Operational Qualification/ Performance Qualification for HPLC Instruments

Operating Instructions

Version:	**6.8**
Date:	**Oktober 2008**

Doc.: HPLC_OQ_PQ_E_Manual.doc

Table of Contents

1 How to Use this Manual

The layout of this manual is designed to provide quick reference to the sections of interest to the user. However, we recommend that you review the manual thoroughly before starting Operational or Performance Qualification in order to obtain full understanding of the procedure.

At various points throughout the manual message of particular importance are indicated by the following symbols whose relevance is as follows:

Tip: Indicates general information to help obtain optimum performance of the instrument.

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

2 Introduction

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.

For a description of the Operational Qualification (OQ) and Performance Qualification (PQ) procedures, refer to the sections below.

2.1 Defining the Limits

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] the following definitions apply:

2.1.1 Operational Qualification (OQ)

The purpose of Operational Qualification 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 his specification, the supplier must therefore define exactly the conditions that must be observed. With varying conditions, e.g. different ambient temperatures, higher limits must be used.

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

2.1.2 Performance Qualification (PQ)

The purpose of Performance Qualification 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 is no longer met. This means: The same procedures are used but the tolerances used for Performance Qualification are less restrictive than those used for Operational Qualification are.

Performance Qualification is usually performed after repair or regular system service procedures have been performed.

Using the same procedures for OQ and PQ simplifies the handling.

2.1.3 System Suitability Check (SSC; also: System Suitability Test, SST)

The purpose of SSC 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 limits that are more restrictive than those used for Performance Qualification are.

2.2 Basic Requirements for Successful OQ and PQ

As described in section 2.1, OQ and PQ are system-specific procedures. The procedures described below apply to the following instruments:

Instrument	Supported Model
Pumps	Dionex ISO-3100A (UltiMate 3000) Dionex LPG-3400A(B) (UltiMate 3000) Dionex LPG-3400M(B) (UltiMate 3000) Dionex DGP-3600A(B) (UltiMate 3000) Dionex DGP-3600M(B) (UltiMate 3000) Dionex HPG-3200A (UltiMate 3000) Dionex HPG-3200M (UltiMate 3000) Dionex HPG-3200RS (UltiMate 3000) Dionex HPG-3400A (UltiMate 3000) Dionex HPG-3400M (UltiMate 3000) Dionex HPG 3400RS (UltiMate 3000) Dionex P680 Dionex P580 Dionex M480 Dionex M300 Agilent 1100/1200 series G1310A Agilent 1100/1200 series G1311B Agilent 1100/1200 series G1311A Agilent 1100/1200 series G1312A Agilent 1100/1200 series G1312B Pump module of the Waters Alliance 2690 Separation Module TSP P2000 TSP P4000 Shimadzu LC-2010 pump Shimadzu LC-10AT*vp* Shimadzu LC-10AD*vp*
UV Detectors	Dionex DAD-3000(RS) (UltiMate 3000) Dionex MWD-3000(RS) (UltiMate 3000) Dionex VWD-3100 (UltiMate 3000) Dionex VWD-3400RS (UltiMate 3000) Dionex PDA-3000 (UltiMate 3000) Dionex PDA-100 Dionex PDA-100U Dionex AD25 Dionex UVD 340U Dionex UVD 170U Dionex UVD 340S Dionex UVD 170S Dionex UVD 160S Dionex UVD 320S Agilent 1100/1200 series G1315A Agilent 1100/1200 series G1315B Agilent 1100/1200 series G1315C Agilent 1100/1200 series G1315D Agilent 1100/1200 series G1314A Agilent 1100/1200 series G1314B Agilent 1100/1200 series G1314C Agilent 1100/1200 series G1365A Agilent 1100/1200 series G1365B Agilent 1100/1200 series G1365C Agilent 1100/1200 series G1365D Waters PDA996 Diode Array Detector Waters PDA2996 Diode Array Detector Waters 2487 Dual Lambda Absorbance Detector

Instrument	Supported Model
UV Detectors *(Cont'd)*	TSP UV1000 Single Lambda Detector TSP UV2000 Dual Lambda Detector TSP UV3000 (analog and digital data acquisition) TSP UV6000 PDA Shimadzu LC-2010 SPD Shimadzu SPD-10A*vp* Shimadzu SPD-10AV*vp*
Samplers	Dionex ACC-3000(T) (UltiMate 3000) Dionex WPS-3000(T)RS (UltiMate 3000) Dionex WPS-3000(T)SL (UltiMate 3000) Dionex WPS-3000(T)PL (UltiMate 3000) Dionex WPS-3000TBPL Analytical (UltiMate 3000) Dionex ASI 100 Dionex GINA 50 Dionex GINA 160 Agilent 1100/1200 series G1313A Agilent 1100/1200 series G1329A Agilent 1100/1200 series G1329B Agilent 1100/1200 series G1367A Agilent 1100/1200 series G1367B Agilent 1100/1200 series G1367C Sampler module of the Waters Alliance 2690 Separation Module Waters WISP 717plus TSP AS3000/AS3500 Shimadzu LC-2010 autosampler Shimadzu SIL-HTA Shimadzu SIL-HTC Shimadzu SIL-10AD*vp*
Column Compartments	Dionex ACC-3000(T) (UltiMate 3000) Dionex TCC-3000RS (UltiMate 3000) Dionex TCC-3000SD (UltiMate 3000) Dionex TCC-3000 (UltiMate 3000) Dionex TCC-3100 (UltiMate 3000) Dionex TCC-3200(B) (UltiMate 3000) Dionex STH 585 Dionex TCC-100 Agilent 1100/1200 series G1316A Agilent 1100/1200 series G1316B Column compartment module of the Waters Alliance 2690 Sep. Module TSP AS3000/AS3500 (optional) Shimadzu LC-2010 column compartment Shimadzu CTO-10A*vp* Shimadzu CTO-10AC*vp* Shimadzu CTO-10AS*vp*
Fluorescence Detectors	Dionex RF2000 Dionex RF1002
Refractive Index Detectors	Shodex RI-101 Agilent 1100/1200 series G1362A
Evaporative Light Scattering Detector	Polymer Laboratories ELS2100

If you use other instruments or a different system configuration, adapt the procedures correspondingly (→ section 3.5).

