CO
9.	Baseline noise, signal height, and wavelength accuracy of the fluorescence detector	Fluorescence or Fluores_V2
10.	Baseline noise and drift of the RI detector	RI_Noise_Drift
11.	Linearity of the RI detector	RI_Linearity
12.	Baseline noise of the evaporative light scattering detector	ELS_Noise
13.	Baseline noise of the electrochemical detector	ECD_Noise
14.	Solvent composition of gradient pumps: accuracy, precision, and ripple	• STD_GRAD(_MTK) • LONG_GRAD(_MTK) • MICRO_GRAD • STD_GRAD_DGP_Left • LONG_GRAD_DGP_Left
15.	Solvent composition for ternary high-pressure gradient pumps: accuracy, precision, and ripple between channels C and B	Tern_Grad_C_B
16.	Solvent composition for quaternary low-pressure gradient pumps: accuracy, precision, and ripple between channels C and B	Quad_Grad_C_D
17.	Temperature accuracy of the Vanquish column compartment (VH-C10-A or VC-C10-A) within a limited temperature range during automatic data acquisition – see section 3.4.6, page 40	Column Oven_LT
18.	Temperature accuracy of the column compartment during automatic data acquisition	Column Oven
19.	Resetting the solvent flow rate to 0.05 mL/min and customer-specific parameters	Stop

TIP When you use a manual injection valve, make sure that no air is injected with the samples. Always inject at least three times the sample loop volume, that is, at least 30 µL.

TIP When you start the batch, the following warnings may appear:
{SOLVENT_CHANGE (91)} SOLVENT_CHANGE (91): Warning P0001: The program start time is undefined.
{SOLVENT_CHANGE - Sampler} Missing inject command.
{Pump} Eluent %A changed from Methanol to Water. Is this correct?
{OQ_COLUMN_OVEN (64) – TemperatureOven} Setting of property 'Average' overrides channel type default.

[Warning] {LONG_GRADIENT (161) - Pump} Ramp step duration (0.000166666666666667 min) is out of range. Minimum supported duration is 0.01 min. Minimum duration will be used.
Warning: The flow ramp between 0.000 and 1.000 min exceeds the maximum flow acceleration.
Warning: Sampler Program script for sample No. x contains no "Method" property assignment.
Message: UV For acquisitions at wavelengths > 345.0 nm and <= 670.0 nm, both lamps should be on.
Warning: ECDRS Cells should be turned on before starting a queue. Otherwise, loss of data will occur.

TIP For systems that are controlled by the Agilent Control Framework, the following warnings may appear (repeatedly):
{GRADIENT - LCSystem} Automatically resolving method inconsistencies. Inconsistencies: Parameters for Thermostat are set, but not supported in current configuration.
Warning: GRADIENT (Instrument Method) LCSystem automatically resolving method inconsistencies. Inconsistencies: Parameters for Emulation Mode are not set.

10. Proceed to one of the following tasks:
    - If qualifying non-Vanquish column compartments in HPLC systems including a Vanquish detector with LightPipe flow cell, proceed to section 3.7.2, page 57.
    - Otherwise, proceed to section 3.8 Evaluating the Test Sequences, page 59.

3.7.2 Non-Vanquish Column Compartments with LightPipe Flow Cell

Qualifying non-Vanquish column compartments in HPLC systems including a Vanquish detector and LightPipe flow cell: To protect the LightPipe flow cells, the qualification of non-Vanquish column compartments in systems with a Vanquish detector and LightPipe flow cell according to the general checks in section 3.7.1, page 54, is not supported. However, if you want to perform also qualification of a column compartment, you need to qualify the system in two steps: With installed LightPipe flow cell and without an installed LightPipe flow cell.

1. Qualify all modules except the column compartment with an installed LightPipe flow cell: Perform qualification according to the general checks (see section 3.7.1, page 54); the test for the qualification of the column compartment will not be offered.
2. Qualify the column compartment without an installed LightPipe flow cell:
   a) Remove the flow cell from the detector.
   b) Disconnect the inlet and outlet capillaries from the flow cell.
   c) Interconnect them with a connecting union.
   d) Close the detector doors.
   e) Start the Qualification Wizard for a second time.
   f) Select only the temperature accuracy test for the column compartment.

g) Add the sequence template to the batch list.

h) Click **Start**.
The sequences **Warm up** and **Stop** will not be repeated.

3. Proceed to section 3.8 Evaluating the Test Sequences, page 59.

3.7.3 Duration

If the column compartment and the non-UV detectors are not included in the check, the entire qualification takes approximately 3.5 hours. The additional duration for the other general checks is as follows (see chapter 4, page 64, for the duration of the special checks):

Module Type	Variant	Additional Duration
Pumps	• Pumps with mixing chamber extension (Summit, UltiMate, Vanquish) • Dual gradient pumps (standard configuration) • UltiMate LPG-3400M(B) pump • UltiMate LPG-3400BM pump • Vanquish VH-P10-A pump	2 hours
	• Dual gradient micro pumps (Summit, UltiMate) • Dual gradient pumps with mixing chamber extension (Summit, UltiMate, Vanquish)	4 hours
Thermostatted column compartment	-	3 hours
Detector	RI detector	1.5 hours
	ELS detector	0.5 hours
	ECD detector	1 hour
	FLD detector	1 hour
Ternary high-pressure gradient system (channels C and B)	-	2 hours
Vanquish Core system with method transfer kit	-	1 hour
System with quaternary, analytical pump (channels C and D)	-	2 hours
System with quaternary, micro pump (channels C and D)	-	4 hours

After the wavelength accuracy of the UV detector has been checked, that is, after approximately 15 min. or 3 h 15 min., you are prompted to change the solvent for channel A(1) from methanol to water. If necessary, connect the fluid components of the RI detector to the system. If an autosampler is installed, OQ/PQ will then run automatically.

TIP It is not necessary to change the solvent manually when qualifying systems with a single wavelength detector (including VWD-3400RS, VF-D40-A and VC-D40-A) as UV detector.

When qualifying the ERC RefractoMax524 RI detector (micro variant), you will be prompted to fluidically disconnect the detector from the system at the end of the **RI Linearity** sequence, as the qualification sequence uses flow rates outside the detector specification.

NOTICE For Agilent systems that are controlled via the Agilent Instrument Control Framework (ICF), the procedure for changing the eluent takes several samples. Check the Audit Trail for log commands that describe the necessary manual tasks. After the "Solvent change step 1" sample, the batch is aborted. You are asked to change the eluent to channel A(1) and restart the batch. No more manual tasks are required afterward.

3.8 Evaluating the Test Sequences

Before evaluating the detector linearity:

1. Enter the actual concentrations for the used standards into the amount columns of the QNT file.
2. Check whether the peak height of the sample with the highest concentration covers the linearity range as described in sections 6.2.6 and 6.2.7 for your detector. If it does not, adapt the injection volume for all samples used for the linearity check in a way that the peak height of the sample with the highest concentration covers the linearity range and repeat the check (see section 6.3.4.3, page 149).

The master sequences on the Chromeleon CD and, thus, all copies made from it for OQ and PQ are linked to the corresponding report.

NOTICE Do not change this report (except certain sheets, see section 3.6.4, page 52). The report contains many references between data sheets. If you insert or delete lines and columns, these references will be lost and the calculations will be wrong.

There are two possibilities to evaluate and sign the report:

- Paper based

- Electronically

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

1. Select the sequence for which you want to print the report.
2. Verify that no sample is selected.
3. Click **File** > **Batch Report**.
4. To start printing, click **OK**.
5. In the lines **Submitter / Operator:** and **Reviewer:** Sign it manually including the date. For each test, the signature area is located at the bottom of the first page.

Electronic Signature

1. Select the sequence that you want to evaluate and sign electronically.
2. Click **File** > **Electronic Signature** > **Submit Results…**.
3. To start signing the results, click **OK**.
4. Check signed results and, if the results are correct, click **OK**.
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…**.

3.9 Repeating Individual Checks

It may be necessary to repeat one or several checks. In this case, refer to section 6.4, page 165. This section provides possible causes for the failure. According to GLP, you have to repeat all checks following the one that failed. The reason is that almost all checks require that the previous check be passed successfully.

Example: If the UV detector linearity check fails, the results regarding the linearity of the injection volume are questionable because the detector linearity is a prerequisite for checking the injection volume.

3.10 Known Restrictions

3.10.1 Thermo Scientific Vanquish System

3.10.1.1 Vanquish Column Compartments

The Vanquish column compartments (VH-C10-A, VC-C10-A) are not supported in Combined Mode. When using more than one column compartment within a timebase, disable the Combined Mode in the Chromeleon Server Configuration program and add the column compartment that you want to test. The column compartments need to be qualified one by one. For the qualification of the first column compartment, use the sequence template from the qualification setup. For all subsequent devices, you have to create a copy of the sequence template manually.

3.10.1.2 Vanquish Detector with LightPipe Flow Cell

The qualification of a column compartment for an HPLC system that includes a Vanquish detector with LightPipe flow cell is only supported for Vanquish column compartments. All other column compartments are not supported, therefore, no qualification sequence is offered for column compartments other than the Vanquish column compartment. For information about how to qualify such systems, see section 3.7.2, page 57.

3.10.1.3 Vanquish Detector with 60 mm LightPipe Flow Cell

The qualification of the Vanquish autosampler linearity with a sample loop volume ≥ 100 µL within an HPLC system that includes a Vanquish detector with 60 mm LightPipe flow cell is only performed up to an injection volume of 50 µL. If the entire volume range should be qualified, a flow cell with 10 mm light path is required.

3.10.1.4 Instrument with Vanquish ISQ Family Mass Spectrometry Detector

Qualifying an HPLC front end (solved with HPLC OQ/PQ revision 9.5)

Trying to qualify an HPLC front end of an instrument containing a Vanquish ISQ Family Mass Spectrometry detector will result in queue ready check errors that prevent you from starting the front end qualification (this restriction is documented with ID CM7-25425 in the Thermo Fisher Scientific tracking system). Perform the following workaround to qualify the front end:

1. Remove the Vanquish ISQ Family driver in the Chromeleon Instrument Configuration Manager.
2. Create the qualification sequences (section 5.3, page 81).

3. Perform the qualification.
4. Add the Vanquish ISQ Family driver to the instrument in the Instrument Configuration Manager.

Editing Instrument Methods (1 to 3 solved with HPLC OQ/PQ revision 9.5)

Editing of instrument methods can result in the following queue ready check errors that prevent you from starting the MS detector qualification (this restriction is documented with ID SWFR-3466 and CM7-25893 in the Thermo Fisher Scientific tracking system). Either avoid editing instrument methods or perform the following workaround:

1. For the following errors, move all MS detector related properties located in the **Inject** stage to the **Run** stage:
   Error: Instrument Method: The ResolutionTuneTable command must be in the Instrument Run stage at retention time = 0.0 s.
2. For the following errors, move all MS detector related properties located in the **Post Run** stage to **Stop Run** stage.
   Error: Instrument Method: Cannot set SpectrumType to Profile in a DoSIMScan command.
   Error: Instrument Method: Command StopAcquisition must be defined in the Instrument StopRun stage.
3. For the following errors, select the specific instrument method device view of the MS detector.
   Error: Instrument Method: Required method property FullScanAcquisitionThreshold is missing.
   Error: Instrument Method: Method property FullScanAcquisitionThreshold must be a value between 1000 and 10000.
4. For the following error, set the correct associated pump info in the Chromeleon 7 instrument configuration manager. The device names of the main and pump device must be identical with the used names in the pump driver. For dual pumps, set the pump device name of the right pump unit.
   Error: Instrument Method: Tuning requires a pump flow rate of 0.050 mL/min.

3.10.2 Agilent ICF

NOTICE
Notes on instrument methods:
When you change the instrument method from the Agilent ICF method editor, this will change the order of the commands in the script view, or commands will be added to the

script view. Therefore, *do not open* the Agilent ICF method editor and *do not save any changes*, as this results in an invalid instrument method.

NOTICE
Notes on compatibility:
When incompatible versions are used (HPLC OQ/PQ versus Chromeleon versus Agilent ICF), the below error or a similar error may occur during Ready Check, depending on the instrument configuration. Therefore, only use supported version combinations (see section 2.2, page 13).
[Error] {OQ_COLUMN_OVEN - MySystem} Manual method resolution required.
Inconsistencies: Xml schema version mismatch (xml version: 1.0.5, expected version: 1.0.3)
No schema version upgrade available for xml version 1.0.5
Invalid xml
Parameters for Column Valve Position are not set.

3.10.3 Miscellaneous

PGM / Instrument methods: The upper pressure limit is set to the value defined in the driver (server configuration). In rare cases, this value can be above the allowed range (due to rounding discrepancies) that results in a ready check error. To solve this issue, do the following:

1. Open the server configuration.
2. Open the pump driver.
3. Select the **Limit** tab.
4. Enter the maximum allowed pressure value and press **OK**.
5. Create a new set of qualification templates via the Wizard.

4 Special Test Procedures for Individual Modules

4.1 Introduction

This section describes test procedures that fundamentally differ from the procedures described in chapter 3, page 15. These special procedures can only be used for certain modules. In addition, all test sequences must be run one after the other, as the tests require different system configurations. The test procedures described in chapter 3, page 15, and section 6.3, page 136, serve as a basis for the descriptions below and this section focuses on the differences in particular. When the test steps are identical, you will find a reference to these sections.

The sequence templates for these tests are available in the **SPECIAL_HPLC_TEMPLATES** directory (see Figure 1, page 46). Start the OQ/PQ Setup from this directory as described in section 3.6.3, page 48.

4.2 UltiMate VWD-3x00 Detectors

4.2.1 Noise and Drift with Dummy Flow Cells

For qualifying the VWD-3100 and VWD-3400 detectors with the dummy flow cell, two sequences will be offered.

- **UV_NOISE_DRIFT_VWD3x00**: Noise and drift measured at a wavelength of 254 nm
- **UV_NOISE_VWD3X00_230nm**: Noise measured at a wavelength of 230 nm

These sequences can be used only for the above detectors and the test procedure requires that the flow cell be changed twice. If you want to perform both tests in a row, you do not have to exchange the flow cell in-between the sequences. However, you still have to manually confirm the related messages.

The following table shows the drift and noise limits for dummy flow cells at a certain wavelength. You need to enter the specifications into the report manually.

Module	Parameter	Description	Limits[1]	
			OQ	PQ
VWD-3100/VWD-3400RS (dummy flow cell)	Baseline Noise	Wavelength: 254 nm Measured with the dummy flow cell that is shipped with the detector (without fluidics).	0.010 mAU	0.020 mAU
	Drift		0.2 mAU/h	0.2 mAU/h
	Baseline Noise	Wavelength: 230 nm Measured with the dummy flow cell that is shipped with the detector (without fluidics).	0.004 mAU	0.008 mAU

[1] OQ limits with optimum measuring conditions, recommended PQ limits.

4.3 Thermo Scientific Autosamplers / Charger

4.3.1 Sample Temperature Accuracy

This section describes how the sample temperature accuracy is determined for the following autosamplers and chargers:

- UltiMate 3000 (all thermostatted WPS- / ACC-3000 autosampler)
- Thermo Scientific Accela
- Vanquish autosampler / Charger

4.3.1.1 Tips for Known Restrictions

TIPS

- *For UltiMate modules with a firmware version ≥ 4.07* and *Vanquish autosampler ≥ 1.03):*
  If the check is interrupted, the carousel movement is not automatically turned on afterward. In this case, repeat the check or use the **Tray_Rotation_On** program to turn it on again. Simply add the program to the batch (object type: Program) and start the batch.
- *For UltiMate modules with firmware version < 4.07:*
  These modules do not support carousel rotation. When you issue a command that relates to carousel rotation, the following error message is displayed in the Audit trail:
  [Error] 13:50:25 0.000 {Sampler} Unknown property. Perform a driver and/or firmware update.
- *For Vanquish autosamplers with firmware version < 1.03:*
  The test is not supported. Perform a firmware update to version 1.03 or a later version.

4.3.1.2 Parts Required

The table lists the materials required for performing the test.

Material Required	Part No.	Remark	Quantity
Column Compartment PQ Kit	6732.0010	-	1
Type K temperature sensor for P600/P700 thermometers	6820.0010	-	1
Standard glass vial (1.8 mL) with water	-	Fill the vial with water and seal it with a slotted septa if available to improve the fixing of the sensor, otherwise do not seal it	1

4.3.1.3 Preparing the System

1. Connect the temperature sensor type K to the thermometer and make the necessary settings (sensor type and calibration values) as described in the Operating Instructions for the thermometer.

2. Fill a standard glass vial (1.8 mL) with water (ideally close it with slotted septa to improve the fixing of the temperature sensor, otherwise let it open) and place it at the following sample position:
   - UltiMate autosampler: RC8.
   - Vanquish autosampler: R:C8
   - Vanquish Charger: shelf position: any; position 2 is recommended due to a favorable temperature sensor cable routing.
   - Accela autosampler: C1.
3. Accela autosampler only: Place another standard glass vial with water at sample position A8.

4.3.1.4 Configuring the System

Read the information in section 3.4.5 on page 38 under " Option A: Using the Column Compartment PQ kit ".

4.3.1.5 Preparing Chromeleon

To qualify the sample temperature accuracy, select the following sequence: For the autosampler, select **SAMPLER_TEMP_ACC**. For the Charger, select **CHARGER_TEMP_ACC**.

4.3.1.6 Performing the Check

1. Start the batch containing the **SAMPLER_TEMP_ACC** and / or **CHARGER_TEMP_ACC** sequence(s).
   The sample temperature is set (ACC-3000T: 15°C, Accela autosampler: 30°C, all others: 10°C) automatically. If necessary, automatic carousel movement is stopped.

NOTICE – General
Movements of the needle arm or carousel may damage the thermometer or module. Do not perform any autosampler or Charger commands during the test.

2. When a message box prompts you to position the temperature sensor, insert the temperature sensor into the vial from above at a right angle until the tip touches the vial bottom.

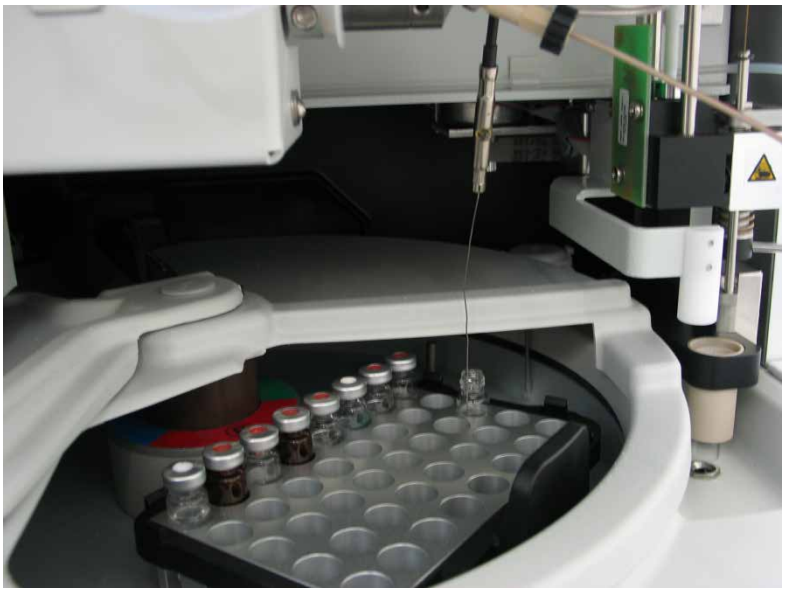

Figure 21: Temperature sensor inserted into the vial (here: an UltiMate autosampler as an example); the sensor cable can be fixed at any available capillary.

Figure 22: Positioning the temperature sensor in the vial (here: Vanquish autosampler); the sensor cable can be led out at the left door side

NOTICE – Vanquish Charger

After closing the Charger door, the Charger performs an inventory scan. To avoid any collision with the mover:

- Place the temperature sensor horizontally and near the door.
- Make sure the tray position is still correct after installing the temperature sensor.

3. Charger only: When a message box prompts you to position the temperature sensor, insert the temperature sensor into the vial as shown in the image.

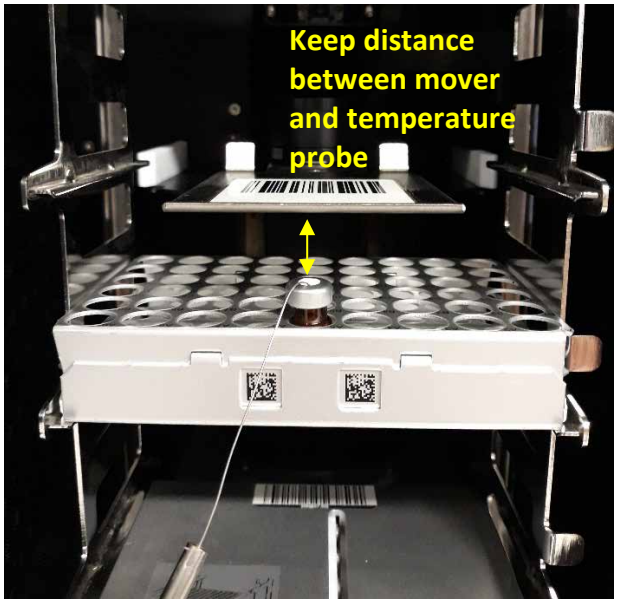

Figure 23: Position of the temperature sensor and tray (Vanquish Charger)

4. Only UltiMate autosampler: Rotate the carousel until the carousel cover closes completely.

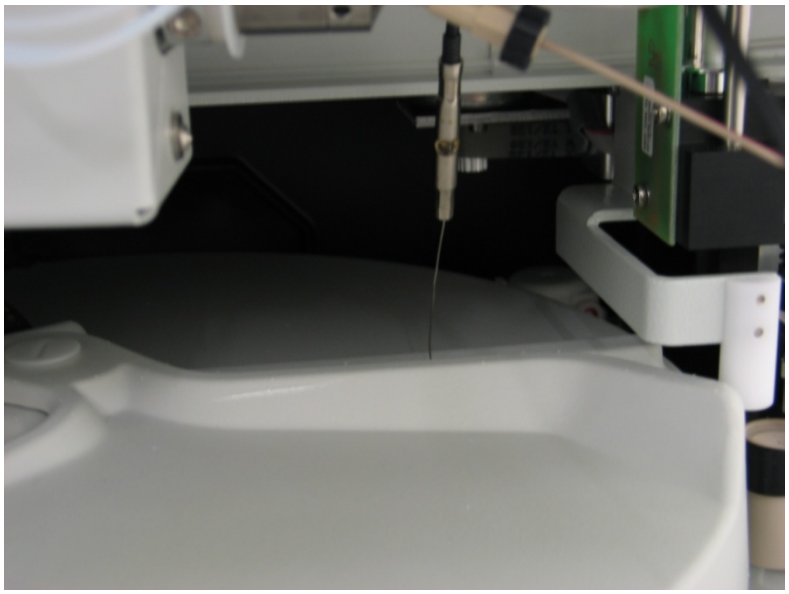

Figure 24: Closed carousel cover (here: UltiMate autosampler)

5. Only Thermo Scientific autosampler: Firmly close the autosampler door(s).

6. Confirm both Chromeleon messages at the start of the check.
   When the nominal temperature is reached, the external thermometer is used to record the sample temperature over a period of 60 minutes.
7. When you are prompted to remove the temperature sensor at the end of the check, remove the temperature sensor.
8. Confirm the Chromeleon message.
   The autosampler movement is restarted.

4.3.1.7 Duration

The test takes approximately 75 minutes for each module (autosampler or charger).

4.4 Thermo Scientific Charged Aerosol / Corona Detector

In addition to the detector to be qualified, this test requires an HPLC pump and autosampler.

TIP A supported UV detector is required to qualify the other system modules.

4.4.1 Prerequisite

Before qualifying the detector, make sure that the other modules in the system (for example, the pump and autosampler) have been qualified successfully. Qualification of the other system modules requires a supported UV detector. In this case, note the information on Corona detectors in section 3.5, page 42.

4.4.2 Parts Required

The table lists all items required for qualifying the detector.

Part	Part No.	Description
Corona Qualification Kit	6081.2250	Includes the following parts: • Shiseido pre-column holder, 20 mm, for 2 and 4 mm ID pre-column cartridges (part number 88-12414) • Shiseido C18 pre-column, 20 x 4.0 mm, 3 µm (see tip below) (part number 88-12307) • Standards kit including vials with five different caffeine solutions (part number 70-6565)

Part	Part No.	Description
Eluent A: water / methanol (80:20% v/v), degassed	-	Approx. 500 mL Water, ultra-pure Type 1 grade rated for resistivity of 18.2 MΩ cm at 25°C, TOC no more than 5 ppm, for example, Water, Ultra Trace Elemental Analysis Grade (part number 12038598) or Water W8-1, Optima UHPLC/MS Grade (part number 15339865). LC-MS-grade methanol, for example, OptimaTM LC/MS Methanol from Fisher Scientific.
Glassware	-	Class A, triple rinsed prior to use

4.4.3 Configuring the System

Ensure that the digital signal of the detector is enabled in the server configuration (unit: pA).

4.4.4 Preparing the System

TIP Equilibrate new pre-columns at a flow rate of 1 mL/min for at least for 15 minutes, using 100% methanol.

1. The standards kit includes vials with five different caffeine solutions. Fill the solutions into 1.8 mL autosampler vials and position them in the autosampler as shown in the table below.

Sample Position – Sampler Variant				Substance	Concentration [µg / mL]
Summit / UltiMate	Vanquish[1]	Vanquish[2]	Any		
RD1	R:C7	R:D1	25	Caffeine in water	5
RD2	R:C8	R:D2	26		25
RD3	R:C9	R:D3	27		125
RD4	R:D1	R:D4	28		250
RD5	R:D2	R:D5	29		500

[1] For sample holder with a capacity of 54 sample vials.

[2] For sample holder with a capacity of 40 sample vials.

2. Ensure that:

   a) Gas flow is on for a sufficient period of time (> 5 min - see *Operating Instructions*).

   b) Gas pressure is within the required range:
      - Corona, Corona ultra series: 35 psi ± 1 psi
      - Corona Veo™ and Vanquish series: 55-65 psi

   c) Pump flow is still turned off.

3. Change the eluent from channel A(1) to water / methanol (80:20% v/v).
4. Purge the system for at least 5 minutes at a flow rate of 5 mL/min.
5. Make sure that the capillary at the column outlet is not connected to the detector, but goes directly to the waste.
6. Install the required column.
7. Equilibrate the system at a flow rate of 1 mL/min for at least 15 minutes.
8. Connect the capillary from the column outlet to the detector inlet.
9. Equilibrate the system for another 30 minutes.

4.4.5 Starting the Tests

Start the batch with the two qualification sequences **CORONA_(VEO)_NOISE_DRIFT_SNR** and **CORONA_(VEO_)RESP_CALIB**.

4.4.6 Duration

The qualification, that is, running the sequence templates, takes about 55 minutes.

TIP The pump flow and gas flow must be turned off manually after qualification. Note that the pump flow should be turned off approx. 15 minutes before the gas flow (also see related description in the Operating Instructions).

4.5 Thermo Scientific Mass Spectrometry Detector

In addition to the detector to be qualified, this test requires an HPLC pump and autosampler.

TIP A supported UV detector is required to qualify the other system modules.

4.5.1 Prerequisites

Before qualifying the mass spectrometry detector, make sure that the other modules in the system (for example, the pump and autosampler) have been qualified successfully. Qualification of the other system modules requires a supported UV detector.

TIP Prior to performing the qualification, completion of a preventive maintenance routine is highly recommended for the mass spectrometry detector.

4.5.2 Parts Required

The table lists all items required for qualifying the mass spectrometry detector.

Part	Part No.	Description
Column	25005-052130	Hypersil™ Gold, 2.1 x 50 mm, 5 µm
Reserpine	HAZMAT-01-00081 or equivalent	Stock solution (100 pg/µL); ≥ 98% purity; LCMS grade reagent
p-Nitrophenol	FM101945 or equivalent	≥ 99% purity; LCMS grade reagent
Calibration solution kit	1R120590-6204	-
Water	W6-4	Fisher Scientific™ Optima™ LC/MS grade or Type I ultra-pure water from a purification system that can provide high resistivity and low conductivity water rated at 18.2 MΩxcm with < 5 ppb total organic content (TOC)
Water and 0.1% formic acid	LS118-4	Optima LC/MS grade
Methanol	A456-4	Optima LC/MS grade
Bottles	-	1 L
Graduated cylinders	-	10, 100, 500, 1000 mL
Precision pipettes	-	Calibrated and capable of measurements in the 0.1, 1, 5 mL ranges.
Miscellaneous	-	Containers for preparing the standard dilutions and vials.

4.5.3 Configuring the System

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 right pump unit in the **Associate pump info** tab (the flow rate of the left pump unit is set automatically to 0 mL/min).

4.5.4 Preparations

4.5.4.1 Cleaning Solvent Bottles

Prior to using a new HPLC solvent bottle, clean it as follows:

1. Rinse with standard laboratory detergent.
2. Rinse thoroughly with Fisher Scientific Water Optima LC/MS Grade (W6-4) or type I ultra-pure water until no soap bubbles are seen.
3. Rinse with ultra-pure water.
4. Rinse with methanol.
5. Rinse with ultra-pure water.
6. Rinse the lid with ultra-pure water.

4.5.4.2 Solvents

Reserpine eluent preparation (for qualification of positive ion mode with HESI probe)

1. Using a clean 500-mL or larger graduated cylinder, prepare a water + 0.1% formic acid / methanol (35:65 v/v %) solution.
2. Transfer to a clean HPLC solvent bottle and ensure that it has been mixed thoroughly.

p-Nitrophenol eluent preparation (for qualification of negative ion mode with HESI probe and positive / negative ion mode with APCI probe)

1. Using a clean 500-mL or larger graduated cylinder, prepare a water / methanol (35:65 v/v %) solution.
2. Transfer to a clean HPLC solvent bottle and ensure that it has been mixed thoroughly.

4.5.4.3 Standards

TIP The reserpine standard is temperature sensitive and should be stored at 4°C.

Reserpine standard preparation (for qualification of positive mode with HESI and APCI probe)

1. Using the prepared eluent, transfer 10-50 mL to a secondary container to use for diluting the reserpine stock (100 pg/µL).
2. Aliquot 1 mL of the prepared eluent into an empty HPLC vial for a solvent blank.

3. Prepare an accurate 1:10 dilution (10 pg/µL final) of the reserpine stock standard and label R1; mix thoroughly.

> **TIP** Pipettes should be calibrated or an accurate dispensing manual pipette should be used. Use adequate volumes for accurate dilutions.

4. Transfer the R1 standard to an HPLC vial.

p-Nitrophenol standard preparation (for qualification of negative mode with HESI and APCI probe)

1. Using the prepared eluent, transfer 10-50 mL to a secondary container to use for diluting the p-nitrophenol stock (1 mg/mL).
2. Aliquot 1 mL of the prepared eluent into an empty HPLC vial for a solvent blank.
3. Prepare an accurate 1:100 dilution (10 ng/µL final) of the p-nitrophenol stock standard and label it N1.
4. Mix the dilution thoroughly.

> **TIP** Use calibrated pipettes or an accurate dispensing manual pipette and use adequate volumes for accurate dilutions.

5. Prepare an accurate 1:100 dilution (100 pg/µL final) of the N1 standard and label it N2.
6. Mix the dilution thoroughly.
7. Prepare an accurate 1:5 dilution (20 pg/µL final) of the N2 standard and label it N3.
8. Mix the dilution thoroughly.
9. Transfer the N3 standard to a suitable HPLC vial.

4.5.4.4 Vial Placement in the Autosampler

Place the solvent blanks and standards into the autosampler at following positions:

Sample Position			Substance	Concentration
Summit / UltiMate [1]	Vanquish	Any other		
RD6	R:D3	30	Reserpine solvent blank	-
RD7	R:D4	31	Reserpine standard R1	10 pg/µL
RD8	R:D5	32	p-Nitrophenol solvent blank	-

Sample Position			Substance	Concentration
RE1	R:D6	33	p-Nitrophenol standard N3	20 pg/µL

(1) Summit / UltiMate autosamplers: ASI-100(T), WPS-3000(T)SL / PL, WPS-3000TBPL Analytical, WPS-3000T(B)FC Analytical, WPS-3000(T)(X)RS, WPS-3000(T)PL RS, and ACC-3000(T)

4.5.5 Preparing the System

1. On the mass spectrometry detector, install a new internal reference calibrant reservoir.
2. On the mass spectrometry detector, ensure that the HESI probe is installed. If a HESI / APCI dual source is used, assure that the corona needle is in HESI position.
3. Ensure that the:
   a) Outlet of the autosampler is directly connected to the column.
   b) Capillary from the column outlet is directly connected to the detector inlet port.
4. Change the eluent from channel A(1) to water and 0.1% formic acid / methanol (35:65% v/v).
5. Purge the system for at least 5 minutes at a flow rate of 5 mL/min.
6. Perform the test (see section 4.5.6, page 77).

4.5.6 Performing the Test

General test for all module variants (with HESI probe)

1. Create the test sequence [select special test: ISQ MS Detector Mass Calibration, Sensitivity (HESI)] for the sensitivity test with the HESI probe according to section 5.3, page 81.
2. Start the queue with the qualification sequence **ISQ_Sensitivity** (requires HESI probe). After finishing the sensitivity test for the positive ion mode (reserpine injections), the queue is aborted after the injection **Solvent change**. Proceed manually with following steps.
3. Change the eluent from channel A(1) to water / methanol (35:65% v/v).
4. Purge the system for at least 5 minutes at a flow rate of 5 mL/min.
5. Start the queue.
6. After the sequence is finished, proceed with the additional test for the module variant with APCI probe if available.

Additional test for module variant with APCI probe option

7. On the mass spectrometry detector, stop pump flow, install the APCI probe and move the corona needle of the source in APCI position.
8. Create the test sequence [select special test: ISQ MS Detector Sensitivity (APCI)] for the sensitivity test with APCI probe according to section 5.3, page 81.
9. Start the queue with the qualification sequence **ISQ_APCI_Sensitivity** (requires APCI probe).

4.5.7 Duration

- The qualification procedure for the HESI source takes about 3 hours.
- The qualification procedure for the APCI source takes an additional hour.

TIP The system has to be set in standby mode manually after qualification.

4.6 Agilent G1321 Fluorescence Detector – Linearity

4.6.1 Parts Required

To determine the linearity of the Agilent 1100/12x0 series G1321 fluorescence detector, you need:

Part	Description
Chromatographic column	For example, Thermo Scientific Acclaim™ 120 (C18, 5 µm, ID: 4.6 mm, length: 100 mm) or similar. This column can be ordered under part no. 059147.
Eluent A: acetonitrile / water (90:10% v/v)	Approximately 200 mL
Standards	Concentration
Acetonitrile / water	90:10 % v/v
Anthracene in acetonitrile	0.5 mg / 100 mL
Anthracene in acetonitrile	0.4 mg / 100 mL
Anthracene in acetonitrile	0.3 mg / 100 mL
Anthracene in acetonitrile	0.2 mg / 100 mL
Anthracene in acetonitrile	0.1 mg / 100 mL

Part		Description
	Anthracene in acetonitrile	0.05 mg / 100 mL
	Anthracene in acetonitrile	0.005 mg / 100 mL

4.6.2 Preparations

Place the standard vials into the positions shown in the table.

Substance	Concentration [mg / 100 mL]	Sample Position
Acetonitrile / water (90:10 % v/v)	-	15
Anthracene in acetonitrile	0.5	16
Anthracene in acetonitrile	0.4	17
Anthracene in acetonitrile	0.3	18
Anthracene in acetonitrile	0.2	19
Anthracene in acetonitrile	0.1	20
Anthracene in acetonitrile	0.05	21
Anthracene in acetonitrile	0.005	22

4.6.3 Performing the Check

1. Start the batch containing the **FLUORES_LINEARITY** sequence.
2. When Chromeleon prompts you in the first sample of the **FLUORES_LINEARITY** sequence, exchange the eluent and install the column.
   In the next sample, the system is prepared for the measurement. Therefore, manual equilibration can be omitted.

   At the end of the sequence, the eluent remains in the system.

TIP
Note on eluent change:
For Agilent systems that are controlled via the Agilent Instrument Control Framework (ICF), the procedure for changing the eluent is divided into several samples. Check the Audit Trail for log commands that describe the necessary manual tasks. After the "Solvent change step 1" sample, the batch is aborted. You are asked to change the eluent on channel A(1) and restart the batch (the system still includes the restriction tubing from the general qualification tests performed earlier). After the "Solvent change step 3" sample, the batch is aborted again. You are asked to install the required column and restart the batch.

5 Chromeleon 7

5.1 Chromeleon 7 Terminology

TIP Note that Chromeleon 7 terminology is different from the terminology used in Chromeleon 6.80. For details, refer to the *Glossary - Chromeleon 7.x* that is available in the Documents folder of your Chromeleon 7 installation.

5.2 Supported Modules

In general, all modules listed in section 6.1 on page 93, are supported, if the driver is shipped with the CM7 version in question.

The device and channel names that are used in the instrument methods of the OQ/PQ sequences can be selected by the user as in CM6.x0 OQ/PQ. Only the additional modules required for the qualification of thermostatted column compartments and column ovens require defined names (see section 3.4.1.1, page 33).

TIP A qualification sequence will be offered for the supported column compartments and ovens only if the signal name for the Dostmann thermometer is **TemperatureOven** (see also section 3.4.5 Column Compartment – General Instructions, page 38.

5.3 Creating the Sequences for the Qualification Tests

For performing OQ and PQ tests in Chromeleon 7, it is not required to create and copy the sequence templates from a Chromeleon CD. An Instrument Qualification Wizard automatically performs these steps for you and creates the sequences to be run. No instrument-specific sequence templates are created.

NOTICE For instruments with a Vanquish ISQ Family Mass Spectrometry detector, see the known issue in section 3.10.1.4, page 61.

1. To start the wizard, on the Chromeleon Console, click **Tools** > **Instrument Qualification**.

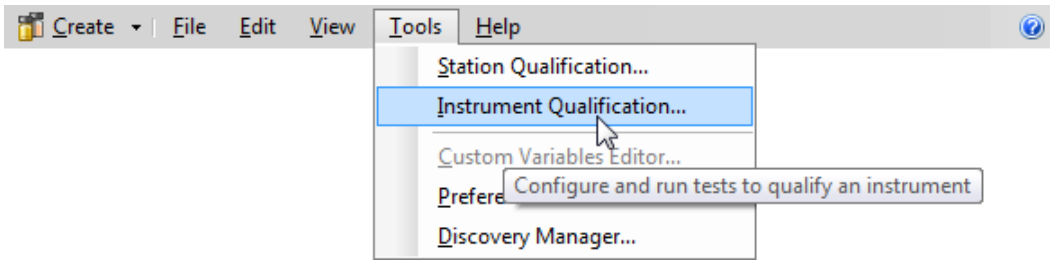

Figure 25: Starting the Instrument Qualification Wizard

2. Select the qualification type:
   - Installation: Qualification of the installation
   - Operational: Qualification in the working environment
   - Performance Qualification: Qualification during routine operation

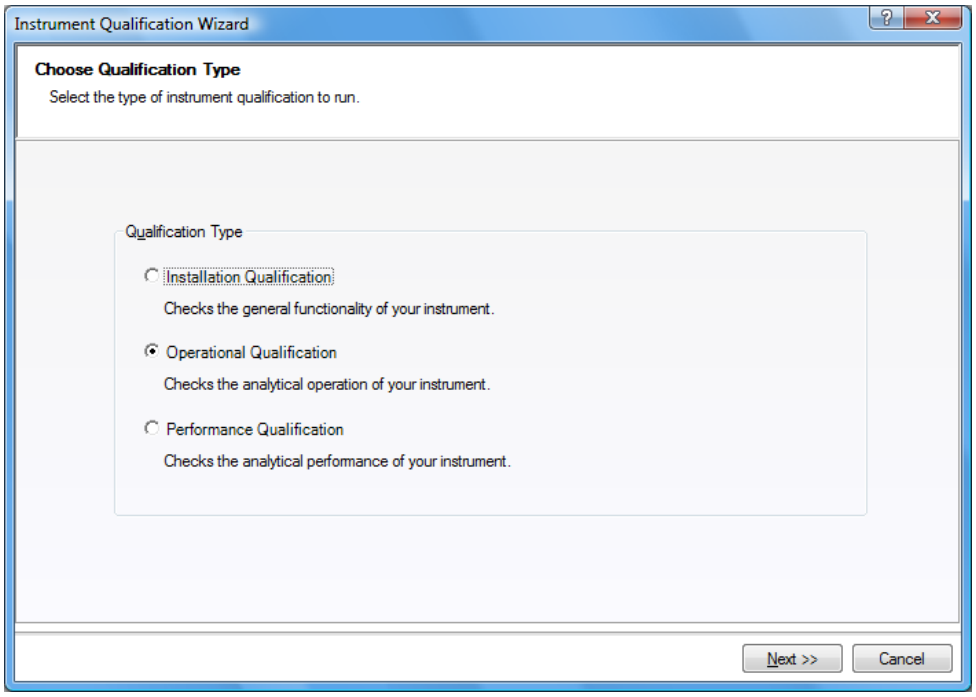

Figure 26: Selecting the qualification type

3. Select the instrument that you want to qualify.

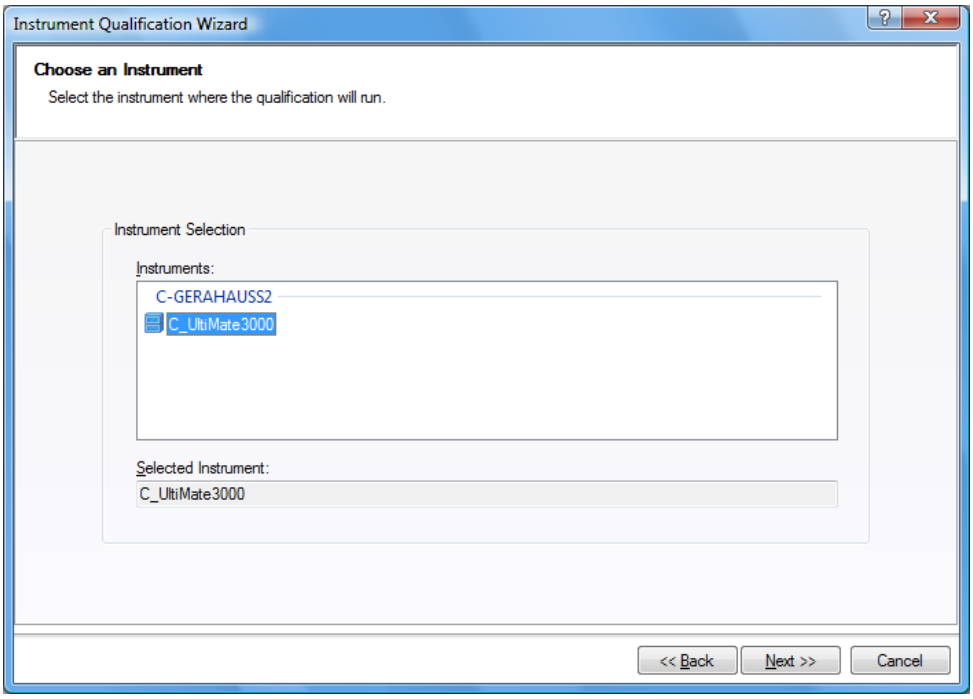

Figure 27: Selecting the instrument

4. To connect the selected instrument to Chromeleon, click **Next >>**.
   The Instrument Connection dialog box shows the connection process.