When qualifying systems that include a Dionex FLM-3x00 Flow Manager, use the NANO_CAP_LC_Templates (not the HPLC_TEMPLATES).

In addition, Chromeleon ≥ 6.50 SP10, 6.60 SP6, or 6.70 SP3 is required.

2.3 Overview of the Checks

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

2.3.1 UV Detectors Using Analytical Flow Cells

Instrument	Parameter	Description	Limits(1) OQ	PQ
VWD-3100, VWD-3400RS (analytical flow cell) (2)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.025 mAU	0.050 mAU
	Drift		0.3 mAU/h	0.3 mAU/h
	Lamp Intensity	The lamp intensity is determined at a measuring wavelength of 230 nm.	> 50 %	> 40 %
	Wavelength Accuracy	Caffeine is injected using water as solvent. The flow rate is 1 ml/min. The characteristic maximum of caffeine is determined at 272.5 nm and compared to its theoretical value.	± 2.0 nm	± 2.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.97 % RSD ≤ 3 % (up to 2.5 AU)	r ≥ 99.90 % RSD ≤ 3 % (up to 2.5 AU)
UVD 340S, UVD 170S, UVD 340U, UVD 170U (analytical flow cell)(2)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.03 mAU	0.05 mAU
	Drift		0.8 mAU/h	2.0 mAU/h
	Lamp Intensity		> 500000 counts/s	> 400000 counts/s
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 0.75 nm	± 0.75 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.98 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
DAD-3000(RS) / MWD-3000(RS) (analytical flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm.	0.03 mAU	0.10 mAU
	Drift		1.0 mAU/h	1.0 mAU/h
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at and 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm

Instrument	Parameter	Description	Limits(1) OQ	PQ
DAD-3000(RS) / MWD-3000(RS) (analytical flow cell) *(Cont'd)*	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.95 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
PDA-3000, PDA 100, PDA-100U	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm.	0.03 mAU	0.10 mAU
	Drift		1.0 mAU/h	1.0 mAU/h
	Lamp Intensity		>13000000 counts/s	>10000000 counts/s
	Wavelength Accuracy	Pyrene is injected using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
PDA-3000, PDA 100, PDA-100U *(Cont'd)*	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
AD25	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm.	0.03 mAU	0.04 mAU
	Drift		0.2 mAU	0.2 mAU
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Caffeine is injected using water as solvent. The flow rate is 1 ml/min. The characteristic maximum of caffeine is determined at 272.5 nm and compared to its theoretical value.	± 2.0 nm	± 2.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)

Instrument	Parameter	Description	Limits(1) OQ	PQ
UVD 320S, UVD 160S, UVD 320, UVD 160 (analytical flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.05 mAU	0.10 mAU
	Drift		1.0 mAU/h	2.0 mAU/h
	Lamp Intensity (16µl volume)(3)		> 400000 counts/s	> 200000 counts/s
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 239 nm and compared to its theoretical value.	± 2 nm	± 3 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The correlation coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.9 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.9 % RSD ≤ 5 % (up to 1.5 AU)
Agilent 1100/1200: G1314A G1314B G1314C G1315A G1315B G1315C G1315D G1365A G1365B G1365C G1365D	Drift	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm.	5.0 mAU/h	5.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
Agilent 1100/1200: G1315A G1315B G1315C G1315D G1365A G1365B G1365C G1365D	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.05 mAU	0.05 mAU
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 2 nm	± 2 nm
G1314A G1314B G1314C	Baseline Noise	Pure water is pumped through the flow cell at a flow rate of 1.0 ml/min. Wavelength: 254 nm	0.04 mAU	0.04 mAU
	Wavelength Accuracy	Caffeine is injected using water as solvent. The flow rate is 1 ml/min. The characteristic maximum of caffeine is determined at 272.5 nm and compared to its theoretical value.	± 2.0 nm	± 2.0 nm

Instrument	Parameter	Description	Limits(1) OQ	PQ
Waters PDA996 Waters PDA2996	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.10 mAU	0.10 mAU
	Drift		1.0 mAU/h	1.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
Waters 2487 Dual Lambda Absorbance Detector	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.05 mAU	0.05 mAU
	Drift		0.5 mAU/h	0.5 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 239 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
TSP UV1000	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.50 mAU	0.10 mAU
	Drift		0.5 mAU/h	1.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy		Not checked	Not checked
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)

Instrument	Parameter	Description	Limits(1)	
			OQ	PQ
TSP UV2000	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.05 mAU	0.10 mAU
	Drift		0.5 mAU/h	1.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 239 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
TSP UV3000	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.08 mAU	0.15 mAU
	Drift		0.5 mAU/h	1.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
TSP UV6000	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.06 mAU	0.10 mAU
	Drift		2.0 mAU/h	4.0 mAU/h
	Lamp Intensity		Not checked	Not checked
TSP UV6000 *(Cont'd)*	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)

Instrument	Parameter	Description	Limits[1] OQ	PQ
Shimadzu - LC-2010 SPD - SPD-10A*vp* - SPD-10AV*vp*	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.05 mAU	0.10 mAU
	Drift		0.8 mAU/h	2.0 mAU/h
	Lamp Intensity		Not checked	Not checked
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 1.0 nm	± 1.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 2.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 2.5 AU)

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

(2) When qualifying a detector with a non-analytical flow cell, such as, a micro, nano, or dummy flow cell, you have to enter the corresponding specifications manually into the report. The reason is that automatic recognition of micro and nano flow cells is not supported. For information about the limits for a non-analytical flow cell, refer to the table in section 2.3.2.