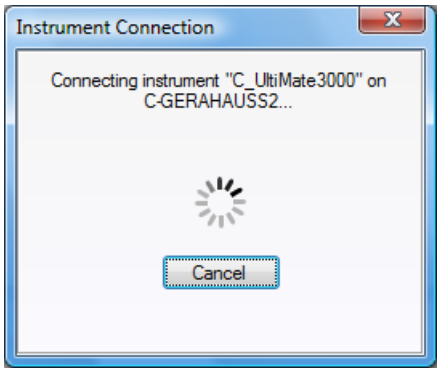

Figure 28: Connecting the instrument

5. On the third wizard page, you can select special test procedures for each module. This option is available only for the modules listed in chapter 4, page 64, and section 5.6, page 90.
   If the selected instrument does not include one of the modules, the wizard page is skipped.

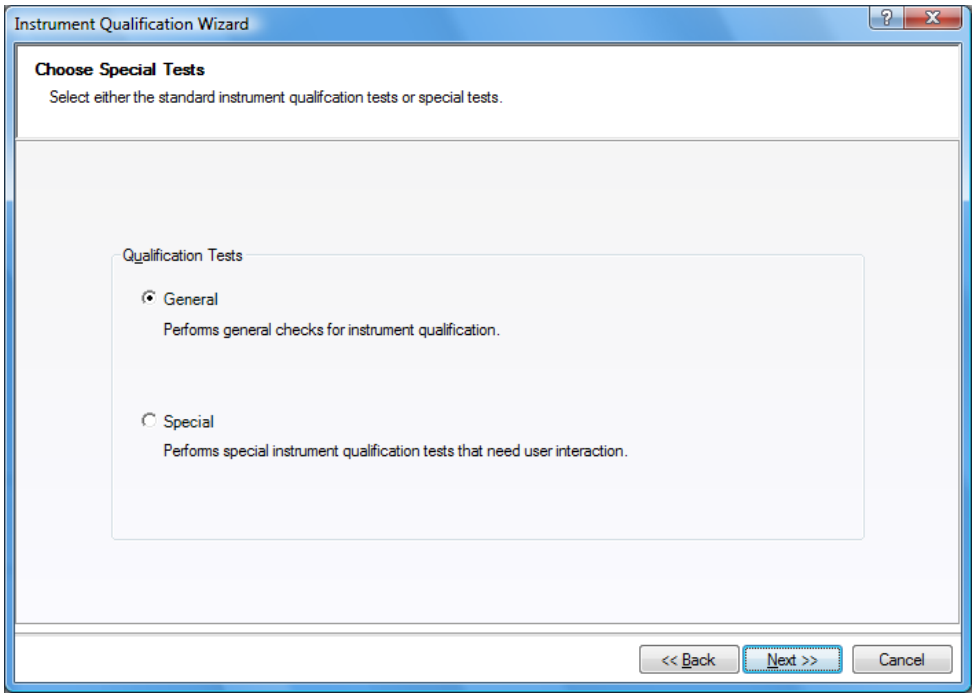

Figure 29: Selecting special tests

6. Click **Next >>**.
   A list of tests is displayed. The list is adapted to the instrument configuration of the selected instrument as defined in the Chromeleon Instrument Configuration Manager.

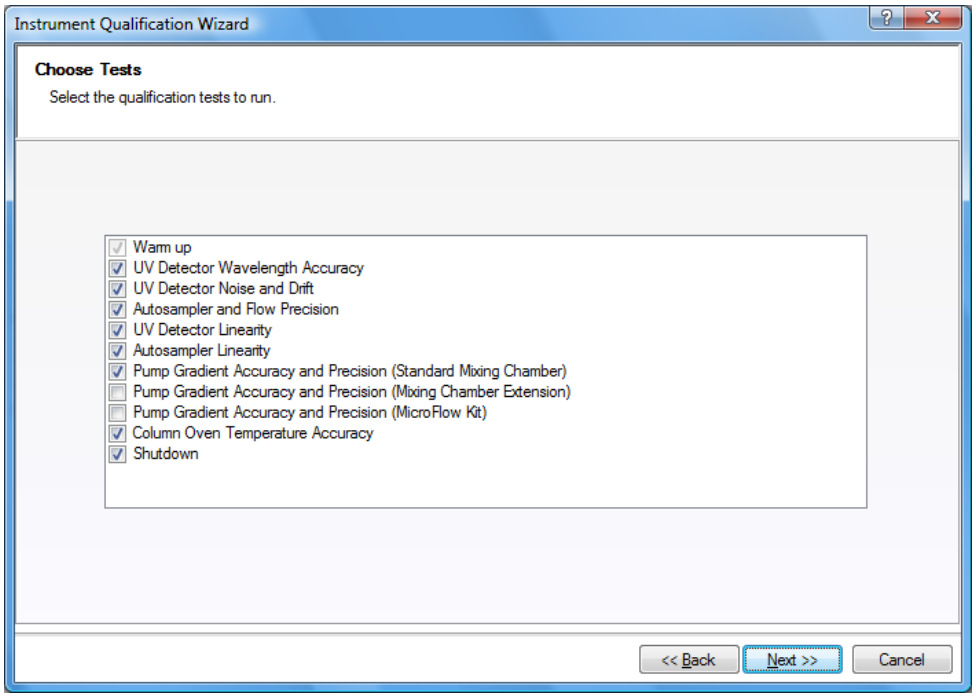

Figure 30: List of tests for the selected instrument

7. Select the sequences that you need for the tests you want to perform.
   Mandatory tests, such as **Warm up**, are shown in the list, but the selection cannot be changed.
8. Click **Next >>**.

9. On the last wizard page, select a unique name under which the OQ and/or PQ sequence directory for this instrument is saved.

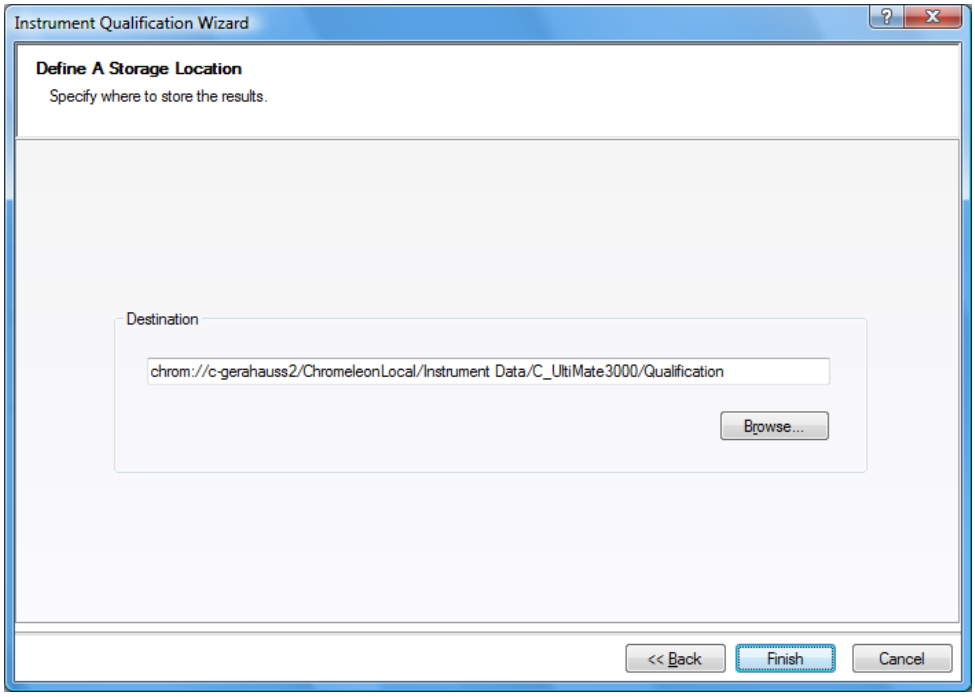

Figure 31: Selecting the storage location

10. Click **Finish**.
    A progress window shows which steps have been performed:

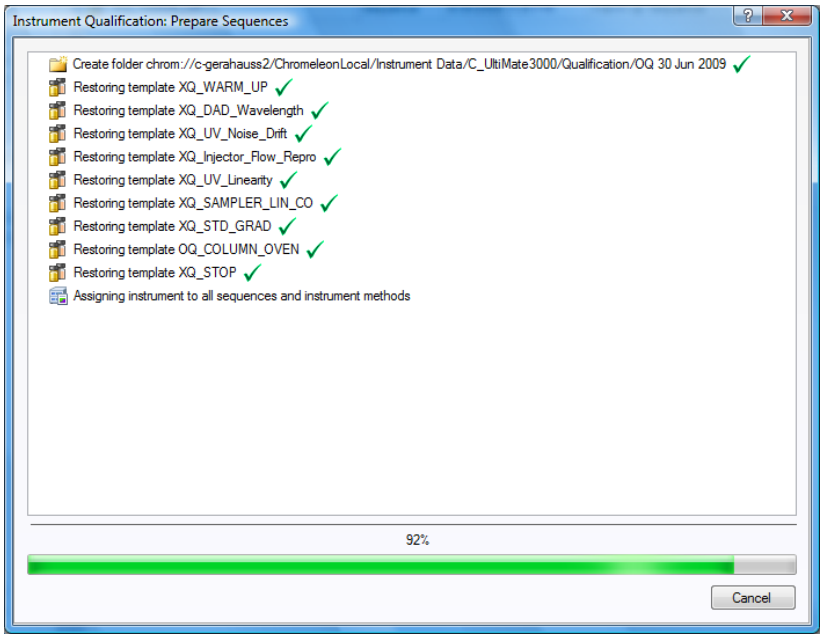

Figure 32: Progress during sequence creation

Finally, the Instrument View dialog box opens, showing the **Queue** tab. It contains all the created sequences.

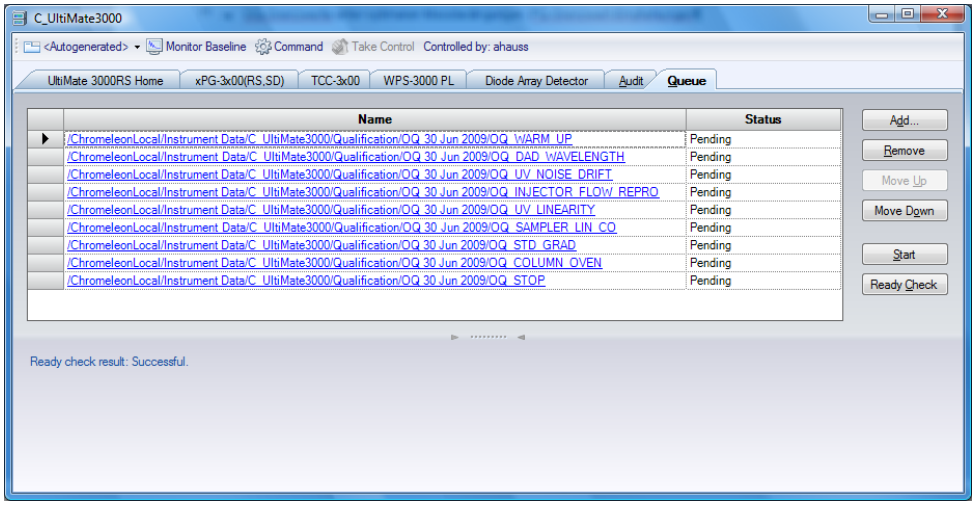

Figure 33: Sequence queue

TIP For ready check warnings that may appear, see section 3.7.1, page 54.

5.4 Performing the Checks

To start the instrument qualification, click **Start** on the **Queue** tab. Chromeleon then runs the sequences.

5.5 Evaluating the Test Sequences

The qualification sequences are saved under the path that was selected in the wizard (see section 5.3, page 81). All sequences are linked to the same report template.

Before evaluating the detector linearity:

1. Enter the actual concentrations for the used standards into the amount columns of the processing method.

2. Check whether the peak height of the injection with the highest concentration covers the linearity range as described in sections 6.2.6 and 6.2.7 for your detector.
   If it does not, adapt the injection volume for all injections used for the linearity test in a way that the peak height of the injection with the highest concentration covers the linearity range and repeat the test (see section 6.3.4.3, page 149).

5.5.1 Adapting the Report

To adapt the report:

TIP Manual inputs are applied automatically to all test sequences.

1. Open the report.

NOTICE Do not change any of the other report sheets. The report contains many references between data sheets. If you insert or delete lines and columns, these references will be lost and the calculations will be wrong.

2. Following cells should be unlocked by default. Otherwise, remove the protection from the SPECIFICATION sheet via the **Home** ribbon and **Protection** group.
3. Enter the following information:
   - Names of customer and tester
   - Sample information such as:
     - Batch number
     - Expiration date
     - Actual concentration of the standard
   - Name of the item that is used to generate the backpressure
     Default: capillary (L: 15 m; ID: 0.18 mm)

TIP Notes regarding the qualification can be documented on the **Specification** sheet of the report on the **Notes** page (field B175). If a remark is entered, delete the text **No remarks**.

4. If applicable, enable the protection of the SPECIFICATION sheet.
5. Save the report.

5.5.2 Evaluation and Signing

There are two main possibilities to evaluate and sign the report:

- Paper based
- Electronically

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

TIP To make sure that Chromeleon reads and processes the data in the report correctly, always print (paper based or electronically) the report from the Chromeleon Console.

To print the report, there are two possibilities:

- In the **Data** category, right-click the sequence for which you want to print the report and then, click **Print Report**.

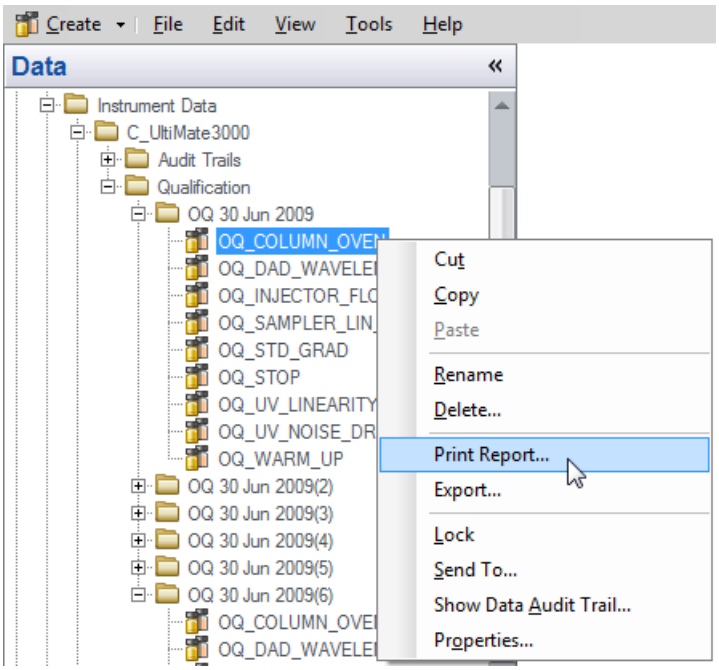

Figure 34: Printing the report via the Data category

- On the **Sequence Editor** toolbar, click **Print**.

Figure 35: Printing the report via the Sequence Editor toolbar

Electronic Signature

1. Select the sequence that you want to evaluate and sign electronically.
2. On the **Sequence Control** toolbar, click **Submit**.
3. To create an electronic report, click **Finish**.
4. Check the results, and, if the tests have passed, enter your signature password and optionally a comment.
5. Click **OK**.
   The names and dates are automatically entered into the line **Submitter / Operator:**.
6. Optionally, repeat all steps above for **Review Results…** and / or **Approve Results…**.

5.6 Selecting Special Test Procedures in Chromeleon 7

In Chromeleon 7, sequence templates for these tests must not be downloaded from the Chromeleon CD - they can be selected directly in the wizard. A wizard page for special tests is displayed for the following modules (also see chapter 4 on page 64 and section 5.3 on page 81):

- VWD-3100
- VWD-3400RS
- Corona (all supported variants)
- Vanquish ISQ Family
- Vanquish autosamplers and chargers (and Dostmann Thermometer with channel **TemperatureOVEN**)
- WPS-3000T (+ Dostmann thermometer with channel **TemperatureOVEN**)
- ACC-3000T (+ Dostmann thermometer with channel **TemperatureOVEN**)
- Accela autosampler (+ Dostmann thermometer with channel **TemperatureOVEN**)
- Agilent G1321A/B

TIP The test for determining the sample temperature accuracy is available only if the instrument configuration includes a Dostmann thermometer with a **TemperatureOVEN** temperature channel.

The tests are prepared and performed as described in chapter 4, page 64. Exception: CM7 includes a new driver for the Dostmann thermometer that allows you to record temperature data directly as a signal channel. Go to the **Signals** tab page in the driver configuration and enter the signal name **TemperatureOVEN**:

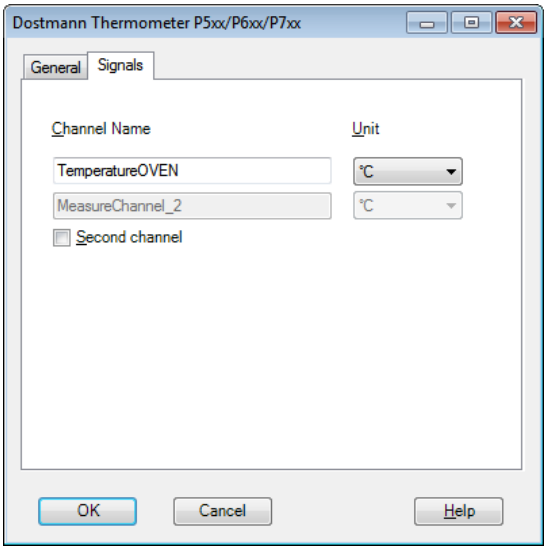

Figure 36: Configuring the Dostmann thermometer

6 Appendix

6.1 Supported Modules

The procedures described below apply to the following modules:

6.1.1 Pumps

The procedures described below apply to the following modules.

6.1.1.1 Thermo Scientific

Series	Variant
Vanquish	• VH-P10-A • VF-P10-A • VF-P20-A • VF-P32-A • VC-P10-A (including the optional method transfer kit) • VC-P20-A (including the optional method transfer kit) • VC-P21-A (including the optional method transfer kit) • VC-P32-A • VC-P33-A • VC-P40-A

Series	Variant
UltiMate 3000	• ISO-3100A • ISO-3100SD • ISO-3100BM • LPG-3400A(B) • LPG-3400XRS • LPG-3400RS • LPG-3400SD(N) • LPG-3400M(B) • LPG-3400BM • DGP-3600A(B) • DGP-3600RS • DGP-3600SD(N) • DGP-3600M(B) • HPG-3200A • HPG-3200M • HPG-3200RS • HPG-3200SD • HPG-3400M • HPG 3400RS • HPG 3400SD
Summit / Gynkotek	• P680 • P580 • M300
Accela	Pump
Spectra System (TSP)	• P2000 • P4000

6.1.1.2 Third-Party Modules

Supplier	Variant
Agilent	• 1100/12x0 series G1310A[1,2] • 1100/12x0 series G1310B[1] • 1100/12x0 series G1311A[1,2] • 1100/12x0 series G1311B[1] • 1100/12x0 series G1311C[1] • 1100/12x0 series G5611A[1] • 1100/12x0 series G1312A[1,2] • 1100/12x0 series G1312B[1,2] • 1100/12x0 series G1312C[1] • 1100/12x0 series G4220A[1] • 1100/12x0 series G4220B[1]
Waters	Pump of the Waters Alliance 2690 Separation Module
Shimadzu	• LC-2010 Pump • LC-10Ai • LC-10AD • LC-10ADvp • LC-10AT • LC-10ATvp • LC-20AD • LC-20ADXR • LC-20AT

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.2 Autosampler / Charger

The procedures described below apply to the following modules.