(3) The lamp intensity is measured only for controlled detectors.

2.3.2 UV Detectors Using Non-Analytical Flow Cells

When qualifying a detector with a non-analytical flow cell, such as, a micro or nano flow cell, you have to enter the corresponding specifications manually into the report. The reason is that automatic recognition of micro and nano flow cells is not supported or not implemented. The table lists the limits that apply for non-analytical flow cells.

Instrument	Parameter	Description	Limits[1] OQ	PQ
VWD-3100, VWD-3400RS (micro flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.035 mAU	0.070 mAU
	Drift		0.3 mAU/h	0.3 mAU/h
	Lamp Intensity	The lamp intensity is determined at a measuring wavelength of 230 nm.	> 50 %	> 40 %
	Wavelength Accuracy	Caffeine is injected using water as solvent. The flow rate is 1 ml/min. The characteristic maximum of caffeine is determined at 272.5 nm and compared to its theoretical value.	± 2.0 nm	± 2.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.95 % RSD ≤ 3 % (up to 1.7 AU)	r ≥ 99.90 % RSD ≤ 3 % (up to 1.7 AU)

VWD-3100, VWD-3400RS (semi-micro flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.035 mAU	0.070 mAU
	Drift		0.3 mAU/h	0.3 mAU/h
	Lamp Intensity	The lamp intensity is determined at a measuring wavelength of 230 nm.	> 50 %	> 40 %
	Wavelength Accuracy	Caffeine is injected using water as solvent. The flow rate is 1 ml/min. The characteristic maximum of caffeine is determined at 272.5 nm and compared to its theoretical value.	± 2.0 nm	± 2.0 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.95 % RSD ≤ 3 % (up to 1.7 AU)	r ≥ 99.90 % RSD ≤ 3 % (up to 1.7 AU)
UVD 340S / UVD 170S / UVD 340U / UVD 170U (micro flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.150 mAU	0.200 mAU
	Drift		1.5 mAU/h	2.0 mAU/h
	Lamp Intensity		> 125000 counts/s	> 100000 counts/s
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 0.75 nm	± 0.75 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.98 % RSD ≤ 5 % (up to 1.5 AU)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.5 AU)
UVD 340S / UVD 170S / UVD 340U / UVD 170U (nano flow cell)	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Wavelength: 254 nm	0.200 mAU	0.300 mAU
	Drift		3.0 mAU/h	4.0 mAU/h
	Lamp Intensity		> 125000 counts/s	> 100000 counts/s
	Wavelength Accuracy	Pyrene is injected, using methanol as solvent. The flow rate is 1 ml/min. The characteristic maximum of pyrene is determined at 333 nm and compared to its theoretical value.	± 0.75 nm	± 0.75 nm
	Linearity	Five caffeine solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 5 % (up to 1.0 AU at 8 µl)	r ≥ 99.90 % RSD ≤ 5 % (up to 1.0 AU at 8 µl)

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

2.3.3 Autosamplers

Instrument	Parameter	Description	Limits(1) OQ	PQ
ACC-3000(T)	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.5 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (see section 5.6.2). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.95 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.02 %	≤ 0.02 %
	Temperature Accuracy	The sample temperature is measured using an external thermometer in a standard vial. The standard vial is filled with water (measured at 15 °C).	± 2 °C	± 4 °C
WPS-3000(T)RS (Analytical version and micro option)	Precision of Injection Volume	Ten injections of the same standard are analyzed. The injection volume is as follows: 5 µl (analytical autosampler), 2 µl (micro autosampler), and 10 µl (autosampler with 250-µl injection volume kit). The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different: Analytical autosampler: 5 µl to 80 µl; Micro option: 1 µl to 20 µl. The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.01 %	≤ 0.01 %
	Temperature Accuracy	The sample temperature is measured using an external thermometer in a standard vial. The standard vial is filled with water (measured at 10 °C).	± 2 °C	± 4 °C

Instrument	Parameter	Description	Limits(1) OQ	PQ
WPS-3000(T)SL (Micro and analytical autosampler versions and 250-µl injection volume kit)	Precision of Injection Volume	Ten injections of the same standard are analyzed. The injection volume is as follows: 5 µl (analytical autosampler), 2 µl (micro autosampler), and 10 µl (autosampler with 250-µl injection volume kit). The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different: Analytical autosampler: 5 µl to 80 µl; Micro autosampler: 1 µl to 20 µl; autosampler with 250-µl injection volume kit: 10 µl to 160 µl. The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromato-gram indicates the carry-over.	≤ 0.01 %	≤ 0.01 %
WPS-3000(T)SL (Micro and analytical autosampler versions and 250 µl injection *volume kit)*	Temperature Accuracy	The sample temperature is measured using an external thermometer in a standard vial. The standard vial is filled with water (measured at 10 °C).	± 2 °C	± 4 °C
WPS-3000(T)PL (only with Upgrade Kit for 250 µl syringe)	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different. The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromato-gram indicates the carry-over.	≤ 0.05 %	≤ 0.05 %