6.1.2.1 Thermo Scientific

Series	Variant
Vanquish	• VH-A10-A • VH-A40-A • VH-A90-A • VF-A10-A • VF-A40-A • VC-A12-A • VC-A13-A
UltiMate 3000	• ACC-3000(T) • OAS-3300TXRS • WPS-3000TXRS • WPS-3000(T)(B)RS • WPS-3000(T)(B)SL • WPS-3000(T)(B)PL(RS) • WPS-3000TBPL Analytical • WPS-3000T(B)FC Analytical
Summit	• ASI 100 • GINA 50
Accela	• Autosampler • Open Autosampler
Spectra System (TSP)	• AS3000 • AS3500

6.1.2.2 Third-Party Modules

Supplier	Variant
Agilent	• 1100/12x0 series G1313A[1,2] • 1100/12x0 series G1329A[1,2] • 1100/12x0 series G1329B[1,2] • 1100/12x0 series G1367A[1,2] • 1100/12x0 series G1367B[1,2] • 1100/12x0 series G1367C[1,2] • 1100/12x0 series G1367D[1] • 1100/12x0 series G1367E[1] • 1100/12x0 series G4226A[1] • 1100/12x0 series G5667A[1]
Waters	• Autosampler of the Waters Alliance 2690 Separation Module • Waters WISP 717plus
Shimadzu	• LC-2010 Autosampler • SIL-10A • SIL-10Ai • SIL-10AF • SIL-HTA • SIL-HTC • SIL-10AD*vp* • SIL-20AHT • SIL-20ACHT • SIL-20AXR • SIL-20ACXR

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.3 Thermostatted Column Compartments

6.1.3.1 Thermo Scientific

Series	Variant
Vanquish	VH-C10-A VC-C10-A

Series	Variant
UltiMate 3000	• ACC-3000(T) • TCC-3000RS • TCC-3000SD • TCC-3000 • TCC-3100 • TCC-3200(B)
Summit	• STH 585 • TCC-100
Accela	Autosampler (Column Compartment)
Spectra System (TSP)	• AS3000 • AS3500 optional

6.1.3.2 Third-Party Modules

Supplier	Variant
Agilent	• 1100/12x0 series G1316A[1,2] • 1100/12x0 series G1316B[1,2] • 1100/12x0 series G1316C[1]
Waters	Column Compartment of the Waters Alliance 2690 Separation Module
Shimadzu	• LC-2010 Column Compartment • CTO-10A • CTO-10A*vp* • CTO-10AC • CTO-10AC*vp* • CTO-10AS*vp* • CTO-20A • CTO-20AC

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.4 UV Detectors

6.1.4.1 Thermo Scientific

Series	Variant
Vanquish	• VH-D10-A • VF-D11-A • VF-D40-A • VC-D11-A • VC-D12-A • VC-D40-A
UltiMate 3000	• DAD-3000(RS) • MWD-3000(RS) • VWD-3100 • VWD-3400RS • PDA-3000
Summit	• PDA-100 • PDA-100U • UVD 340U • UVD 170U • UVD 340S • UVD 170S
Dionex	AD25
Accela	Accela PDA
Spectra System (TSP)	• UV1000 Single Lambda Detector • UV2000 Dual Lambda Detector • UV3000 (analog and digital data acquisition) • UV6000 PDA

6.1.4.2 Third-Party Modules

Supplier	Variant
Agilent	• 1100/12x0 series G1315A[1,2] • 1100/12x0 series G1315B[1,2] • 1100/12x0 series G1315C[1,2] • 1100/12x0 series G1315D[1,2] • 1100/12x0 series G4212A[1] • 1100/12x0 series G4212B[1] • 1100/12x0 series G1314A[1,2] • 1100/12x0 series G1314B[1,2] • 1100/12x0 series G1314C[1,2] • 1100/12x0 series G1314D[1] • 1100/12x0 series G1314E[1] • 1100/12x0 series G1314F[1] • 1100/12x0 series G1365A[1,2] • 1100/12x0 series G1365B[1,2] • 1100/12x0 series G1365C[1,2] • 1100/12x0 series G1365D[1,2]
Waters	• PDA996 Diode Array Detector • PDA2996 Diode Array Detector • 2487 Dual Lambda Absorbance Detector
Shimadzu	• LC-2010 SPD • SPD-10A • SPD-10A*vp* • SPD-10AV • SPD-10AV*vp* • SPD-20A • SPD-20AV

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.5 Florescence Detectors

6.1.5.1 Thermo Scientific

Series	Variant
Vanquish	• VF-D50-A • VF-D51-A • VC-D50-A • VC-D51-A
UltiMate 3000	• FLD-3100 • FLD-3400RS
Summit	• RF2000 • RF1002

6.1.5.2 Third-Party Modules

Supplier	Variant
Agilent	• 1100/12x0 series G1321A[1,2] • 1100/12x0 series G1321B[1]

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.6 Corona Detectors

6.1.6.1 Thermo Scientific

Series	Variant
Vanquish	• VH-D20-A • VF-D20-A
-	• Corona Veo SD • Corona Veo RS • Corona ultra • Corona ultra RS • Corona • Corona plus

6.1.7 Mass Spectrometry Detectors

6.1.7.1 Thermo Scientific

Series	Variant
Vanquish	Vanquish ISQ Family: Device Model: • EC (Source Type: HESI) • EM (Source Type: HESI / APCI)

6.1.8 Electrochemical Detectors

6.1.8.1 Thermo Scientific

Series	Variant
UltiMate 3000	ECD-3000RS

6.1.9 Refractive Index Detectors

6.1.9.1 Third-Party Modules

Supplier	Variant
Agilent	1100/12x0 series G1362A[1,2]
ERC	• RefractoMax521 • RefractoMax524
Shodex	RI-101

[1] Supported for control via Agilent Instrument Control Framework (ICF).

[2] Supported for control via 1100/1200 HPLC system driver.

6.1.10 Evaporative Light Scattering Detectors

6.1.10.1 Third-Party Modules

Supplier	Variant
Polymer Laboratories	• ELS2100 • ELS 2100 Ice
Varian	• 380-LC ELS detector • 385-LC ELS detector

6.1.11 Modules not Requiring any Re-Calibration

6.1.11.1 Thermo Scientific

Series	Variant
Thermo Scientific	A2D Analog to USB Adapter
Thermo Scientific	Vanquish Solvent Monitor

6.2 Overview of the Checks and Limits

The following tables provide an overview of the parameters checked by Chromeleon and list the recommended PQ limits for each HPLC module.

6.2.1 Pumps

For a detailed description of the test procedures, see section 6.3.1, page 136.

6.2.1.1 Thermo Scientific

The Vanquish Core binary and quaternary pump qualification includes also the qualification of the optional method transfer kit.

Module	Parameter	Nominal values and conditions	Limits[1]	
			OQ	PQ
VH-P10-A VF-P10-A VF-P20-A VF-P32-A VC-P10-A VC-P20-A VC-P21-A VC-P32-A VC-P33-A VC-P40-A ISO-3100A ISO-3100SD ISO-3100BM LPG-3400A(B) LPG-3400XRS LPG-3400RS LPG-3400SD(N) LPG-3400M(B) LPG-3400BM DGP-3600A(B) DGP-3600RS DGP-3600SD(N) DGP-3600M(B) DGP-3600BM HPG-3200A HPG-3200RS HPG-3200SD HPG-3200M HPG-3400A HPG-3400M HPG-3400RS HPG-3400SD P680 and P580 with analytical pump heads	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 0.05 % or SD ≤ 0.01 min	RSD ≤ 0.1 % or SD ≤ 0.02 min

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
LPG-3400A(B) DGP-3600A(B) HPG-3200A HPG-3200M HPG-3400A HPG-3400M P580 (HPG und LPG) and P680 (HPG, LPG, and DGP: all mixing chamber variants with analytical pump heads	Gradient Accuracy	Step gradient channel A/B and C/D (C/D: only LPG-3400A(B)): Steps: 1, 50, 99% channel B or D Flow rate: 2 mL/min	HPG: ≤ 0.2 % LPG/DGP: ≤ 1.0 % (A/B) ≤ 2.0 % (C/D)	HPG: ≤ 0.5 % LPG/DGP: ≤ 2.0 %
	Gradient Precision		SD ≤ 0.2 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
LPG-3400M(B) DGP-3600M(B)	Gradient Accuracy	Step gradient channel A/B and C/D (C/D: only LPG-3400LPG-3400M(B)): Steps: 10, 50, 90% channel B or D Flow rate: 1 mL/min	≤ 2.0 % (A/B) ≤ 3.0 % (C/D)	≤ 3.0 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
VF-P10-A VF-P20-A VF-P32-A VC-P10-A VC-P20-A VC-P21-A VC-P32-A VC-P33-A	Gradient Accuracy	Step gradient channel A(1)/B(1) and C/D (C/D: only quaternary pumps: Steps: 1, 50, 99% channel B or D Flow rate: 2 mL/min	P10: ≤ 0.2 % <> P10: ≤ 1.0 %	P10: ≤ 0.5 % <> P10: ≤ 2.0 %
	Gradient Precision		SD ≤ 0.15%	SD ≤ 0.5%
	Ripple		≤ 0.5 %	≤ 0.5 %
Vanquish Core Method transfer kit (Extension valve test)	Gradient Accuracy	Step gradient channel A(1)/B(1) Flow rate: 2 mL/min	Equal to Vanquish Core Pump	Equal to Vanquish Core Pump
	Ripple			
	Loop Volume		≥ 100 µL	≥ 100 µL
HPG-3200RS HPG-3200SD HPG-3400RS HPG-3400SD LPG-3400RS LPG-3400SD(N) DGP-3600RS DGP-3600SD(N)	Gradient Accuracy	Step gradient channel A(1)/B(1) and C/D (C/D: only quaternary pumps: Steps: 1, 50, 99% channel B or D Flow rate: 2 mL/min	HPG: ≤ 0.2 % LPG/DGP: ≤ 1.0 % (A/B) ≤ 2.0 % (C/D)	HPG: ≤ 0.5 % LPG/DGP: ≤ 2.0 %
	Gradient Precision		SD ≤ 0.15%	SD ≤ 0.5%
	Ripple		≤ 0.5 %	≤ 0.5 %
LPG-3400BM DGP-3600BM	Gradient Accuracy	Step gradient channel A/B and C/D	≤ 1.0 % (A/B) ≤ 2.0 % (C/D)	≤ 2.0 %

Module	Parameter	Nominal values and conditions	Limits[1] OQ	PQ
	Gradient Precision	(C/D: only LPG-3400BM) Steps: 10, 50, 90% channel B or D Flow rate: 1 mL/min	SD ≤ 0.3 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
LPG-3400XRS	Gradient Accuracy	Step gradient channel A/B and C/D: Steps: 10, 50, 90% channel B or D Flow rate: 1 mL/min	≤ 1.0 % (A/B) ≤ 2.0 % (C/D)	≤ 2.0 %
	Gradient Precision		SD ≤ 0.2 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
VH-P10-A	Gradient Accuracy	Step gradient channel A1/B1: Steps: 10, 50, 90% channel B1 Flow rate: 1 mL/min	≤ 0.2 %	≤ 0.4 %
	Gradient Precision		SD ≤ 0.15 %	SD ≤ 0.3 %
	Ripple		≤ 0.5 %	≤ 0.5 %
Accela Pump	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 0.05 % or SD ≤ 0.01 min	RSD ≤ 0.1 % or SD ≤ 0.02 min
	Gradient Accuracy	Step gradient channels A/B and C/D Steps: 10, 50, 90% channel B or D Flow rate: 1 mL/min	≤ 2.0 %	≤ 2.0 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
TSP P2000[2] TSP P4000	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 1.5 % or SD ≤ 0.04 min	RSD ≤ 2.0% or SD ≤ 0.06 min
	Gradient accuracy	Step gradient channel A/B Step: 50% channel B Flow rate: 2 mL/min	≤ 1.0 %	≤ 2.0 %
	Gradient precision		SD ≤ 1.0 %	SD ≤ 2.0 %
	Ripple		≤ 0.5 %	≤ 0.5 %
TSP P4000	Gradient accuracy	Step gradient channel C/D Step: 50% channel D Flow rate: 2 mL/min	≤ 1.0 %	≤ 2.0 %
	Gradient precision		SD ≤ 1.0 %	SD ≤ 2.0 %

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
	Ripple		≤ 0.5 %	≤ 0.5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) To determine the gradient accuracy and the gradient precision for the TSP P2000 pump, the solvent composition must be as follows: 0, 50, and 100% of solvent B. This is because the pump does not support a gradient program with more than 9 steps.

6.2.1.2 Third-Party Modules – Agilent

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1100/12x0 Series: G1310A/B G1311A/B/C G5611A G1312A/B/C	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 0.07 % or SD ≤ 0.02 min	RSD ≤ 0.07 % or SD ≤ 0.02 min
1100/12x0 Series: G1311A/B/C G5611A G1312A/B	Gradient Accuracy	Step gradient channel A/B (G1312A/B only): Steps: 1, 50, 99% channel B Step gradient channel A/B (G1311A/B/C and G5611A only): Steps: 20, 50, 80% channel B Flow rate: 2 mL/min Step gradient channel C/D (G1311A/B/C only): Steps: 20, 50, 80% channel D Flow rate: 2 mL/min	G1311 / G5611: ≤ 1.5 % G1312: ≤ 0.7 %	G1311 / G5611: ≤ 1.5 % G1312: ≤ 0.7 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %
1290 Series: G4220A/B	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 0.07 % or SD ≤ 0.005 min	RSD ≤ 0.07 % or SD ≤ 0.005 min
	Gradient Accuracy	Step gradient channel A/B Steps: 1, 50, 99% channel B Flow rate: 2 mL/min	≤ 0.7 %	≤ 0.7 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.1.3 Third-Party Modules – Waters

Module	Parameter	Nominal values and conditions	Limits[1]	
			OQ	PQ
Pump module of the Alliance 2690 Separation Module	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 4.0 % or SD ≤ 0.1 min	RSD ≤ 4.0% or SD ≤ 0.1 min
	Gradient Accuracy	Step gradient channel A/B Steps: 1, 50, 99% channel B Flow rate: 2 mL/min	≤ 0.5 %	≤ 0.5 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.1.4 Third-Party Modules – Shimadzu

Module	Parameter	Nominal values and conditions	Limits[1]	
			OQ	PQ
LC-2010 LC-10Ai LC-10AD LC-10ADvp LC-10AT LC-10ATvp LC-20AD(XR) LC-20AT	Flow Precision	Flow rate: 0.3 mL/min Determined using the retention time precision (standard deviation and relative standard deviation) of caffeine. The greater value is the valid limit.	RSD ≤ 0.075 % or SD ≤ 0.02 min	RSD ≤ 0.15 % or SD ≤ 0.04 min
	Gradient Accuracy	Step gradient channel A/B Steps: 1, 50, 99% channel B Flow rate: 2 mL/min	≤ 1.0 %	≤ 2.0 %
	Gradient Precision		SD ≤ 0.5 %	SD ≤ 0.5 %
	Ripple		≤ 0.5 %	≤ 0.5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.2 Manual Injection Valve

For a detailed description of the test procedure, see section 6.3.2, page 140.

Device	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Manual injection valve	Precision of Injection Volume	Injection volume: 10 µL	RSD ≤ 0.30 %	RSD ≤ 0.50 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.3 Autosamplers

For a detailed description of the test procedures, see section 6.3.2, page 140.

6.2.3.1 Thermo Scientific

Vanquish

Module	Parameter	Nominal values and conditions	Limits[1] OQ	Limits[1] PQ
VH-A10-A VH-A40-A VF-A10-A VF-A40-A	Precision of Injection Volume	Injection volume: 1 µL	RSD ≤ 0.25 %	RSD ≤ 0.50 %
	Linearity of Injection Volume	Inject volume range: 1 – 25 µL (25 µL sample loop, – part no. 6850.1911) Inject volume range: 1 – 10 µL (10 µL sample loop – part no. 6850.1915) Inject volume range: 1 – 100 µL (100 µL sample loop – part no. 6850.1913 and flow cell with 10 mm light path) Inject volume range: 1 – 50 µL (100 µL sample loop – and Vanquish 60-mm-LightPipe flow cell)	r ≥ 99.999 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (10 and 25 µL sample loop) Injection volume: 20 µL (100 µL sample loop)	≤ 0.01 %	≤ 0.01 %
	Temperature Accuracy	Temperature: 10°C	- 2 / + 4 °C[2]	± 4 °C[2]
VC-A12-A	Precision of Injection Volume	Injection volume: 3 µL	RSD ≤ 0.25 %	RSD ≤ 0.50 %

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VC-A13-A	Linearity of Injection Volume	Inject volume range: 3 – 100 µL (100 µL sample loop – part no. 6850.1913 and flow cell with 10 mm light path) Inject volume range: 1 – 10 µL (10 µL sample loop – part no. 6850.1915) Inject volume range: 3 – 25 µL (25 µL sample loop, – part no. 6850.1911) Inject volume range: 3 – 250 µL (250 µL sample loop – part no. 6851.1970 and flow cell with 10 mm light path) Inject volume range: 3 – 1000 µL (1000 µL sample loop – part no. 6851.1980 and flow cell with 10 mm light path) Inject volume range: 3 – 50 µL (≥ 100 µL sample loop – and Vanquish 60-mm-LightPipe flow cell)	r ≥ 99.999 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (10 and 25 µL sample loop) Injection volume: 20 µL (100 µL sample loop)	≤ 0.01 %	≤ 0.01 %
	Temperature Accuracy	Temperature: 10°C	- 2 / + 4 °C(2)	± 4 °C(2)

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) Valid for ambient temperatures of < 33 °C (nominal test temperature: 10 °C, cooling performance: ≥ 23 K below ambient temperature) and a relative humidity of ≤ 80%.

UltiMate 3000

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
ACC-3000(T)	Precision of Injection Volume	Injection volume: 5 µL (20 and 50 µL sample loop) Injection volume: 20 µL (200 µL sample loop)	RSD ≤ 0.5 %	RSD ≤ 1.0 %

Module	Parameter	Nominal values and conditions	Limits[1] OQ	PQ
	Linearity of Injection Volume	Inject volume range: 1 – 10 µL (20 µL sample loop) Inject volume range: 5 – 25 µL (50 µL sample loop) Inject volume range: 5 – 80 µL (200 µL sample loop)	r ≥ 99.95 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (20 and 50 µL sample loop) Injection volume: 20 µL (200 µL sample loop)	≤ 0.02 %	≤ 0.02 %
	Temperature Accuracy	Temperature: 15°C	± 2°C	± 4°C
OAS-3300TXRS	Precision of Injection Volume	Injection Volume: 10 µL	RSD ≤ 0.5 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Inject volume range: 4 – 12 µL	r ≥ 99.99 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 10 µL	≤ 0.10 %	≤ 0.10 %
WPS-3000(T)(B)RS WPS-3000(T)(B)SL (Analytical variant, micro option and 250 µL injection volume kit) WPS-3000TXRS	Precision of Injection Volume	Injection volume: 5 µL (analytical) Injection volume: 2 µL (micro + XRS) Injection volume: 10 µL (250 µL kit)	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Inject volume range: 5 – 90 µL (analytical) Inject volume range: 1 – 20 µL (micro + XRS) Inject volume range: 10 – 160 µL (250 µL kit)	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (micro + XRS) Injection volume: 20 µL (other variants)	≤ 0.01 %	≤ 0.01 %
	Temperature Accuracy	Temperature: 10°C	± 2°C[2]	± 4°C[2]
WPS-3000(T)(B)PL WPS-3000(T)(B) PLRS	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 0.3 %	RSD ≤ 0.5 %

Module	Parameter	Nominal values and conditions	Limits[1] OQ	PQ
(only with upgrade kit for 250 µL syringe)	Linearity of Injection Volume	Inject volume range: 1 – 12 µL (20 µL sample loop) Inject volume range: 1 – 20 µL (50 µL sample loop) Inject volume range: 5 – 50 µL (100 µL sample loop) Inject volume range: 5 – 80 µL (125 µL sample loop)	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (20 and 50 µL sample loops) Injection volume: 20 µL (other variants)	≤ 0.05 %	≤ 0.05 %
	Temperature Accuracy	Temperature: 10°C	± 2°C[2]	± 4°C[2]
WPS-3000TBPL Analytical (Standard and Large Volume configuration) WPS-3000T(B)FC Analytical (Standard and Large Volume configuration)	Precision of Injection Volume	Injection volume: 5 µL (standard) Injection volume: 20 µL (large volume)	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Inject volume range: 5 – 25 µL (standard) Inject volume range: 20 – 140 µL (large volume)	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection volume: 10 µL (standard) Injection volume: 20 µL (large volume)	TBPL: ≤ 0.03 % T(B)FC: ≤ 0.05 %	TBPL: ≤ 0.05 % T(B)FC: ≤ 0.10 %
	Temperature Accuracy	Temperature: 10°C	± 2°C[2]	± 4°C[2]

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) Valid for ambient temperatures of ≤ 25 °C and a relative humidity of ≤ 50%.

Accela

Module	Parameter	Nominal values and conditions	Limits[1] OQ	PQ
Accela Autosampler (25 µL sample loop)	Precision of Injection Volume	Injection Volume: 10 µL	RSD ≤ 1.0 %	RSD ≤ 2.0 %
	Linearity of Injection Volume	Inject volume range: 2.5 – 12.5 µL	r ≥ 99.95 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 10 µL	≤ 0.1 %	≤ 0.1 %

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
	Temperature Accuracy	Temperature: 30°C	± 2°C	± 4°C

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Summit

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
ASI 100 (250 µL syringe)	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.1 %	≤ 0.1 %
Gina 50	Precision of Injection Volume	Injection Volume: 10 µL	RSD ≤ 0.4 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Inject volume range: 10 – 80 µL	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.1 %	≤ 0.1 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

TSP

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
TSP AS3000/3500	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 1.0 %	RSD ≤ 2.0 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL	r ≥ 99.90 % RSD ≤ 1.5 %	r ≥ 99.90 % RSD ≤ 1.5 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.1 %	≤ 0.1 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.3.2 Third-Party Modules – Agilent

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1100/12x0 Series: G1313A G1329A/B G1367A/B/C/D	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL Inject volume range: 5 – 40 µL (G1367D only)	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.2 %	≤ 0.2 %
12x0 Series: G1367E G5667A G4226A	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 0.5 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL Inject volume range: 1 – 20 µL (G4226A only)	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 10 µL	≤ 0.2 %	≤ 0.2 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.3.3 Third-Party Modules – Waters

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Sampler module of the Alliance 2690 Separation Module	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.1 %	≤ 0.1 %
WISP 717plus autosampler	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Inject volume range: 5 – 80 µL	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.1 %	≤ 0.1 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.3.4 Third-Party Modules – Shimadzu

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
SIL-10A SIL-10Ai SIL-10AF	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 1.0 %	RSD ≤ 2.0 %
	Linearity of injection volume	Inject volume range: 5 – 50 µL	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.02 %	≤ 0.02 %
LC-2010 SIL-10HTA SIL-10HTC SIL-10ADvp SIL-20A(C)HT SIL-20A(C)XR	Precision of Injection Volume	Injection Volume: 5 µL	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of injection volume	Inject volume range: 5 – 50 µL (SIL-10ADvp, SIL-20A(C)XR) Inject volume range: 5 – 80 µL (Other autosamplers)	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	Injection Volume: 20 µL	≤ 0.02 %	≤ 0.02 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.4 Charger

For a detailed description of the test procedure, see section 6.3.2.4, page 145.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VH-A90-A	Tempera-ture Accuracy	Temperature: 10 °C	- 2 / + 4 °C(2)	± 4 °C(2)

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) Valid for ambient temperatures of < 32 °C (nominal test temperature: 10 °C, cooling performance: ≥ 22 K below ambient temperature) and a relative humidity of ≤ 80%.

6.2.5 Thermostatted Column Compartments and Column Ovens

For a detailed description of the test procedure, see section 6.3.3, page 145.

6.2.5.1 Thermo Scientific

Module	Parameter	Nominal values and conditions	Limits[1]	
			OQ	PQ
VH-C10-A	Temperature Accuracy	Without post-column cooler, but with VH-D10-A LightPipe flow cell: OQ: Measured at: 5, 20, 35, 50 °C PQ: Measured at: 10, 20, 35, 50 °C With post-column cooler and VH-D10-A LightPipe flow cell or without LightPipe flow cell: OQ: Measured at: 5, 40, 80, 120 °C PQ: Measured at: 10, 40, 80, 100 °C	± 1 °C	± 2 °C
VC-C10-A	Temperature Accuracy	With VH-D10-A LightPipe flow cell: OQ: Measured at: 5, 20, 35, 50 °C PQ: Measured at: 10, 20, 35, 50 °C Without LightPipe flow cell: OQ: Measured at: 5, 30, 60, 85 °C PQ: Measured at: 10, 30, 45, 60 °C	± 1 °C	± 2 °C
Column compartment module of the ACC-3000(T) autosampler	Temperature Accuracy	OQ: Measured at[2]: 35, 40, 50°C PQ: Measured at[2]: 35, 40, 45°C	± 2°C	± 3°C
Column compartment of the ECD-3000RS detector	Temperature Accuracy	OQ: Measured at[3]: 35, 40°C PQ: Measured at[3]: 35, 40°C	± 2°C	± 3°C
TCC-3000RS	Temperature Accuracy	OQ: Measured at: 10, 30, 60, 105°C PQ: Measured at: 15, 30, 60, 90°C	± 1°C	± 2°C
TCC-3000SD	Temperature Accuracy	OQ: Measured at (firmware < 1.30): 10, 30, 50, 65°C OQ: Measured at (firmware ≥ 1.30): 10, 30, 50, 80°C PQ: Measured at: 15, 30, 45, 60°C	± 1°C	± 2°C
TCC-3000 TCC-3100 TCC-3200(B) TCC-100	Temperature Accuracy	OQ: Measured at: 10, 30, 60, 80°C PQ: Measured at: 15, 30, 45, 60°C	± 1°C	± 2°C

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
STH 585	Temperature Accuracy	OQ: Measured at: 5, 20, 60, 85°C PQ: Measured at: 15, 30, 45, 60°C	± 1°C	± 2°C
Column compartment of the Accela autosampler	Temperature Accuracy	OQ: Measured at(4): 30°C PQ: Measured at(4): 30°C	± 2°C	± 3°C
Column oven of the TSP AS3000/AS3500 autosamplers	Temperature Accuracy	OQ: Measured at: 20, 40, 60, 80°C PQ: Measured at: 25, 35, 45, 60°C	± 2°C	± 3°C

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) According to the specification of the column compartment module, only target temperatures above ambient are permitted. That is why measuring points below 35°C are not evaluated.

(3) Due to the supported temperature range, the temperature accuracy is tested at two measuring points only.

(4) According to the specification of the column compartment, a target temperature can only be set within the context of a sample. Therefore, the temperature accuracy is tested at a single measuring point only.

6.2.5.2 *Third-Party Modules – Agilent*

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1100/12x0 Series: G1316A G1316B	Temperature Accuracy	OQ: Measured at(2): 5, 20, 60, 80°C PQ: Measured at(2): 15, 30, 45, 60°C	± 2°C	± 2°C
1290 Series: G1316C	Temperature Accuracy	OQ: Measured at(3): 20, 60, 95°C PQ: Measured at(3): 30, 50, 75°C	± 2°C	± 2°C

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) Qualification of the first measuring point is omitted for the above-mentioned Agilent column thermostats when controlled by Agilent ICF (also see footnote (3)).

(3) It is not possible to set the temperature on the column compartment module when the retention time is negative. The first measurement reading is 10 minutes after the sample has been started. At this time, equilibration of the column compartment may not be complete. Therefore, the same temperature is set for the second measuring point. The column compartment module has passed the check even if the target temperature is reached only for the second measuring point.

6.2.5.3 Third-Party Modules – Waters

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Column compartment module of the Alliance 2690 Separation Module	Temperature Accuracy	OQ: Measured at(2.3): 35, 45, 55°C PQ: Measured at(2.3): 35, 45, 55°C	± 1°C	± 2°C

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) It is not possible to set the temperature on the column compartment module when the retention time is negative. The first measurement reading is 10 minutes after the sample has been started. At this time, equilibration of the column compartment may not be complete. Therefore, the same temperature is set for the second measuring point. The column compartment module has passed the check even if the target temperature is reached only for the second measuring point.

(3) According to the specification of the column compartment module, only target temperatures above ambient are permitted. That is why measuring points below 35°C are not evaluated.

6.2.5.4 Third-Party Modules – Shimadzu

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Thermostatted column compartment • LC-2010 • CTO-10AS*vp*	Temperature Accuracy	OQ: Measured at(2): 20, 40, 60°C PQ: Measured at(2): 25, 35, 50°C	± 3°C	± 3°C
Thermostatted column compartment • CTO-10A • CTO-10A*vp* • CTO-20A	Temperature Accuracy	OQ: Measured at(2.3): 35, 60, 80°C PQ: Measured at(2.3): 35, 45, 60°C	± 3°C	± 3°C
Thermostatted column compartment • CTO-10AC • CTO-10AC*vp* • CTO-20AC	Temperature Accuracy	OQ: Measured at(2): 20, 60, 80°C PQ: Measured at(2): 25, 45, 60°C	± 3°C	± 3°C

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) It is not possible to set the temperature on the column compartment module when the retention time is negative. The first measurement reading is 10 minutes after the sample has been started. At this time, equilibration of the column compartment may not be complete. Therefore, the same temperature is set for the second measuring point. The column compartment module has passed the check even if the target temperature is reached only for the second measuring point.

(3) According to the specification of the column compartment module, only target temperatures above ambient are permitted. That is why measuring points below 35°C are not evaluated.

6.2.6 UV Detectors with Analytical Flow Cells

For a detailed description of the test procedures, see section 6.3.4, page 146.

When qualifying a detector with a non-analytical flow cell, see section 6.2.7, page 127.

6.2.6.1 Thermo Scientific

Vanquish

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VH-D10-A (10-mm-LightPipe flow cell)	Baseline Noise	Measuring wavelength: 230 nm	≤ 0.030 mAU	≤ 0.050 mAU
	Drift		≤ 0.5 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1:272.1 nm Nominal wavelength 1:333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.0 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
VF-D11-A VC-D11-A VC-D12-A (standard (bio) flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.030 mAU	≤ 0.050 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1:272.1 nm (D11 only) Nominal wavelength 1:333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.2 AU	r ≥ 99.97 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
VF-D40-A VC-D40-A (standard (bio) flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.025 mAU	≤ 0.050 mAU
	Drift		≤ 0.1 mAU/h	≤ 0.2 mAU/h
	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 2.5 AU	r ≥ 99.97 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

UltiMate 3000

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VWD-3100 VWD-3400RS (analytical flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.025 mAU	≤ 0.050 mAU
	Drift		≤ 0.3 mAU/h	≤ 0.3 mAU/h
	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 2.5 AU	r ≥ 99.97 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
DAD-3000(RS) MWD-3000(RS) (analytical flow cell)	Baseline Noise	Measuring wavelength: 254 nm.	≤ 0.03 mAU	≤ 0.10 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (DAD only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Summit

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
UVD 340S UVD 170S UVD 340U UVD 170U (analytical flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.03 mAU	≤ 0.05 mAU
	Drift		≤ 0.8 mAU/h	≤ 2.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (UVD-340x only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 0.75 nm	± 0.75 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.98 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %
PDA-3000 PDA 100 PDA 100U	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.03 mAU	≤ 0.10 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Accela

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Accela PDA (analytical flow cell with 1 cm path length)	Baseline Noise	Measuring wavelength: 254 nm.	≤ 0.30 mAU	≤ 0.50 mAU
	Drift		≤ 2.0 mAU/h	≤ 2.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 1.2 AU	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Dionex

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
AD25	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.03 mAU	≤ 0.04 mAU
	Drift		≤ 0.2 mAU/h	≤ 0.2 mAU/h
	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Spectra System (TSP)

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
TSP UV1000	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.50 mAU	≤ 0.10 mAU
	Drift		≤ 0.5 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Not checked	Not checked	Not checked
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%
TSP UV2000	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.05 mAU	≤ 0.10 mAU
	Drift		≤ 0.5 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength: 239 nm (maximum of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
TSP UV3000	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.08 mAU	≤ 0.15 mAU
	Drift		≤ 0.5 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength: 333.3 nm (maximum of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%
TSP UV6000	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.06 mAU	≤ 0.10 mAU
	Drift		≤ 2.0 mAU/h	≤ 4.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.2 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.6.2 Third-Party Modules – Agilent

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1100/12x0 Series: G1314A/B/C/D/E/F G1315A/B/C/D G1365A/B/C/D	Drift	Measuring wavelength: 254 nm	≤ 5.0 mAU/h	≤ 5.0 mAU/h
	Linearity	Absorption range: up to 1.5 AU Absorption range: up to 2.5 AU (G1314D/E/F only)	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %
G1315A/B/C/D G1365A/B/C/D	Baseline Noise	Measuring wavelength: 254 nm.	≤ 0.05 mAU	≤ 0.05 mAU
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (DAD only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 2.0 nm	± 2.0 nm
G1314A/B/C/D/E/F	Baseline Noise	Measuring wavelength: 254 nm	G1314A-E: 0.04 mAU G1314F: 0.05 mAU	G1314A-E: 0.04 mAU G1314F: 0.05 mAU
	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1290 Series: G4212A/B	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.03 mAU	≤ 0.05 mAU
	Drift		≤ 3.0 mAU/h	≤ 3.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.0 AU	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.6.3 Third-Party Modules – Waters

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Waters PDA996 Waters PDA2996	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.10 mAU	≤ 0.10 AU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%
Waters 2487 Dual Lambda Absorbance Detector	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.05 mAU	≤ 0.05 mAU
	Drift		≤ 0.5 mAU/h	≤ 0.5 mAU/h
	Wavelength Accuracy	Nominal wavelength: 239 nm (maximum of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.6.4 *Third-Party Modules – Shimadzu*

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Shimadzu LC-2010 SPD SPD-10A(V) SPD-10A(V)*vp* SPD-20A(V)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.05 mAU	≤ 0.10 mAU
	Drift		≤ 0.8 mAU/h	≤ 2.0 mAU/h
	Wavelength Accuracy	Nominal wavelength: 333.3 nm (maximum of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.5 AU	r ≥ 99.90% RSD ≤ 5%	r ≥ 99.90% RSD ≤ 5%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.7 UV Detectors with Non-Analytical Flow Cells

When qualifying a detector with a non-analytical flow cell, such as, a micro, nano, or dummy flow cell, you may have to enter the corresponding specifications manually into the report, depending on the detector type. The reason is that automatic recognition of micro and nano flow cells is not always possible or not supported. For information about the limits for non-analytical flow cells, refer to the table below.

TIP When a detector of the Vanquish and UltiMate series (VWD-3x00 or DAD / MWD-3000) is qualified, the flow cell type is automatically detected and specifications are automatically entered in the report. When a detector of the Summit series (UVD) is qualified, the specifications must be entered manually.

For a detailed description of the test procedures, see section 6.3.4, page 146.

6.2.7.1 Thermo Scientific

Vanquish

Module	Parameter	Nominal values and conditions	Limits(1) OQ	Limits(1) PQ
VH-D10-A (60-mm-LightPipe flow cell)	Baseline Noise	Measuring wavelength: 230 nm Response Time: 4 s; Slit / bandwidth: 4 nm.	≤ 0.030 mAU	≤ 0.050 mAU
	Drift		≤ 3.0 mAU/h	≤ 5.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.0 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
VF-D11-A VC-D11-A VC-D12-A (semi-micro (bio), semi-analytic flow cell)	Baseline Noise	Measuring wavelength: 254 nm Response Time: 4 s; Slit / bandwidth: 4 nm.	≤ 0.030 mAU	≤ 0.050 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (D11 only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 2.2 AU	r ≥ 99.97 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
VF-D40-A VC-D40-A (semi-micro (bio) flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.035 mAU	≤ 0.070 mAU
	Drift		≤ 0.1 mAU/h	≤ 0.2 mAU/h
	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 2.5 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

UltiMate 3000

Module	Parameter	Nominal values and conditions	Limits(1) OQ	Limits(1) PQ
VWD-3100 VWD-3400RS (micro flow cell	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.035 mAU	≤ 0.070 mAU
	Drift		≤ 0.3 mAU/h	≤ 0.3 mAU/h

Module	Parameter	Nominal values and conditions	Limits(1) OQ	PQ
semi-micro flow cell)	Wavelength Accuracy	Nominal wavelength: 272.5 nm (maximum of caffeine)	± 2.0 nm	± 2.0 nm
	Linearity	Absorption range: up to 1.7 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
DAD-3000(RS) MWD-3000(RS) (semi-analytical flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.04 mAU	≤ 0.15 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (DAD only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %
DAD-3000(RS) MWD-3000(RS) (semi-micro flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.06 mAU	≤ 0.20 mAU
	Drift		≤ 1.0 mAU/h	≤ 1.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (DAD only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 1.0 nm	± 1.0 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.95 % RSD ≤ 3 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

Summit

Module	Parameter	Nominal values and conditions	Limits(1) OQ	PQ
UVD 340S(2) UVD 170S(2) UVD 340U(2) UVD 170U(2) (micro flow cell)	Baseline Noise	Measuring wavelength: 254 nm.	≤ 0.15 mAU	≤ 0.20 mAU
	Drift		≤ 1.5 mAU/h	≤ 2.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (UVD-340x only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 0.75 nm	± 0.75 nm
	Linearity	Absorption range: up to 1.5 AU	r ≥ 99.98 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

Module	Parameter	Nominal values and conditions	Limits(1) OQ	PQ
UVD 340S(2) UVD 170S(2) UVD 340U(2) UVD 170U(2) (nano flow cell)	Baseline Noise	Measuring wavelength: 254 nm	≤ 0.20 mAU	≤ 0.30 mAU
	Drift		≤ 3.0 mAU/h	≤ 4.0 mAU/h
	Wavelength Accuracy	Nominal wavelength 1: 272.1 nm (UVD-340x only) Nominal wavelength 2: 333.3 nm (maxima of pyrene)	± 0.75 nm	± 0.75 nm
	Linearity	Absorption range: up to 1.0 AU at 8 µL	r ≥ 99.90 % RSD ≤ 5 %	r ≥ 99.90 % RSD ≤ 5 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) The specifications have to be entered into the report manually.

6.2.8 Fluorescence Detectors with Analytical Flow Cells

For a detailed description of the test procedures, see section 6.3.5, page 151.

6.2.8.1 Thermo Fisher

Module	Parameter	Nominal values and conditions	Limits(1) OQ	PQ
VF-D50-A VF-D51-A VC-D50-A VC-D51-A FLD-3100 FLD-3400RS (analytical flow cell)	Signal-to-Noise Ratio	Excitation wavelength 350 nm; emission wavelength 397 nm for the first 20 minutes and 450 nm for another 20 minutes.	ASTM: ≥ 550 Dark Signal: ≥ 2100	ASTM: ≥ 225 Dark Signal: ≥ 1100
	Wavelength Accuracy Excitation	Emission wavelength 397 nm. Nominal excitation wavelength: 350 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm
	Wavelength Accuracy Emission	Excitation wavelength: 350 nm. Nominal emission wavelength: 397 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
RF2000	Baseline Noise	Excitation wavelength: 350 nm; emission wavelength: 397 nm	≤ 0.30 mV	≤ 0.30 mV
	Signal Minimum	Excitation wavelength: 350 nm; Emission wavelength range: 450 - 397 nm	≥ 40 mV	≥ 40 mV
	Signal Maximum		≤ 80 mV	≤ 80 mV
	Wavelength Accuracy(2)	Excitation wavelength: 350 nm Emission wavelength range: 380 - 410 nm (step: 1 nm) Nominal emission wavelength: 397 nm (maximum)	± 10 nm	± 10 nm
RF1002	Baseline noise	Excitation wavelength: 350 nm; emission wavelength: 397 nm	≤ 0.60 mV	≤ 0.60 mV
	Signal Minimum	Excitation wavelength: 350 nm; Emission wavelength range: 450 - 397 nm	≥ 40 mV	≥ 40 mV
	Signal Maximum		≤ 80 mV	≤ 80 mV
	Wavelength Accuracy(2)	Excitation wavelength: 350 nm Emission wavelength range: 380 - 410 nm (step: 1 nm) Nominal emission wavelength: 397 nm (maximum)	± 10 nm	± 10 nm

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) The manufacturer specification of ± 2 nm for the excitation and emission wavelengths can be checked only by using a special flow cell and a mercury lamp. For OQ and PQ, the module should preferably be checked with the components used for the measurements.

6.2.8.2 Third-Party Modules – Agilent

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
1100/12x0 Series G1321A (standard flow cell)	Signal-to-Noise Ratio	Excitation wavelength 350 nm; emission wavelength 397 nm for the first 20 minutes and 450 nm for another 20 minutes.	Dark Signal: ≥ 500	Dark Signal: ≥ 200
G1321B	Signal-to-Noise Ratio	Excitation wavelength 350 nm; emission wavelength 397 nm for the first 20 minutes and 450 nm for another 20 minutes.	ASTM: ≥ 500 Dark Signal: ≥ 2000	ASTM: ≥ 200 Dark Signal: ≥ 1000

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
G1321A/B	Wavelength accuracy Excitation	Emission wavelength 397 nm. Nominal excitation wavelength: 350 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm
	Wavelength accuracy Emission	Excitation wavelength: 350 nm. Nominal emission wavelength: 397 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm
	Linearity	Excitation wavelength: 250 nm. Emission wavelength: 400 nm Absorption range: up to approx. 10 LU	r ≥ 99.8 % RSD≤1.5% Offset: ≤1.5 %	r ≥ 99.0 % RSD ≤ 3 % Offset: ≤3 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.9 Fluorescence Detectors with Non-Analytical Flow Cells

For a detailed description of the test procedures, see section 6.3.5, page 151.