Instrument	Parameter	Description	Limits[1] OQ	PQ
WPS-3000(T)PL (only with Upgrade Kit for 250 µl syringe) *(Cont'd)*	Temperature Accuracy	The sample temperature is measured using an external thermometer in a standard vial. The standard vial is filled with water (Measured at 10 °C).	± 2 °C	± 4 °C
WPS-3000TBPL Analytical (Standard and Large Volume configuration)	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl (Standard Configuration) or 20 µl (250 µl injection *volume kit*) of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different. The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.03 %	≤ 0.05 %
	Temperature Accuracy	The sample temperature is measured using an external thermometer in a standard vial. The standard vial is filled with water (Measured at 10 °C).	± 2 °C	± 4 °C
ASI-100 (250 µl syringe)	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.1 %	≤ 0.1 %

Instrument	Parameter	Description	Limits(1) OQ	PQ
GINA 50, GINA 160 (250 µl syringe)	Precision of Injection Volume	Six injections are analyzed. Each of them contains 10 µl of the same standard sample. The relative standard deviation of the peak areas indicates the precison of the injection volume.	RSD ≤ 0.4 %	RSD ≤ 0.5 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (10 µl to 80 µl). Injection volume and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r ≥ 99.99 % RSD ≤ 0.5 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.1 %	≤ 0.1 %
Agilent 1100/1200: G1313A G1329A G1329B G1367A G1367B G1367C	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.99 % RSD ≤1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.2 %	≤ 0.2 %
Sampler module of the Waters Alliance 2690 Separation Module	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %

Instrument	Parameter	Description	Limits(1) OQ	PQ
Sampler module of the Waters Alliance 2690 Separation Module *(Cont'd)*	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.1 %	≤ 0.1 %
Waters WISP 717plus	Precison of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 1.0 %	RSD ≤ 1.0 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.1 %	≤ 0.1 %
TSP AS3000/3500	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 1.0 %	RSD ≤ 2.0 %
	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 1.5 %	r ≥ 99.90 % RSD ≤ 1.5 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.1 %	≤ 0.1 %
Shimadzu - LC-2010 - SIL-10HTA - SIL-10HTC - SIL-10Ad*vp*	Precision of Injection Volume	Ten injections are analyzed. Each of them contains 5 µl of the same standard. The relative standard deviation of the peak areas indicates the precision of the injection volume.	RSD ≤ 0.3 %	RSD ≤ 0.5 %

Instrument	Parameter	Description	Limits[1] OQ	PQ
Shimadzu - LC-2010 - SIL-10HTA - SIL-10HTC - SIL-10Ad*vp* *(Cont'd)*	Linearity of Injection Volume	Five injections of the same standard sample are analyzed. The injection volumes are different (5 µl to 80 µl; 5 µl to 50 µl for the SIL-10AD*vp*, respectively). The injection volume and the peak area are represented in a graph. The regression coefficient of the resulting line and the deviation from it indicate the linearity.	r ≥ 99.90 % RSD ≤ 1.0 %	r ≥ 99.90 % RSD ≤ 1.0 %
	Carry-Over	After a highly concentrated test sample has been injected, a blind sample is injected. The blind sample contains only solvent. The peak area measured in this chromatogram indicates the carry-over.	≤ 0.02 %	≤ 0.02 %

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

2.3.4 Pumps

Instrument	Parameter	Description	Limits[1] OQ	PQ
ISO-3100A, LPG-3400A(B), LPG-3400M(B), DGP-3600A(B), DGP-3600M(B), HPG-3200RS, HPG-3200A, HPG-3200M, HPG3400RS, HPG-3400A, HPG-3400M, P680 and P580 with analytical pump heads	Flow Precision	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. The greater value is the valid limit.	RSD ≤ 0.05 % or SD ≤ 0.01 min	RSD ≤ 0.1 % or SD ≤ 0.02 min
LPG-3400A(B), LPG-3400M(B), DGP-3600A(B), DGP-3600M(B), HPG-3200A, HPG-3200M, HPG-3200RS, HPG-3400A, HPG-3400M, HPG-3400RS, P580 (HPG and LPG) and P680 (HPG, LPG, and DGP: all mixing chamber types); all pumps with analytical pump heads	Gradient Accuracy	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channel A, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	HPG: ≤ 0.2 % LPG/DGP (analytical): ≤ 1.0 % LPG/DGP (micro): ≤ 2.0 %	HPG: ≤ 0.5 % LPG/DGP (analytical): ≤ 2.0 % LPG/DGP (micro): ≤ 2.0 %
	Gradient Precision		STD ≤ 0.5 %	STD ≤ 0.5 %
	Ripple	For each step of the above gradient runs, the ripple is determined relative to the absorption of solvent B.	≤ 0.5 %	≤ 0.5 %