TIP When a detector of the Vanquish or UltiMate series is qualified, the flow cell type is automatically detected and specifications are automatically entered in the report.

6.2.9.1 Thermo Scientific

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VF-D50-A VF-D51-A VC-D50-A VC-D51-A FLD-3100 FLD-3400RS (micro flow cell)	Signal-to-Noise Ratio	Excitation wavelength 350 nm; emission wavelength 397 nm for the first 20 minutes and 450 nm for another 20 minutes.	ASTM: ≥ 225 Dark Signal: ≥ 1025	ASTM: ≥ 110 Dark Signal: ≥ 500
	Wavelength accuracy Excitation	Emission wavelength 397 nm. Nominal excitation wavelength: 350 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm
	Wavelength Accuracy Emission	Emission wavelength 350 nm. Nominal excitation wavelength: 397 nm (maximum of the Raman signal of water)	± 3 nm	± 3 nm

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.10 Corona Detectors

For a detailed description of the test procedures, see section 6.3.6, page 155.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
VH-D20-A VF-D20-A Corona Veo SD Corona Veo RS	Baseline noise	Filter: 5 s	≤ 20.0 fA	≤ 20.0 fA
	Height of spikes		≤ 60.0 fA	≤ 60.0 fA
	Drift		≤ 40.0 fA/min	≤ 40.0 fA/min
	Signal-to-Noise Ratio	Caffeine concentration: 5 µg/mL	≥ 10	≥ 10
	Precision (height)		RSD ≤ 10.0 %	RSD ≤ 10.0 %
	Signal calibration	Signal range: up to approx. 40 pA	r2 ≥ 99.90 %	r2 ≥ 99.90 %

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Corona Corona plus Corona ultra (RS)	Baseline noise	Filter: None (Corona, Corona plus) Filter: Corona (Corona ultra) Filter: 4 (Corona ultra RS)	≤ 40.0 fA	≤ 40.0 fA
	Height of spikes		≤ 200.0 fA	≤ 200.0 fA
	Drift		≤ 40.0 fA/min	≤ 40.0 fA/min
	Signal-to-Noise Ratio	caffeine concentration: 25 µg/mL	≥ 10	≥ 10
	Precision (height)		RSD ≤ 10.0 %	RSD ≤ 10.0 %
	Signal calibration	Signal range: up to approx. 40 pA	r2 ≥ 99.90 %	r2 ≥ 99.90 %

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.11 Mass Spectrometry Detectors

For a detailed description of the test procedure, see section 6.3.7, page 158.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Vanquish ISQ EC	Autotune	Positive and negative ion mode	ok(2)	ok(2)
	Sensitivity	HESI positive mode	≥ 400 : 1 RMS	≥ 400 : 1 RMS
		HESI negative mode	≥ 500 : 1 RMS	≥ 500 : 1 RMS
Vanquish ISQ EM	Autotune	Positive and negative ion mode	ok(2)	ok(2)
	Sensitivity	HESI positive mode	≥ 400 : 1 RMS	≥ 400 : 1 RMS
		HESI negative mode	≥ 500 : 1 RMS	≥ 500 : 1 RMS
		APCI positive mode (if available)	≥ 1000 : 1 RMS	≥ 1000 : 1 RMS
		APCI negative mode (if available)	≥ 80 : 1 RMS	≥ 80 : 1 RMS

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) See section 6.3.7.1, page 158.

6.2.12 Electrochemical Detectors

For a detailed description of the test procedure, see section 6.3.7, page 158.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
ECD-3000RS	Baseline Noise	DC mode, Filter: 10 s	< 0.75 pA	< 1.50 pa
		Pulse mode, Filter: medium	< 5.00 pC	< 10.00 pC

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.13 Refractive Index Detectors

For a detailed description of the test procedures, see section 6.3.9, page 162.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
ERC RefractoMax521 / RefractoMax524 Shodex RI-101 Agilent 1100/1200 G1362A	Baseline noise	Temperature: 35°C	≤ 50 nRIU	≤ 50 nRIU
	Drift		≤ 500 nRIU/h	≤2500 nRIU/h
	Linearity	Signal range: up to approx. 500 µRIU	r ≥ 99.9%	r ≥ 99.9%

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

6.2.14 Evaporative Light Scattering Detectors

For a detailed description of the test procedure, see section 6.3.10, page 163.

Module	Parameter	Nominal values and conditions	Limits(1)	
			OQ	PQ
Polymer Laboratories ELS 2100 / ELS 2100 Ice Varian 380/385-LC ELS detector	Baseline noise	Evaporator temperature: 90°C Carrier gas flow: 1.6 SLM(2) at 4.1 bar	≤ 0.300 mV	≤ 0.300 mV

(1) OQ limits with optimum measuring conditions, recommended PQ limits.

(2) SLM: Standard liter per minute.

6.3 Test Design

6.3.1 Pump

6.3.1.1 Flow Precision

Theory

The flow precision can be determined very exactly by weighing out which quantity of solvent is delivered over a specific period. For statistic evaluation of the results, repeat this measurement several times. However, this requires a lot of work: The measuring time must be at least five minutes if it is not electronically linked to the weighing process. Otherwise, inaccuracies in the timing affect the results. An additional disadvantage is that the procedure cannot be automated and that the used scales must be very exact.

As an alternative, the flow precision can be determined by injecting the same sample standard multiple times. The flow precision primarily affects the precision of the retention time. This method is used during automated OQ and PQ.

Procedure and evaluation

The precision of the flow and the precision of the injection volume are established with the **Injector_Flow_Repro** sequence. Standard 4 is injected ten times, using an injection volume of 5 µL for each injection (deviations see the table in section 6.3.2.1, page 140).

The relative standard deviation (RSD) or the standard deviation (SD) of the retention times of the ten injections indicates the flow precision. The larger of the values is the valid limit.

The expression “The greater value is the valid limit” refers to comparable values. This means that either the RSD must be converted to an SD value or vice versa. This conversion takes the absolute retention time t_R of the peak of the interest into account

For the pumps of the UltiMate 3000 series with analytical pump head, the values are RSD ≤ 0.05% or SD ≤ 0.01 min. We assume that caffeine elutes at about 1.5 min.

including

$RSD = SD / t_R$ or $SD = RSD * t_R$:

RSD ≤ 0.05 % corresponds to SD ≤ 0.00075 min
SD ≤ 0.01 min corresponds to RSD ≤ 0.67 %
This means that SD ≤ 0.01 min is greater than RSD ≤ 0.05%. The test is passed when the measured result for SD is below or equal to 0.01 min.

6.3.1.2 Solvent Composition of the Gradient Pump, Accuracy, Precision, and Ripple (includes the qualification of the extension valve of the method transfer kit)

Theory

If the gradient pump composes the solvent inaccurately, this will mainly effect the retention times. To keep the measuring effort low, different compositions are checked based on the ASTM instructions. Use 100% water for solvent A. Solvent B is a mixture of water and acetone (0.1% Vol. for flow cells with 1 cm light path). Acetone is highly absorbing in the range of λ = 265 nm. The gradient can be observed in a chromatogram. There are no sample injections required.

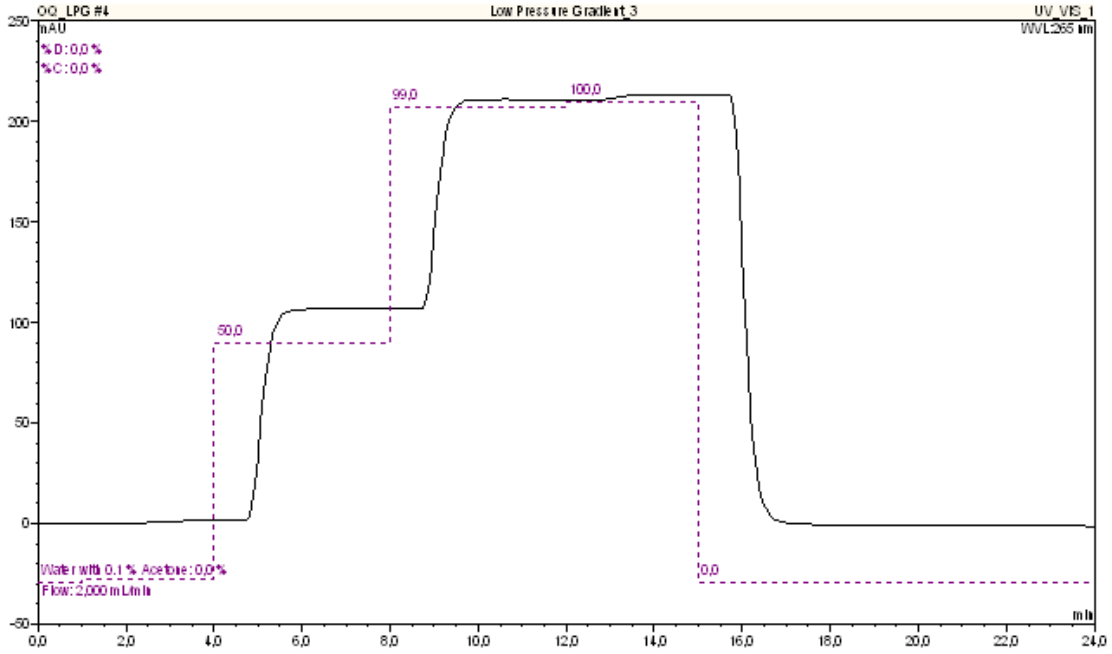

Figure 37: Theoretical (broken line) and real gradients (STD_GRAD standard sequence for gradient pumps)

Procedure

The following solvent compositions (in %B) are mixed: 0, 1, 50, 99, and 100 (example for the standard step gradient). However, some pumps are qualified using different step gradients, for example, due to restricted specifications. Section 6.2.1 on page 104 provides more details on the eluent composition that is used for each of the supported pumps.

For the described arrangement and non-changing solvent composition, the ripple is indicated by the signal noise.

The Vanquish Core extension valve of the method transfer kit is qualified by evaluating the extension valve loop volume.

To qualify a gradient pump, the **STD_GRAD** or **Long_GRAD** sequence is automatically selected by the Wizard. Dependent on the pump type, additional or optional sequences are offered (see below).

For the qualification of the Vanquish Core method transfer kit extension valve, the Wizard automatically selects the sequence **STD_GRAD_MTK** or **Long_GRAD_MTK** (supported only for Vanquish Core systems with binary or quaternary pump) instead of the **STD_GRAD** or **Long_GRAD** sequence.

For the ternary high-pressure gradient systems from Shimadzu, the Wizard automatically selects the additional sequence **TERN_GRAD_C_B**. This sequence is used to determine the accuracy and the precision of the gradient and the ripple between the solvent channels C and B.

For the quaternary low-pressure gradient systems from Thermo Scientific (Vanquish, UltiMate, Accela, and Spectra System), and Agilent, the Wizard automatically selects the additional sequence **QUAT_GRAD_C_D**. This sequence is used to determine the accuracy and the precision of the gradient and the ripple between the solvent channels C and D.

For dual gradient pumps, the qualification is performed for both pump units. The sequence **STD_GRAD** is used to check the right pump unit. The sequence **STD_GRAD_DGP_Left** is used to check the left pump unit.

For specific Summit P680, UltiMate, and Vanquish pumps, up to two optional sequences may be offered. According to the installed pump option (MicroFlow kit or mixer volume), select the correct sequence manually.

- **MICRO_GRAD** (offered only for Summit and UltiMate pumps)
  The **MICRO_GRAD** sequence is used for pumps with an installed MicroFlow kit. The gradient composition timetable corresponds to the program of the **STD_GRAD** sequence. However, because of the lowered mixing volume compared to a pump with standard mixer, the detector signal is shifted to earlier retention times. The evaluation of the check considers this time shift.
- **LONG_GRAD**
  The **LONG_GRAD, LONG_GRAD_DPG_LEFT**, **LONG_QUAT_GRAD** sequences are used if a mixer with a volume above 400 µL is installed. A larger mixing chamber volume increases the equilibration time of the gradient. The gradient composition timetable and the evaluation were adapted accordingly.

TIP For the UltiMate LPG-3400M(B) and LPG-3400BM micro pumps, only the **LONG_GRAD** sequence is available.

TIP For the UltiMate DGP-3600M(B) and DGP-3600BM micro pumps, only the **LONG_GRAD** and **LONG_GRAD_DGP_Left** sequences are available.

Evaluation

To facilitate the comparison, the absorption values are converted and expressed as %B (quaternary pumps also as %D). To compensate the detector drift, the absorption of the pure solvent A (quaternary pumps as %C) is measured at the beginning and at the end of the gradient. These values are the basis for the regression line that is used to correct the baseline of the entire chromatogram.

To define the gradient accuracy, the measured step height is compared to the height that must theoretically result from the solvent composition.

To define the precision, three gradients are recorded. The standard deviations of the step heights indicate the precision.

The ripple is determined for all steps. A 0.4 minutes interval is defined for each step. For each interval, Chromeleon uses the data to calculate a regression line, based on the method of least squares. Parallel to the regression line, two lines are drawn through the measured minimum and maximum value. The noise values in relation to the absorption signal when 100% eluent B or D is pumped, is an indication for the ripple in [%].

To qualify the extension valve of the method transfer kit, the step gradient accuracy test is performed with and without extension valve loop. The gradient delay of the step gradient runs with and without extension valve loop is evaluated and converted, with help of the used flow rate, into a volume. This reflects the theoretical loop volume of the extension valve. The calculated loop volume of the extension valve, the gradient accuracy and the ripple are a measure for the qualification of the extension valve.

6.3.2 Autosampler

6.3.2.1 Precision of Injection Volume

Theory

The precision of the injection volume is mainly influenced by the quality of the autosampler syringe / metering device and the volume that has been adjusted to the injection volume. In addition, the mechanics for the syringe movement / metering device is a decisive factor for the accuracy and precision of the injection volume.

Especially when you use a manual injection valve:

- Verify that there are no air bubbles in the sample.
- Inject at least five times the sample loop volume; that is, inject at least 50 µL.

Varying injection volumes affect the peak areas even if the same standard is injected.

Procedure and evaluation

With the **Injector_Flow_Repro** sequence, a caffeine standard (solvent: water at a flow rate of 0.3 mL/min; wavelength: 272 nm) is injected six or ten times. The autosampler type determines the injection volume and the standard to be used (see the table below). The relative standard deviation of the peak areas of the ten injections indicates the precision of the injection volume.

Autosampler	Standard[(1)] used	Injection volume / µL
Other	Standard 4	5
Summit Gina 50	Standard 3	10
UltiMate ACC-3000(T) Sample loop volume: 200 µL	Standard 3	20
UltiMate OAS-3300TXRS	Standard 3	10
UltiMate WPS-3000(T)SL		
Micro	Standard 5	2
with 250 µL injection volume kit	Standard 3	10
UltiMate WPS-3000TBPL / WPS3000T(B)FC Analytical Large Volume Configuration	Standard 3	20
UltiMate WPS-3000(T)RS Micro option	Standard 5	2
UltiMate WPS-3000TBRS	Standard 5	2
UltiMate WPS-3000TXRS	Standard 5	2

Autosampler	Standard(1) used	Injection volume / µL
Accela Autosampler	Standard 3	10
Vanquish Horizon and Flex Autosampler with any flow cell except 60-mm-LightPipe flow cell of Vanquish VH-D10	Standard 6	1
Vanquish Horizon and Flex Autosampler and Vanquish VH-D10 with 60-mm-LightPipe flow cell	Standard 3	1
Vanquish Core Autosampler and Vanquish VH-D10 with 60-mm-LightPipe flow cell	Standard 2	3

(1) Also, see section 3.1, page 16.

6.3.2.2 Carry-Over

Theory

After a highly concentrated sample, a sample containing only solvent is injected. Ideally, only the signal for the solvent is displayed in the chromatogram. However, if a signal for the sample is displayed, this indicates the carry-over by the autosampler. As the highly concentrated sample exceeds the linearity range of the detector, a reference sample with a considerably lower concentration is also injected.

Procedure and evaluation

The carry-over by the autosampler is measured with samples 6 to 9 of the **Sampler_Lin_CO** sequence (solvent: water at a flow rate of 1.0 mL/min, wavelength: 272 nm).

Sample no.	Content	Concentration
6	Water (same vial as sample 9)	-
7	Solution of caffeine in water	10 µg/mL (standard 2 - reference sample)
8	Solution of caffeine in water	2000 µg/mL (standard 7)
9	Water (same vial as sample 6)	-

The carry-over (CO in [%]) is calculated as follows:

$$CO = \frac{Area_{Water,corr}}{Area_{Conc:2000\,\mu g/ml}} = \frac{Area_{Water,CarryOver} - Area_{Water}}{Area_{Reference} \times \frac{c_{HighConcentratedSample}}{c_{Reference}}}$$

$$= \frac{Area_{Water,CarryOver} - Area_{Water}}{Area_{Reference}} \times \frac{c_{Reference}}{c_{Conc\,2000\,\mu g/ml}}$$

with:

Symbol	Description
$Area_{water,corr}$	Area of the caffeine peak in the water sample (sample 9 – sample 6)
$Area_{Conc:2000\mu g/mL}$	Peak area of the highly concentrated caffeine sample (sample 8)
$Area_{water,CarryOver}$	Peak area of the water injection (sample 9: solvent and caffeine peaks) after the carry-over sample (sample 8)
$Area_{water}$	Peak area of the water injection (sample 6: solvent peak) before the carry-over sample (sample 8)
$Area_{Reference}$	Peak area of the reference sample (sample 7)
$c_{Reference}$	Caffeine concentration of the reference solution (conc.: 10 µg/mL)
$c_{Conc:2000\mu g/mL}$	Caffeine concentration of the carry-over solution (conc.: 2000 µg/mL)

TIP The detection parameter settings for automatic peak integration aim to reliably and precisely determine the integration line. However, as the peak shape of water injections may be very small and noisy, it is not always possible to ensure a correct automatic integration. In this case, we recommend correcting peak integration manually.

6.3.2.3 *Linearity of Injection Volume*

Theory

The linearity of the injection volume and its precision depend on the quality of the syringe and the syringe volume that has been adjusted to the injection volume. Besides, the quality of the autosampler mechanics also affects the result.

Select the concentration of the standard, which is injected in different volumes, in such a way that the detector works in the linear range for all injections, usually between 10 mAU and 1000 mAU.

Procedure and evaluation

With the **Sampler_Lin_CO** sequence, a caffeine standard (solvent: water at a flow rate of 1 mL/min, wavelength: 272 nm) is injected five times. The autosampler type determines the injection volume and the standard (see table).

Autosampler	Standard[1] used	Injection volume / µL
Other	Standard 2	5 / 10 / 20 / 40 / 80
Agilent G1367D	Standard 2	5 / 10 / 20 / 30 / 40
Agilent G4226A	Standard 3	1 / 5 / 10 / 15 / 20
Shimadzu SIL-10A / SIL-10Ai / SIL-10AF / SIL-10ADvp / SIL-20A(C)XR	Standard 2	5 / 10 / 20 / 40 / 50
Summit Gina 50	Standard 2	10 / 20 / 40 / 60 / 80
UltiMate ACC-3000(T)		
Sample loop volume: 20 µL	Standard 3	1 / 3 / 5 / 7 / 10
Sample loop volume: 50 µL	Standard 3	5 / 10 / 15 / 20 / 25
UltiMate OAS-3300TXRS	Standard 3	4 / 6 / 8 / 10 / 12
UltiMate WPS-3000(T)SL / WPS-3000(T)RS		
Analytical	Standard 2	5 / 10 / 20 / 40 / 90
Micro	Standard 3	1 / 5 / 10 / 15 / 20
with 250 µL injection volume kit	Standard 2	10 / 20 / 40 / 80 / 160
UltiMate WPS-3000(T)PL / WPS-3000(T)PLRS		
Sample loop volume: 20 µL	Standard 3	1 / 3 / 6 / 9 / 12
Sample loop volume: 50 µ	Standard 3	1 / 5 / 10 / 15 / 20
Sample loop volume: 100 µL	Standard 2	5 / 10 / 20 / 40 / 50
UltiMate WPS-3000TBPL / WPS-3000T(B)FC Analytical		
Standard configuration	Standard 3	5 / 10 / 15 / 20 / 25
Large Volume configuration	Standard 2	20 / 50 / 80 / 110 / 140
UltiMate WPS-3000(T)RS Micro option	Standard 3	1 / 5 / 10 / 15 / 20
UltiMate WPS-3000TBRS	Standard 3	1 / 5 / 10 / 15 / 20
UltiMate WPS-3000TXRS	Standard 3	1 / 5 / 10 / 15 / 20
Accela Autosampler	Standard 3	2.5 / 5 / 7.5 / 10 / 12.5
Vanquish Horizon and Flex Autosampler and flow cell with 10-mm light path		
Sample loop volume: 10 µL	Standard 3	1 / 3 / 5 / 7 / 10
Sample loop volume: 25 µL	Standard 3	1 / 5 / 12.5 / 20 / 25

Autosampler		Standard(1) used	Injection volume / µL
	Sample loop volume: 100 µL	Standard 2	1 / 25 / 50 / 75 / 100
Vanquish Horizon and Flex Autosampler and 60-mm-LightPipe flow cell			
	Sample loop volume: 10 µL	Standard 2	1 / 3 / 5 / 7 / 10
	Sample loop volume: 25 µL	Standard 2	1 / 5 / 12.5 / 20 / 25
	Sample loop volume: 100 µL	Standard 2	1 / 10 / 25 / 40 / 50
Vanquish Core Autosampler and flow cell with 10-mm light path			
	Sample loop volume: 10 µL	Standard 3	1 / 3 / 5 / 7 / 10
	Sample loop volume: 25 µL	Standard 3	3 / 5 / 12.5 / 20 / 25
	Sample loop volume: 100 µL	Standard 2	3 / 25 / 50 / 75 / 100
	Sample loop volume: 250 µL	Standard 2	3 / 50 / 100 / 200 / 250
	Sample loop volume: 1000 µL	Standard 2	3 / 200 / 400 / 800 / 1000
Vanquish Core Autosampler and 60-mm-LightPipe flow cell			
	Sample loop volume: 10 µL	Standard 2	1 / 3 / 5 / 7 / 10
	Sample loop volume: 25 µL	Standard 2	3 / 5 / 12.5 / 20 / 25
	Sample loop volume: ≥ 100 µL	Standard 2	3 / 10 / 25 / 40 / 50

(1) Also, see section 3.1, page 16.