Instrument	**Parameter**	**Description**	**Limits(1)** OQ	PQ
M 480 with analytical pump heads	Flow Precision	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. The greater value is the valid limit.	RSD ≤ 0.1 % or SD ≤ 0.02 min	RSD ≤ 0.1 % or SD ≤ 0.02 min
M 480 with analytical pump heads – low pressure gradient	Gradient Accuracy	Two different step gradients of two channels are programmed. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channels A and C, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	≤ 1.5%	≤ 2.0%
	Gradient Precision		Not checked	Not checked
	Ripple	For each step of the above gradient runs, the ripple is determined relative to the absorption of solvent B.	≤ 0.5 %	≤ 0.5 %
M480 and M300 High pressure gradients with analytical pump heads	Gradient Accuracy	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precsion. For channels A and C, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	≤ 1.5 %	≤ 2.0 %
	Gradient Precision		Not checked	Not checked
	Ripple	For each step of the above gradient runs, the ripple is determined relative to the absorption of solvent B.	≤ 0.5 %	≤ 0.5 %
M 300	Flow Precision	Six injections are analyzed. All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. The greater value is the valid limit.	RSD ≤ 0.1 % or SD ≤ 0.02 min	RSD ≤ 0.1 % or SD ≤ 0.02 min

Instrument	Parameter	Description	Limits[1]	
			OQ	PQ
Agilent 1100/1200: G1310A G1311A G1312A G1312B	Flow Precison	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. The greater value is the valid limit.	RSD ≤ 0.07 % or SD ≤ 0.02 min	RSD ≤ 0.07 % or SD ≤ 0.02 min
Agilent 1100/1200: G1311A G1312A G1312B	Gradient Accuracy	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channel A, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	G1311A: ≤ 1.5 % G1312A/B: ≤ 0.7 %	G1311A: ≤ 1.5 % G1312A/B: ≤ 0.7 %
	Gradient Precision		STD ≤ 0.5 %	STD ≤ 0.5 %
	Ripple	For each step of the above gradient runs, the ripple is determined relative to the absorption of solvent B.	≤ 0.5 %	≤ 0.5 %
Pump module of the Waters Alliance 2690 Separation Module	Flow Precision	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. 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	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channel A, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	≤ 0.5 %	≤ 0.5 %
	Gradient Precision		STD ≤ 0.5 %	STD ≤ 0.5 %
	Ripple	The ripple is determined relative to the absorption of solvent B for each step of the above gradient runs.	≤ 0.5 %	≤ 0.5 %

Instrument	Parameter	Description	Limits[1] OQ	PQ
P2000[2] P4000	Flow Precision	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. 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	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channel A, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	≤ 1.0 %	≤ 2.0 %
	Gradient Precision		STD ≤ 1.0 %	STD ≤ 2.0 %
	Ripple	The ripple is determined relative to the absorption of solvent B for each step of the above gradient runs.	≤ 0.5 %	≤ 0.5 %
Shimadzu - LC-2010 - LC-10AT*vp* - LC-10AD*vp*	Flow Precision	Ten injections are analyzed. (When the Dionex GINA 50 or GINA 160 autosampler is used, six injections are analyzed.) All of them contain the same standard sample. The relative standard deviation and the standard deviation of the retention times indicate the flow precision. 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	A step gradient of two channels is programmed and measured three times. The deviation between the measured and theoretical signal heights indicates the accuracy with which the pump forms the gradient. The deviations between the three gradient runs indicate the gradient precision. For channel A, water is used as solvent; for channel B, water with 0.1 % Vol. acetone is used.	≤ 1.0 %	≤ 2.0 %
	Gradient Precision		STD ≤ 0.5 %	STD ≤ 0.5 %
	Ripple	The ripple is determined relative to the absorption of solvent B for each step of the above gradient runs.	≤ 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.

2.3.5 Thermostatted Column Compartments and Column Ovens

Instrument	Parameter	Description	Limits(1)	
			OQ	PQ
Column compartment module of the ACC-3000(T) autosampler	Temperature Accuracy	A 3-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 2 °C Measured at(3): 35 °C, 40 °C, 50 °C	± 3 °C Measured at(3): 35 °C, 40 °C, 45 °C
TCC-3000RS	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 1 °C Measured at: 10 °C, 30 °C, 60 °C, 105 °C	± 2 °C Measured at: 15 °C, 30 °C, 60 °C, 90 °C
TCC-3000SD	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 1 °C Measured at: 10 °C, 30 °C, 50 °C, 65 °C	± 2 °C Measured at: 10 °C, 30 °C, 45 °C, 60 °C
TCC-3000, TCC-3100, TCC-3200(B), TCC-100	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 1 °C Measured at: 10 °C, 30 °C, 60 °C, 80 °C	± 2 °C Measured at: 15 °C, 30 °C, 45 °C, 60 °C
STH 585	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 1 °C Measured at: 5 °C, 20 °C, 60 °C, 85 °C	± 2 °C Measured at: 15 °C, 30 °C, 45 °C, 60 °C
Agilent 1100/1200: G1316A G1316B	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 2 °C Measured at: 5 °C, 20 °C, 60 °C, 80 °C	± 2 °C Measured at: 15 °C, 30 °C, 45 °C, 60 °C

Instrument	**Parameter**	**Description**	**Limits[1]** OQ	PQ
Column compartment module of the Waters Alliance 2690 Separation Module	Temperature Accuracy	A 3-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 1 °C Measured at[2,3]: 35 °C, 45 °C, 55 °C	± 2 °C Measured at[2,3]: 35 °C, 45 °C, 55 °C
Column oven of the TSP AS3000/AS3500 Autosamplers	Temperature Accuracy	A 4-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 2 °C Measured at: 20 °C, 40 °C, 60 °C, 80 °C	± 3 °C Measured at: 25 °C, 35 °C, 45 °C, 60 °C
Shimadzu Column Compartments: - LC-2010 - CTO-10AS*vp*	Temperature Accuracy	A 3-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 3 °C Measured at[2]: 20 °C, 40 °C, 60 °C	± 3 °C Measured at[2]: 25 °C, 35 °C, 45 °C, 60 °C
Shimadzu Column Compartment: - CTO-10A*vp*	Temperature Accuracy	A 3-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 3 °C Measured at[2,3]: 35 °C, 60 °C, 80 °C	± 3 °C Measured at[2,3]: 35 °C, 45 °C, 60 °C
Shimadzu Column Compartment: - CTO-10AC*vp*	Temperature Accuracy	A 3-step temperature gradient is programmed. An externally calibrated thermometer is used to measure the temperature that is reached. If technically possible, the data are automatically read by Chromeleon. Else, enter the values manually during the measurement.	± 3 °C Measured at[2]: 20 °C, 60 °C, 80 °C	± 3 °C Measured at[2]: 25 °C, 45 °C, 60 °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 also 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.

2.3.6 Fluorescence Detectors

Instrument	Parameter	Description	Limits[(1)] OQ	PQ
RF 2000	Baseline Noise	Pure water is pumped through the flow cell: The flow rate is 1 ml/min. Excitation wavelength: 350 nm emission wavelength: 394 nm	≤ 0.30 mV	≤ 0.30 mV
	Signal Minimum	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Excitation wavelength: 350 nm. It is observed how the signal changes when the emission wavelength changes from 450 nm to 394 nm.	> 40 mV	> 40 mV
	Signal Maximum		≤ 80 mV	≤ 80 mV
	Wavelength Accuracy[(2)]	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. At the excitation wavelength of 350 nm, the emission wavelength is varied from 380 nm to 410 nm in 1 nm increments. The relative signal maximum is compared to the theoretical maximum.	± 10 nm	± 10 nm
RF 1002	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Excitation wavelength: 350 nm emission wavelength: 394 nm	≤ 0.60 mV	≤ 0.60 mV
	Signal Minimum	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Excitation wavelength: 350 nm. It is observed how the signal changes when the emission wavelength changes from 450 nm to 394 nm.	> 40 mV	> 40 mV
	Signal Maximum		≤ 80 mV	≤ 80 mV
	Wavelength Accuracy[(2)]	Pure water is pumped through the flow cell at 1.0 ml/min At an excitation wavelength of 350 nm, the emission wavelength is varied from 380 nm to 410 nm in 1 nm increments. The relative signal maximum is compared to the theoretical 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 using a special flow cell and a mercury lamp. For OQ and PQ, the instrument should preferably be checked with the components used for the measurements.

2.3.7 Refractive Index Detectors

Instrument	Parameter	Description	Limits(1) OQ	PQ
Shodex RI-101 Agilent 1100/1200 G1362A	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min. Temperature: 35 °C	50 nRIU	50 nRIU
	Drift		500 nRIU/ h	2500 nRIU/h
	Linearity	Five glycerin solutions are injected in different concentrations. Concentration and peak area are represented in a graph. The regression coefficient of the resulting line and the deviations from it indicate the linearity.	r $\geq$ 99.9 %	r $\geq$ 99.9 %

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

2.3.8 Evaporative Light Scattering Detectors

Instrument	Parameter	Description	Limits(1) OQ	PQ
Polymer Laboratories ELS 2100	Baseline Noise	Pure water is pumped through the flow cell. The flow rate is 1 ml/min.	0.3 mV	0.3 mV

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

3 Process

3.1 General Test Procedure

All required materials are provided in the Performance Qualification kit (part no. **4832.5000A**).

Article No.	Description	Quantity
All kits		
709.7021	10 µl sample loop (1/16")	1
2261.0102	VS - 2 VA 1/16" connecting unit	1
2200.5502	Single-part, hand-tight fitting	2
3323.0010	Standards kit (caffeine and Pyrene)	1
709.6000.066	Finger-tight 33 mm fitting set (5 sets: ferrule and fitting screws)	0.2
Kits with pressure regulator (available until spring 2007) also include		
2251.6010A	Pressure regulator (complete)	1
Kits with restriction tubing (available since spring 2007) also include		
5035.3000	Restriction tubing (ID: 0.18 mm; length: 15 m)	1
2261.0102	connecting unit (VS - 2 VA 1/16")	1
2251.6001	PEEK tubing (ID: 0.25 mm; length: 0.10 m)	1

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

The Standards kit (part no. **3323.0010**) contains the seven required caffeine/pyrene standards. The standard at position RA8 (or 8) contains water as solvent. Due to legal shipping restrictions, the pyrene standard is shipped in solid form. Before you can use the standard, dissolve the solid pyrene in 1 ml of methanol (HPLC-grade):

- Unscrew the cap from the 2 ml vial labeled *3 µg Pyrene*.
- Add about 1 ml of methanol (HPLC-grade), which is about half the vial volume.
- Screw the cap onto the vial. Make sure that the cap seals tight.
- Shake the vial for about 10 seconds to dissolve the solid pyrene.
- 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 Dionex VWD-3100 and VWD-3400 detectors, the AD25 detector, and any other supported single wavelength detectors; see Section 3.2). Concentrations deviating by ±30 % do not affect the test results.