The peak area and injection volume are represented in a graph and the regression line is determined. The correlation coefficient and the standard deviation of this line indicate the linearity.

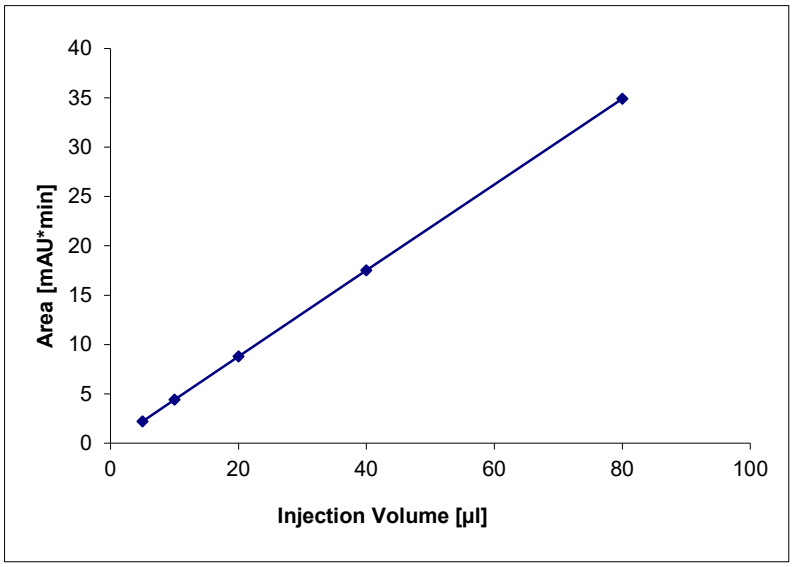

Figure 38: Linearity of injection volume

6.3.2.4 Sample Temperature Accuracy of Autosamplers / Chargers

Special test for certain devices – see also section 4.3, page 66.

Theory

The sample temperature accuracy mainly depends on the cooling and heating accuracy of the autosampler / Charger, the insulation of the sample compartment, and the thermal transfer to the vial.

Procedure and evaluation

The sample temperature accuracy is determined with the help of an external thermometer. The temperature sensor is placed in a standard vial (1.8 mL) filled with water. The carousel cover or autosampler / Charger door must be closed during the test. The autosampler temperature is set to a nominal temperature (e.g., 10°C), depending on the autosampler type. The sample (water) temperature is recorded over a period of 60 minutes. Within the 60 minutes, the sample temperature reaches a stable value. The temperature accuracy is the temperature difference between the sample temperature and the nominal autosampler temperature.

6.3.3 Column Compartment

6.3.3.1 Temperature Accuracy

Theory

Depending on the type of application, temperature fluctuations of the solvent and especially of the column can result in considerable retention time fluctuations. In addition to the precision of the temperature achieved with the column compartment, the accuracy is important as well. Only high accuracy allows transferring applications to different systems.

Procedure and evaluation

Four measuring points are used to check the temperature accuracy of the column compartment. The check is performed with the **Column_Oven(_LT)** sequence. An external, calibrated thermometer is used to measure the achieved temperature.

The achieved temperatures are compared to the set values. The difference indicates the temperature accuracy.

Remarks:

TIP If it is not allowed to modify the Chromeleon Server Configuration due to customer restrictions, perform the manual qualification according to the description in section 3.7.2, page 57.

- Depending on the system configuration, the **Column_Oven** and the **Column_Oven_LT** sequence might be offered, e.g, for Vanquish column compartments (see also section 3.4.6, page 40).
- It is not possible to set the temperature on certain column compartment modules (Waters Alliance 2690 Separation Module, Shimadzu, and Agilent G1316) when the retention time is negative. The first measurement reading is 10 minutes after the sample has been started. At this time, equilibration of the column compartment may not be complete. Therefore, the same temperature is set for the second measuring point (50 minutes). The column compartment module has passed the check even if the target temperature is reached only for the second measuring point. This means that evaluation is performed for three measuring points only.
- Due to its small temperature range, evaluation for the UltiMate ACC-3000(T) and ECD-3000RS column oven is performed for three and / or two measuring points only.
- For the column compartment of the Accela autosampler, a target temperature can only be set within the context of a sample. Therefore, the temperature accuracy is tested at a single measuring point only.

6.3.4 UV Detector

6.3.4.1 Baseline Noise, and Drift

Theory

Drift and baseline noise are important factors for UV detectors. Increased baseline noise considerably reduces the sensitivity, as it is not possible to distinguish between low-level signals and noise. With increased drift, it is more difficult to integrate the signals correctly because the less stable the baseline is, the more inaccurate is integration. The baseline noise of the detector mainly depends on the condition of the lamp.

There is a considerable increase in noise if an old lamp with poor light intensity is used. This is also true when the flow cell is dirty. In addition, make sure that the measuring and ambient conditions are constant and that the flow cell is free from gas bubbles.

To measure the drift of a UV detector, also make sure that the measuring and ambient conditions are constant. In addition, it is very important that the lamp has been turned on for several hours. In the detector environment, avoid drafts and direct sunlight.

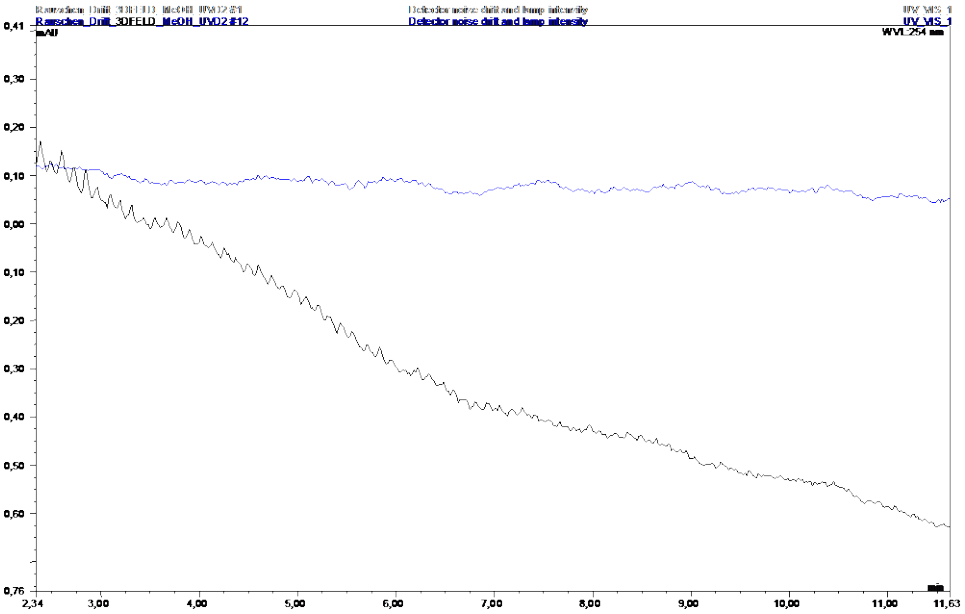

Figure 39: Lamp drift directly after the lamp has been turned on (bottom chromatogram) and after it has been turned on for six hours (top chromatogram)

The lamp intensity decreases while the lamp is in operation. In addition, lamps age when turned on and off frequently.

Procedure and evaluation

The checks for noise, and drift are included in the **UV_Noise_Drift** sequence.

For those checks, water is pumped through the cell at a flow rate of 1 mL/min. The UV signal is recorded at 254 nm (Vanquish diode array detectors: 230 nm).

To calculate noise, the measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression based on measured values, using the method of least squares. Parallel to the regression line, two lines are drawn through the values with maximum distance from this regression line. The noise is the distances of these lines. The calculated values are averaged for all 20 intervals to establish the final value.

To calculate the drift, Chromeleon calculates a regression line from all data points within a range of 1 to 21 minutes based on the method of least squares. The slope of the regression line is the calculated drift. The absolute drift value must not exceed the limit.

6.3.4.2 Wavelength Accuracy

Procedure and evaluation

The following sequences are automatically selected by the Wizard:

- *Single-wavelength detectors, UltiMate VWD-3400RS, and Vanquish VF-D40-A, VC-D40-A*: **Wavelength_Single**.
  The wavelength accuracy is determined using caffeine in water (c = 60 µg/mL) at a flow rate of 1 mL/min. As water is used as solvent, it is not necessary to change the solvent manually.

Recorded wavelength(s)	Evaluation
270 nm, 272 nm and 274 nm	A parabola is calculated from the signal heights of the caffeine signal and the wavelengths. The maximum of the parabola is determined and compared to the theoretical value of the spectral maximum of caffeine (272.5 nm)

- *Multi-wavelength detectors, photodiode array detectors, and others*:
  - Multi-wavelength UV detectors without PDA option: **UV_Wavelength**.
  - Photodiode array detectors: **DAD_Wavelength**.
    Separate sequences are available for the following detectors: TSP UV 2000, Waters 2487, Shimadzu LC-2010 SPD, SPD-10A(V), and SPD-10A(V)vp detectors. Wavelength accuracy is determined using pyrene in methanol (c = 3 µg/mL) at a flow rate of 1 mL/min.

Detector types	Recorded wavelength(s)	Evaluation
Multi-wavelength and Shimadzu detectors (two channels)	331 nm, 333 nm, and 335 nm	A parabola is calculated from the signal heights of the pyrene signal and the wavelengths. The parabola maximum is determined and compared to the theoretical value of the spectral maximum of pyrene (333.3 nm).
Photodiode array detectors	The UV spectrum for pyrene is recorded between 250 nm and 350 nm.	Chromeleon determines the spectral maxima between 250 nm and 290 nm and between 330 nm and 350 nm and compares them to their theoretical values (272.1 nm and 333.3 nm).
TSP UV2000 and Waters 2487 detectors (two channels)	235 nm, 240 nm, and 245 nm	A parabola is calculated from the signal heights of the pyrene signal and the wavelengths. The maximum of the parabola is determined and compared to the theoretical value of the spectral maximum for pyrene (239.4 nm).

The following image shows the typical UV spectrum of pyrene recorded by a photodiode-array detector.

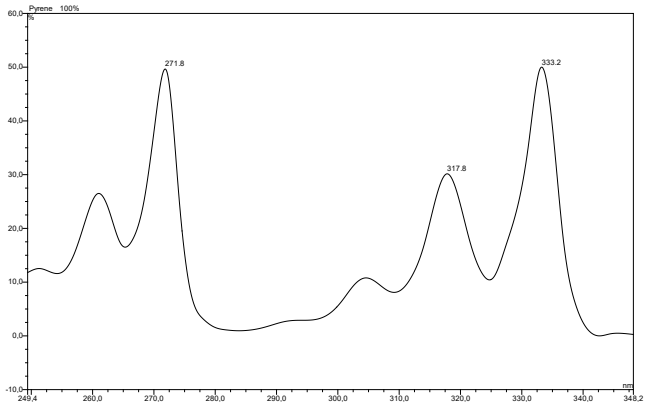

Figure 40: UV spectrum of pyrene in methanol

6.3.4.3 Linearity

Theory

The linearity of a detector mainly depends on the optical and electronic systems. With electronic systems, non-linearity is caused by dark current and dark current drift. Dark measurements can be used to compensate the influence of these factors.

However, as the light intensity decreases due to lamp ageing or absorption of the eluent or sample, the influence of the dark current on the linearity increases. The influence of the eluent is insignificant in this case, as water is used for the test procedure. The influence of the sample is fully used in this test procedure to determine the detector linearity. Consider that the resulting deviations of the linear behavior are only important with extremely high absorption (> 1.5 AU).

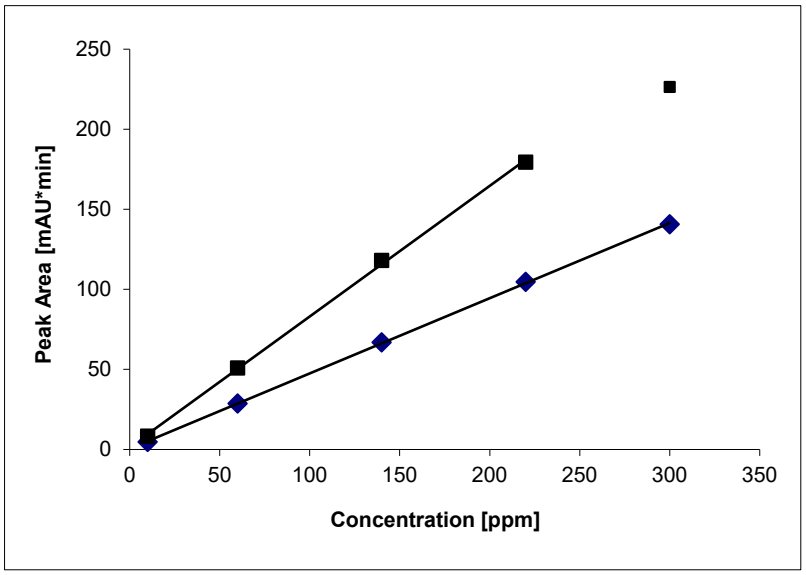

Figure 41: Linearity of the detector signal depending on the peak area

Procedure and evaluation

The detector linearity is measured under the following conditions:

Parameter	Description
Sequence used	**UV_LINEARITY**
Standards used	5 different caffeine standards
Set concentrations	10 µg/mL, 60 µg/mL, 140 µg/mL, 220 µg/mL, and 300 µg/mL, dissolved in water; the actual concentrations are entered into the QNT file and taken into account.
Eluent	Water
Flow rate	1 mL/min.
Wavelength	272 nm

Concentration and peak area are represented in a graph. The regression coefficient and the relative standard deviation of this line indicate the linearity.

TIP The peak height of the sample with the highest concentration has to cover the linearity range as described in sections 6.2.6 and 6.2.7 for each of the detectors. The recommended injection volume is listed in the following table. If the sample with the highest concentration is outside the linearity range, adapt the injection volume for all samples used for the linearity check: Adapt it so that the peak height of the sample with the highest concentration matches the requested value.

Detector	Injection volume / µL
Other	10.0
PDA-100 / PDA-3000 / AD25	8.0
VWD-3100 / VWD-3400RS	13.0
VH-D10-A (60-mm-LightPipe flow cell)	1.7
VF-D11-A / VC-D11-A / VC-D12-A (with standard bio flow cell)	11.0
VF-D11-A / VC-D11-A / VC-D12-A (with semi-micro bio and semi-analytic flow cell)	13.0
VF-D40-A / VC-D40-A (with semi-micro bio flow cell)	10.5
VF-D40-A / VC-D40-A (with standard bio flow cell)	11.5

6.3.5 Fluorescence Detector

6.3.5.1 Baseline Noise / Signal Height

Theory

Drift and baseline noise are important factors for UV detectors. Increased baseline noise considerably reduces the sensitivity, as it is not possible to distinguish between low-level signals and noise.

The baseline noise of the detector mainly depends on the condition of the lamp. There is a considerable increase in noise if an old lamp with poor light intensity is used. This is also true when the flow cell is dirty. In addition, make sure that the measuring and ambient conditions are constant and that the flow cell is free from gas bubbles.

In addition to the absolute value of the baseline noise, the signal height to noise ratio is important. The signal height mainly depends on the condition of the lamp and the flow cell. A contaminated flow cell may result in a higher fluorescence signal.

Procedure and evaluation

The following sequences are used:

- *All fluorescence detectors (except Summit RF1002and RF2000 detectors)*:
  The **FLUORES_V2** sequence is used to determine the signal-to-noise ratio (SNR). Water is pumped through the flow cell at a flow rate of 1 mL/min. The excitation wavelength is 350 nm. The emission signal is recorded for 20 min at an emission wavelength of 397 nm (Raman signal of water) and for another 20 min at 450 nm (dark current).

  The signal-to-noise ratio (SNR) is evaluated as follows:

  - Noise evaluation at 450 nm – SNR (Dark Current)

    $$\mathrm{SNR(Dark\ Current)} = \frac{\mathrm{Average\,Signal\ Value}_{397\,\mathrm{nm}} - \mathrm{Average\,Signal\ Value}_{450\,\mathrm{nm}}}{\mathrm{Noise}_{450\,\mathrm{nm}}}$$

  - According to ASTM with noise evaluation at 397 nm (only for UltiMate and Vanquish detectors)

    $$SNR(ASTM) = \frac{Average\ Signal\ Value_{397\,nm} - Average\ Signal\ Value_{450\,nm}}{Noise_{397\,nm}}$$

  To determine the noise, the measuring signal is split into 40 intervals of 30 seconds each. For each interval, Chromeleon calculates a regression line, based on the method of least squares. The noise value is the distance between two parallel lines and the regression line through the lowest and highest values. For the calculated values, the 40 interval values are averaged.

- *Summit RF1002 and RF2000 fluorescence detectors*:
  The **Fluorescence** sequence is used to determine the noise and the signal height. When testing the Summit RF2000, make sure that the detector's ZWAVE parameter is set to 1 (see section 3.4, page 33). Water is pumped through the flow cell at a flow rate of 1 mL/min. The excitation wavelength is 350 nm; the emission wavelength is 397 nm.

  To determine the noise, the measuring signal is split into 30 intervals of 30 seconds each. For each interval, Chromeleon calculates a regression line, based on the method of least squares. The noise value is the distance between two parallel lines and the regression line through the lowest and highest values. For the calculated values, the 30 interval values are averaged.

6.3.5.2 Wavelength Accuracy

Procedure and evaluation

The following sequences are used:

- *All fluorescence detectors (except Summit RF1002 and RF2000 detectors):*
  The **FLUORES_V2** sequence is used to determine the wavelength accuracy (emission and excitation) by using spectra. Water is pumped through the flow cell at a flow rate of 1 mL/min. The emission spectrum is recorded in the range around 397 nm (excitation wavelength: 350 nm). The excitation spectrum is recorded in the range around 350 nm (emission wavelength: 397 nm). The relative signal maximum of both spectra is determined and compared to the theoretical maximum.

TIP Remark on the manufacturer specification: It is only possible to check the manufacturer specification of ± 2 nm for the excitation and the emission wavelengths by using a special flow cell and a mercury lamp. For OQ and PQ, the module should preferably be checked with the components used for the measurements.

- *Summit RF1002 and RF2000 fluorescence detectors*:
  The **Fluorescence** sequence is used to determine the wavelength accuracy of the emission spectrum. Water is pumped through the flow cell at a flow rate of 1 mL/min. For an excitation wavelength of 350 nm, the emission wavelength changes in 1 nm increments from 380 nm to 410 nm. The relative signal maximum is compared to the theoretical maximum.