Sample Position		**Substance**	**Concentration**	**Checks**
Dionex Sampler(1)	*Any Sampler*			
RA1	1	Pyrene in methanol	3 µg/ml	• Wavelength accuracy of UV detector (see section 3.2 for a list of excluded detectors)
RA2	2	Caffeine in water	10 µg/ml	• Linearity of injection volume (for sample loop volumes > 50 µl) • Detector linearity • Carry over by the autosampler

Sample Position		Substance	Concentration	Checks
Dionex Sampler[(1)]	*Any Sampler*			
RA3	3	Caffeine in water	60 µg/ml	• Wavelength accuracy (VWD-3400RS, single wavelength detectors) • Precision of injection volume and flow (Gina 50, ACC-3000, and WPS-3000 with sample loop volumes > 200 µl) • Linearity of injection volume (for sample loop volumes ≤ 50 µl) • Detector linearity
RA4	4	Caffeine in water	140 µg/ml	• Precision of injection volume and flow (not for Gina 50) • Detector linearity
RA5	5	Caffeine in water	220 µg/ml	• Precision of injection volume and flow (WPS-3000(T)SL Micro) • Detector linearity
RA6	6	Caffeine in water	300 µg/ml	• Detector linearity
RA7	7	Caffeine in water	2000 µg/ml	• Carry-over by the autosampler
RA8	8	Water (solvent)		• Carry-over by the autosampler

(1) Dionex autosamplers: ASI-100(T), WPS-3000(T)SL / PL, WPS-3000TBPL Analytical, WPS-3000(T)RS and ACC-3000(T)

For the five standards required for qualifying the RI detector, order part no. **3325.0010**

Sample Position			Substance	Concentration	Checks
ASI-100	*WPS-3000xx*[(2)]	*Any Sampler*			
RA9	RB1	9	Glycerin in water	5 mg/ml	RI detector linearity
RA10	RB2	10	Glycerin in water	10 mg/ml	RI detector linearity
RA11	RB3	11	Glycerin in water	15 mg/ml	RI detector linearity
RA12	RB4	12	Glycerin in water	25 mg/ml	RI detector linearity
RA13	RB5	13	Glycerin in water	35 mg/ml	RI detector linearity

(2) xx: WPS-3000(T)SL / PL, WPS-3000TBPL Analytical, WPS-3000(T)RS und ACC-3000(T)

In addition, the following solvents are required:

Solvent	Quantity	Checks
Methanol (HPLC grade) – Channel A	Approx. 100 ml	Wavelength accuracy of UV Detector (exceptions see section 3.2)
Water (HPLC grade) – Channel A	Approx. 600 – 1200 ml	All tests except wavelength accuracy of the UV detector (exceptions see section 3.2)
Water (HPLC grade) with 0.1 % Vol. acetone – Channel B	Approx. 300 ml	Gradient accuracy, gradient precision, and ripple

For qualifying the column compartment, a calibrated thermometer is required. The thermometer is provided in the Column Thermostat PQ kit (part no. **5705.0050A**).

For qualifying the column compartment of the ACC-3000(T), a flexible temperature sensor (Temperature Sensor Type K for Thermometer P600, part no. **6820.0010**) is required in addition to the Column Thermostat PQ kit.

3.2 Test Procedure for Single Wavelength and VWD-3400RS Detectors

Some important steps in the test procedure for single wavelength detectors and Dionex VWD-3400RS detectors differ from the steps described in section 3.1:

The Standards kit (part no. **3323.0010**) includes seven caffeine and pyrene standards. However, as caffeine is used for the wavelength accuracy check, you do not have to prepare (that is, dissolve) the pyrene standard. Sample position RA1 (or 1) is not used.

In addition, the solvent used for the wavelength accuracy check is water (not methanol). Therefore, only the following solvents are required:

Solvent	Quantity	Checks
Water (HPLC-grade) – Channel A	Approx. 700 – 1300 ml	All
Water (HPLC-grade) with 0.1 % Vol. acetone – Channel B	Approx. 300 ml	Gradient accuracy, gradient precision, and ripple

3.3 Connecting and Configuring the System

The steps below describe the fluid connections of the HPLC system and 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.

3.3.1 System Connections

- **System**
  Remove the column from the system. In both positions of the motorized switching valve of the autosampler, thoroughly rinse all fluid components of the autosampler and injection valve with water. Rinse also the pump thoroughly with water. Only then, connect the pressure regulator or restriction tubing from the Performance Qualification kit to the injection valve and the UV detector, using the fitting screws shipped with the kit. Use the 33 mm finger-tight fitting for the injection valve and the single-part hand-tight fitting for the UV detector. With the restriction tubing, install the PEEK tubing from the Performance Qualification kit between the injection valve and the restriction tubing, using the connecting units from the kit if necessary (→ Figure 1).

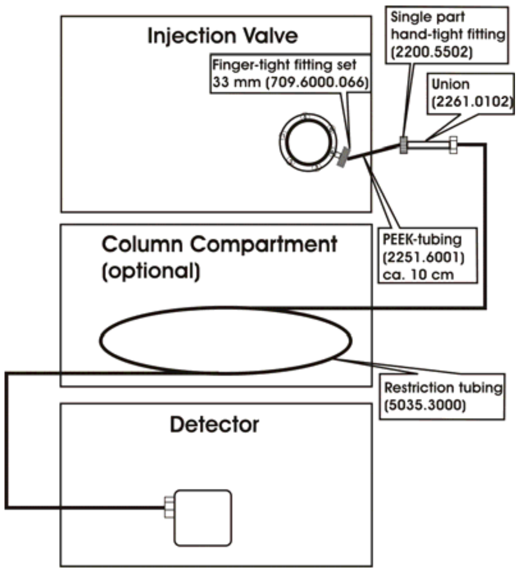


Figure 1: Restriction tubing installed between injection valve and detector

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

- **Qualifying a Dionex P680 DGP, DGP-3600A(B), or DGP-3600M(B) pump**
  To qualify both pumps of a Dionex dual gradient pump with the same autosampler, pressure regulator or restriction tubing, and detector, you have to use an external motorized switching valve, such as, a valve in the Dionex TCC-100, TCC-3100, TCC-3200(B) or TCC-3000RS/SD.. (For a list of the supported valves, see section 3.5). For information about the fluid connections, see the images below.