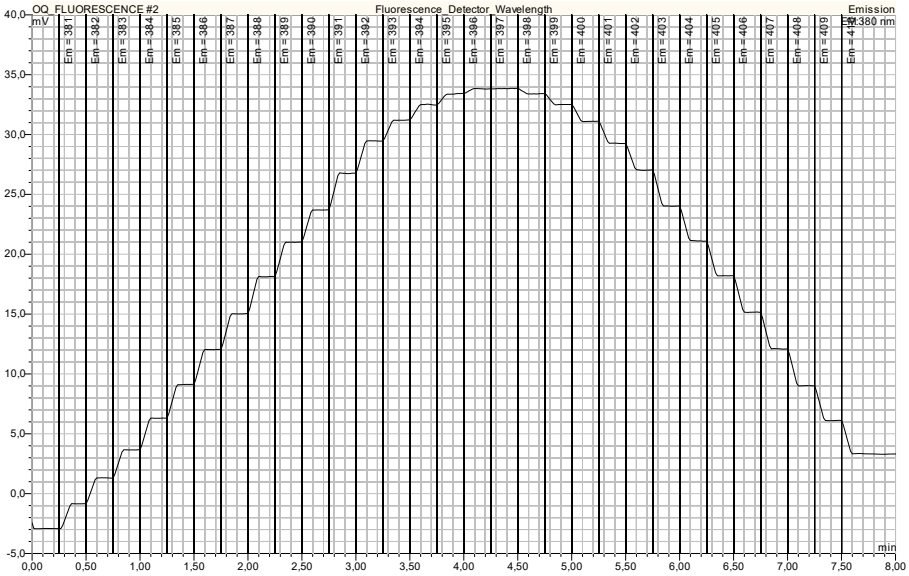

Figure 42: Chromatogram for defining the relative maximum of the emission spectra between 380 nm and 410 nm

6.3.5.3 Linearity

Special test for certain devices only – also see sections 6.2.8 and 6.2.9.

Theory

The linearity of a fluorescence detector mainly depends on the optical and electronic systems, the sample concentration and the eluent. With electronic systems, non-linearity is caused by dark current and dark current drift. Dark measurements can be used to compensate the influence of these factors. Contamination in the flow cell or optics and extremely high sample concentrations or the eluent may cause stray light that influences the detector linearity. In addition, adsorption from the sample on the cell walls can be found with very low sample concentrations. This effect also influences detector linearity.

The influence of the sample in a suitable concentration range is fully used in this test procedure to determine the detector linearity. Consider that the resulting deviations of the linear behavior are only important with extremely high or very low sample concentrations. Therefore, the test results reflect the influence of the detector itself on linearity.

Procedure and evaluation

The detector linearity is measured under the following conditions:

Parameter	Description
Sequence used	**FLUORES_LINEARITY**
Standards used	7 anthracene standards
Concentrations	0.5 mg/100 mL, 0.4 mg/100 mL, 0.3 mg/100 mL, 0.2 mg/100 mL, 0.1 mg/100 mL, 0.05 mg/100 mL, and 0.005 mg/100 mL, dissolved in acetonitrile. The actual concentrations are entered into the QNT file and are taken into account.
Eluent	Acetonitrile / water (90:10% v/v)
Flow rate	1 mL/min.
Inject volume	10 µL

Concentration and peak area are represented in a graph. The regression coefficient, the relative standard deviation, and the relative y-axis intercept (relative to the peak area of the sample with the highest concentration) of the graph indicate the linearity.

6.3.6 Corona Detector

6.3.6.1 Baseline Noise/Signal Height/Drift/Spikes/Precision

Theory

Baseline noise is an important specification for a detector. Increased baseline noise considerably reduces the detection sensitivity, as it is not possible to distinguish between small signals and noise. In addition to the absolute value of the baseline noise, the signal height to noise ratio is important.

The causes that may influence these specifications are diverse and are described in detail in the Operating Instructions for the module.

Procedure

The **CORONA_(VEO)_NOISE_DRIFT_SNR** sequence performs the following checks:

- Noise
- Height of spikes
- Drift
- Signal-to-noise ratio
- Precision of height

The checks are performed under the following conditions:

Parameter	Description
Standard	Caffeine dissolved in water
Standard concentration	Corona and Corona Ultra series: 25 µg/mL Corona Veo and Vanquish series: 5 µg/mL
Eluent	Water / methanol (80:20% v/v)
Flow rate	1 mL/min.
Inject volume	10 µL

The settings for recording the detector signal are as follows:

Parameter	Variant	Setting
Filter	Corona	None
	Corona plus	
	Corona ultra	Corona
	Corona ultra RS	4
	Corona Veo /Vanquish series	5 s
Nebulizer temperature	Corona	Off
	Corona plus	
	Corona ultra (RS)	25 °C
	Corona Veo / Vanquish series	35 °C
Data collection rate	All Corona detectors	10 Hz
Power function	All Corona detectors	1

To perform the noise, drift and height of spike test, eluent is pumped through the detector. For the signal-to-noise ratio and the height precision tests, six caffeine injections are performed.

Evaluation - Noise and Spikes Height

The measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression line, based on the method of least squares. Parallel to the regression line, two lines are drawn through the smallest and largest values. The noise is the distance of these lines. The calculated values are averaged for all 20 intervals to establish the final value.

To calculate the height of the largest spike, the measuring signal is also split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates the signal average value, the minimum, and the maximum signal. The height of the positive spikes within an interval is calculated according to the following formula:

$$\text{Spike Height}_{\text{Int. X}} = \text{Signal Maximum}_{\text{Int. X}} - \left(\text{Signal Average}_{\text{Int. X-1}} + \text{Signal Average}_{\text{Int. X+1}}\right) / 2$$

The height of the negative spikes within an interval is calculated according to the above formula; however, the signal minimum is used instead of the signal maximum. The absolute greatest height of a spike of all intervals corresponds to the height of the largest spike. The signal average values of the first and last interval outside of the measuring signal are extrapolated.

Evaluation - Drift

To calculate the drift, Chromeleon calculates a regression line from all data points within a range of 2 to 22 minutes based on the method of least squares. The slope of the regression line is the calculated drift. The absolute drift value must not exceed the limit.

Evaluation - Signal-to-Noise Ratio

The signal-to-noise ratio (SNR) is calculated as follows:

$$\mathrm{SNR} = \frac{\text{Peak Height Average}}{\text{Signal Noise}}$$

Evaluation - Precision of Height

The relative standard deviation of the peak heights of the six injections indicates the precision of the peak height.

6.3.6.2 Signal Calibration

Theory

The mass of the analyte and the corresponding detector response (peak area) are proportional to the square root. Therefore, the calibration function used here is a quadratic regression.

Procedure and evaluation

The signal calibration is measured under the following conditions:

Parameter	Description
Sequence used	**CORONA_(VEO)_Resp_Calib**
Standard	Caffeine dissolved in water
Standard concentrations	Corona, Corona Ultra series: 25 µg/mL, 125 µg/mL, 250 µg/mL und 500 µg/mL Corona Veo / Vanquish series: 5 µg/mL, 25 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL The actual concentrations are entered into the QNT file and are taken into account.
Eluent	Water / methanol (80:20% v/v)
Flow rate	1 mL/min.
Inject volume	10 µL

Concentration and peak area are represented in a graph and the quadratic regression is determined. The coefficient of determination of this quadratic regression indicates the calibration.

6.3.7 Mass Spectrometry Detector

6.3.7.1 Autotune

Theory

The system tune and diagnostic procedure (autotune) is performed prior to the sensitivity test with HESI source to ensure that the instrument is working properly.

Procedure

The sequence **ISQ_Sensitivity** is used to perform the autotune in HESI positive and HESI negative mode. It is run with a low flow rate (0.05 mL/min) infusion of the internal reference calibrant using the LC pump to deliver the calibrant to the detector. The following source settings are used:

Parameter	Setting	
	Positive Ion Mode	Negative Ion Mode
Vaporizer temperature	0 °C	0 °C
Ion transfer tube temperature	350 °C	350 °C
Source voltage	3000 V	-2500 V
Sheath gas pressure	30.0 psig	42.0 psig
Aux. gas pressure	2.0 psig	6.7 psig
Sweep gas pressure	0.0 psig	0.0 psig

Evaluation

The Chromeleon audit trail is evaluated. An empty audit trail entry for the property “Auto tune error message:” is indicated as **ok**, i.e., that the autotune has passed.

6.3.7.2 Sensitivity

Theory

The sensitivity is an important parameter for a detector. A low sensitivity considerably reduces the limit of detection, for example, by an increased noise or an insufficient mass accuracy.

Procedure

With HESI and, if available with APCI source, the positive and negative ion mode is qualified with two different sequences dependent on the source type. The sensitivity test is performed with three eluent injections followed by four analyte injections (the first analyte injection is only performed for system equilibration).

With HESI source, the sensitivity is measured under the following conditions:

Parameter	Description	
	Positive Ion Mode (HESI)	**Negative Ion Mode (HESI)**
Sequence used	**ISQ_Sensitivity**	
HPLC parameter		
Standard	Eluent as blank Reserpine diluted with eluent (10 pg/µL)	Eluent as blank p-Nitrophenol diluted with eluent (20 pg/µL)
Eluent	Water and 0.1% formic acid / Methanol (35:65 v/v%)	Water / Methanol (35:65 v/v%)
Flow rate	0.4 mL/min	
Inject volume	1 µL	
Sample temperature	4 °C	
Column temperature	20 °C	
Source Parameter		
Vaporizer temperature	450 °C	
Ion transfer tube temperature	350 °C	
Source voltage	3000 V	-2000 V
Sheat gas pressure	40 psig	
Aux gas pressure	3 psig	
Sweep gas pressure	0 psig	
Scan Parameter		
Mode	Scan	
Type	SIM	
Scan name	Reserpine	p-Nitrophenol
SIM mass	609.3 m/z	138.1 m/z
Ion polarity	Positive	Negative
SIM width	0.1 amu	
Dwell time	0.3 s	
Chrom Filter	7	
Source CID voltage	0 V	

With APCI source, the sensitivity is measured under the following conditions:

Parameter	Description	
	Positive Ion Mode (APCI)	**Negative Ion Mode (APCI)**
Sequence used	**ISQ_APCI_Sensitivity**	
HPLC parameter		
Standard	Same as used for positive ion mode test with HESI	Same as used for negative ion mode test with HESI
Eluent	Water / Methanol (35:65 v/v%)	
Flow rate	0.4 mL/min	0.9 mL/min
Inject volume	1 µL	
Sample temperature	4 °C	
Column temperature	20 °C	
Source Parameter		
Vaporizer temperature	550 °C	500 °C
Ion transfer tube temperature	350 °C	
Source current	0.01 µA	7 µA
Sheat gas pressure	55 psig	10 psig
Aux gas pressure	2 psig	
Sweep gas pressure	0 psig	
Scan Parameter		
Mode	Scan	
Type	SIM	
Scan name	Reserpine	p-Nitrophenol
SIM mass	609.3 m/z	138.1 m/z
Ion polarity	Positive	Negative
SIM width	0.1 amu	
Dwell time	0.2 s	
Chrom Filter	7	
Source CID voltage	0 V	

Evaluation

For each injection (analyte injections number two to four), the signal-to-noise ratio for the target analyte is calculated. The signal-to-noise ratio is determined using the noise algorithm root mean square (RMS) with an average of 50 consecutive minimum noise points in the

baseline. The calculated values of all three injections are averaged to establish the final value.

6.3.8 Electrochemical Detector

6.3.8.1 Baseline Noise

Theory

Baseline noise is an important specification for a detector. Increased baseline noise considerably reduces the detection sensitivity, as it is not possible to distinguish between small signals and noise.

Procedure

The sequence **ECD_NOISE** is used to measure the detector noise. The check is performed using a simulator cell (QualifierRS for DC mode or PulseQualifierRS for pulse mode) for each potentiostat.

For each potentiostat, (maximum four DC potentiostats or one pulse potentiostat), the settings for recording the ECD signal for DC mode is shown in the table below.

Parameter	Setting
Potential	300 mV
Data collection rate	10 Hz
Filter	10 s

The settings for recording the ECD signal for pulse mode is shown in the table below.

Parameter	Setting
E1 Potential	100 mV
E1 PulseWidth	800 ms
E2/E3/E4 Potential	0 mV
E2/E3/E4 PulseWidth	10 ms
Aquisistion Delay	300 ms
GainRaingePulse	Medium

Evaluation

To calculate noise, the measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression line, based on the method of least squares.

Parallel to the regression line, two lines are drawn through the smallest and largest values. The noise is the distance of these lines. The calculated values are averaged for all 20 intervals to establish the final value.

6.3.9 RI Detector

6.3.9.1 Baseline Noise and Drift

Theory

Drift and baseline noise are important factors for RI detectors. Increased baseline noise considerably reduces the sensitivity, as it is not possible to distinguish between low-level signals and noise. With increased drift, it is more difficult to integrate the signals correctly because the less stable the baseline is, the more inaccurate is integration.

The baseline noise of the detector mainly depends on the condition of the lamp. There is a considerable increase in noise if an old lamp with poor light intensity is used. The noise also increases when the reference cell or flow cell is dirty.

To measure the drift of a RI detector, make sure that:

- The measuring and ambient conditions are constant.
- The lamp has been burning for several hours and the entire optical bench is heated up sufficiently.
- The sample and reference part of the flow cell has been rinsed sufficiently.
- The flow cell is free from gas bubbles.

Procedure and evaluation

The **RI_NOISE_DRIFT** sequence includes both the checks of the noise and the drift. Water is pumped through the sample cell at a flow rate of 1 mL/min; the reference cell, too, has been rinsed with water before. The RI signal is recorded at a temperature of 35°C.

To calculate drift and noise, the measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression based on measured values, using the method of least squares. The slope of this curve corresponds to the drift of the measuring signal; the value of the slope is the value of the drift. Parallel to the regression line, two lines are drawn through the smallest and largest values. The noise is the distance of these lines. The calculated values are averaged for all 20 intervals to establish the final value.

6.3.9.2 Linearity

Theory

The linearity of a detector mainly depends on the optical and electronic systems. With electronic systems, non-linearity is caused by dark current and dark current drift. Dark measurements can be used to compensate the influence of these factors. However, as the light intensity decreases due to lamp ageing or refraction by the sample, the influence of the dark current on the linearity increases. The influence of the eluent is insignificant in this case, as the water used during the test procedure is present in the sample flow cell as well as the reference cell. The influence of the sample is fully used in this test procedure to determine the detector linearity. Consider that the resulting deviations of the linear behavior are only important at extremely high sample concentrations due to the strong refraction of the light beam (signals > 600 µRIU).

Procedure and evaluation

The detector linearity is measured under the following conditions:

Parameter	Description
Sequence used	**RI_LINEARITY**
Standard	5 glycerin standards
Standard concentrations	5 mg/mL, 10 mg/mL, 15 mg/mL, 25 mg/mL, and 35 mg/mL, dissolved in water The actual concentrations are entered into the QNT file and taken into account.
Eluent	Water
Flow rate	1 mL/min.
Inject volume	10 µL
Detector temperature	35 °C

Concentration and peak area are represented in a graph. The regression coefficient of this line indicates the linearity.

6.3.10 Evaporative Light Scattering Detector

6.3.10.1 Baseline Noise

Theory

Baseline noise is an important specification for a detector. Increased baseline noise considerably reduces the detection sensitivity, as it is not possible to distinguish between small signals and noise. The baseline noise of the detector mainly depends on the condition

of the lamp. There is a considerable increase in noise if an old lamp with poor light intensity is used. The evaporator temperature and carrier gas flow also affect the noise. Therefore, make sure that the measuring and ambient conditions are kept constant.

Procedure and evaluation

The noise is measured under the following conditions:

Parameter	Description
Sequence used	**ELS_NOISE**
Eluent	Water
Flow rate	1 mL/min.

The conditions for recording the ELS signal are as follows:

Parameter	Description
Nebulizer temperature	50 °C
Evaporator temperature	90 °C
Carrier gas flow	1.6 SLM at 4.1 bar

To calculate noise, the measuring signal is split into 20 intervals of 1 minute each. For each interval, Chromeleon calculates a regression based on measured values, using the method of least squares. Parallel to the regression line, two lines are drawn through the smallest and largest values. The noise is the distance of these lines. The calculated values are averaged for all 20 intervals to establish the final value.

6.4 Troubleshooting

6.4.1 General Notes

A system pressure that is well above 130 bar for a flow rate of 1 mL/min (solvent: water) after the restriction tubing has been connected indicates that a capillary is contaminated. Inspect and exchange the capillaries (including the restriction tubing) to ensure that OQ and PQ are correctly performed.

If problems occur during the checks that cannot be solved observing the notes below, also refer to the respective sections in the operating manuals.

6.4.2 Failure of Individual Checks

6.4.2.1 Pump

Test	Possible Cause	Remedial Action
Flow precision	Autosampler draws air from the vial.	Either there is too little sample volume in vial or the value set for the **Needle Depth** parameter is too low.
	Air bubbles in the syringe	Prime the syringe and buffer tubing sufficiently.
	Autosampler leaking	See *Autosampler Manual*
	Injection valve leaking	See *Autosampler Manual*
Gradient accuracy	Air in the system	Prime the system.
	System not equilibrated	Rinse the system.
	Composition of solvent B or D not correct	Make sure that the solvent composition is correct.
Gradient precision	Air in the system	Prime the system.
	System not equilibrated	Rinse the system.
	Air in the system	Prime the system.
Ripple	Air in the system	Prime the system.
	System not equilibrated	Rinse the system.
Method transfer kit – extension valve test	Loop volume too small	Ensure that a loop with sufficient volume is installed (currently supported: 200 µL).
	Loop volume is negative	Ensure that the loop is installed correctly in the extension valve (valve position 1_2 includes the loop).

6.4.2.2 Autosampler

Test	Possible Cause	Remedial Action
Precision of injection volume	Autosampler draws air from the vial.	Either there is too little sample volume in vial or the value set for the **Needle Depth** parameter is too low.
	Air bubbles in the syringe	Prime the syringe.
	Autosampler leaking	See *Autosampler Manual*
	Injection valve leaking	See *Autosampler Manual*
Linearity of injection volume	Detector linearity check failed	See above.
	Syringe old	Replace the syringe.

6.4.2.3 UV Detector

Test	Possible Cause	Remedial Action
Wavelength accuracy	Spectrum calibration not successful.	Dependent on the detector: First, disconnect and then, reconnect the detector in Chromeleon to trigger a wavelength calibration. Perform a manual wavelength calibration with the flow cell filled with water while making sure that the lamp was turned on for at least the time required for your detector (refer to the *operating manual* for the detector).
	Increased drift	See below.
Baseline noise	Solvent contaminated	Exchange the solvent.
	Lamp too old	Replace the lamp.
	Air bubbles in the flow cell	Prime the flow cell.
Drift	Detector not yet warmed up	Allow the detector sufficient time to warm up.
	Vanquish LightPipe flow cell contaminated	Rinse the flow cell at a high flow rate (1 – 3 mL/min), using water and acetonitrile or methanol
	System not equilibrated	Rinse the system.
	Lamp defective	Replace the lamp.
	Fluctuations in the ambient temperature	If necessary, close the windows and shield the module from the air conditioning system.
	Draft	If necessary, close the windows and shield the module from the air conditioning system.

Test	Possible Cause	Remedial Action
Detector linearity	Lamp too old	Replace the lamp.
	Concentration of standards not correct	Use new standards.
	Peak height of the sample with the highest concentration not in the linearity range specified for the detector (see sections 6.2.6 and 6.2.7)	Reduce the injection volume for all samples used for the detector linearity check so that the peak height of the sample with the highest concentration is in the linearity range of the detector.
	Flow cell contaminated	Clean flow cell. Replace flow cell.

6.4.2.4 RF2000 Fluorescence Detector

Test	Possible Cause	Remedial Action
Wavelength accuracy	The Raman peak of water not visible because the module performs an Autozero whenever the wavelength is changed.	On the module, set the **ZWAVE** parameter to 1 (see section3.5, page 42).

6.4.2.5 Charged Aerosol / Corona detector

The possible causes for errors are diverse and are described in detail and using many examples in the Operating Instructions for the module. Therefore, this manual does not give a list of errors.

6.4.2.6 Mass Spectrometry Detector

Test	Possible Cause	Remedial Action
Autotune	Test failed	See *Operating Manual*.
Sensitivity	Composition of eluent for ESI positive and / or ESI negative mode is not correct	Make sure that the solvent composition is correct (see section 4.5.4.2, page 75).
	Concentration of the standard not correct	Prepare fresh standards.
	Routine and preventive maintenance necessary	See *Operating Manual*.

6.4.2.7 Electrochemical detector

Test	Possible Cause	Remedial Action
Baseline noise	Wrong simulator cell	Check whether a QualifierRS for DC mode or a PulseQualifierRS simulator cell for Pulse mode was used.

6.4.2.8 RI Detector

Test	Possible Cause	Remedial Action
Baseline noise	Air bubbles in the flow cell	Rinse the sample and reference cells for up to one hour, using degassed water (flow rate: 1 mL/min). Repeatedly press the Purge key. If necessary, repeat the procedure with methanol.
Drift	Solvent contaminated	Use new solvent.
	Fluctuations in the ambient temperature	Position the detector at a location with few temperature fluctuations.
	Air bubbles in the flow cell	Rinse the sample and reference cells with degassed water (see above).
Detector linearity	Concentration of the standard not correct	Use fresh standards.

6.4.2.9 ELS Detector

Test	Possible Cause	Remedial Action
Baseline noise	Pump pulsation too high	Purge the pump and all channels, if necessary.
Baseline spikes	Gas supply contaminated	Replace the gas supply.

www.thermofisher.com

Thermo Fisher Scientific Inc.
168 Third Avenue
Waltham
Massachusetts 02451
USA