  - *Case a: 6-port/2-position valve*

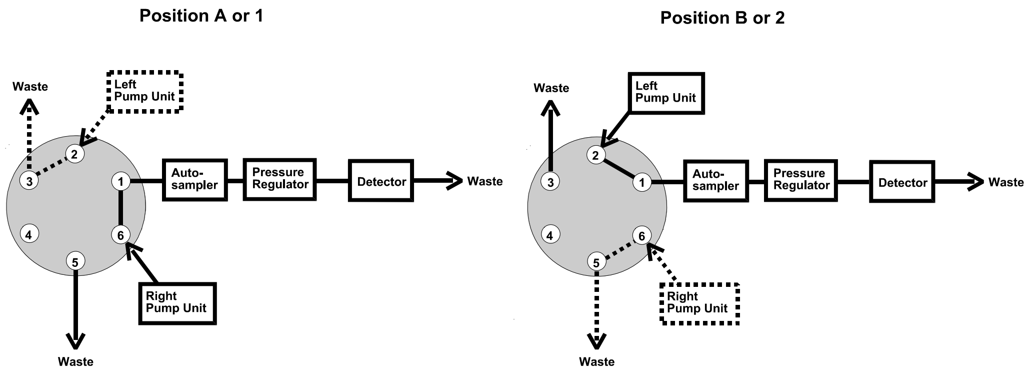


Figure 2: Fluid connection for testing the Dionex P680 DGP and DGP-600A, using a 6-port/2-position valve
Left: The valve is in position A or 1, depending on the valve type
Right: The valve is in position B or 2, depending on the valve type

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

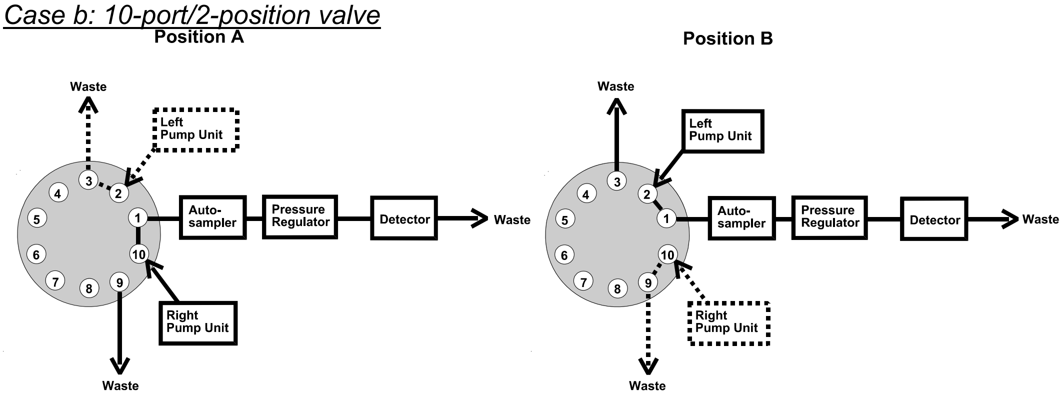


Figure 3: Fluid connection for testing the Dionex P680 DGP and DGP-600A, using a 10-port/2-position valve
Left: The valve is in position A
Right: The valve is in position B

- **Manual injection valve**
  Verify that the injection valve is fitted with a 10-µl sample loop.
- **Autosampler**
  Position the standards as shown in the tables on page 27 and the following pages.
- **Column compartment**
  When qualifying the column compartments, the temperature sensor of the thermometer must be securely attached to the heating block.
  - On the supported Shimadzu column compartments, loosen a fastening screw, insert the sensor between the screw and the metal block, and carefully retighten the screw.
  - When qualifying the Dionex ACC-3000(T) column compartment, install the temperature sensor as shown in Figure 4. Be sure to use the type K temperature sensor (and not the sensor from the Column Thermostat PQ Kit). 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.

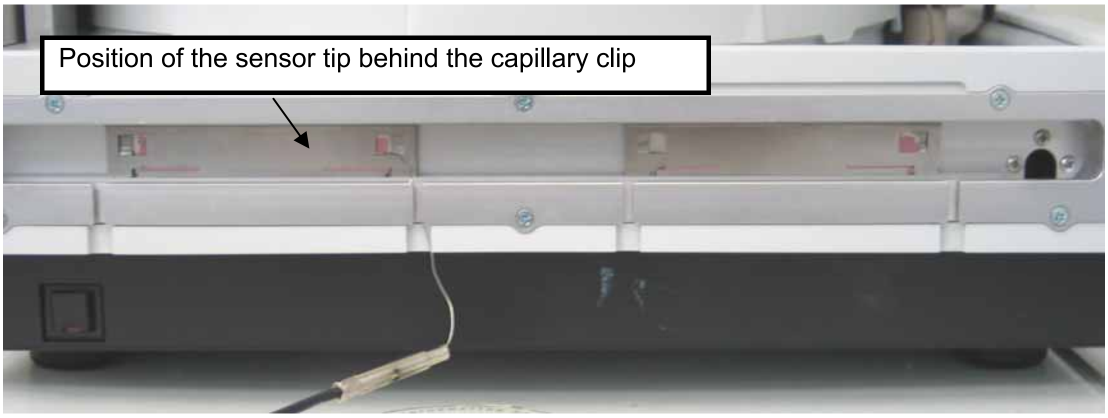
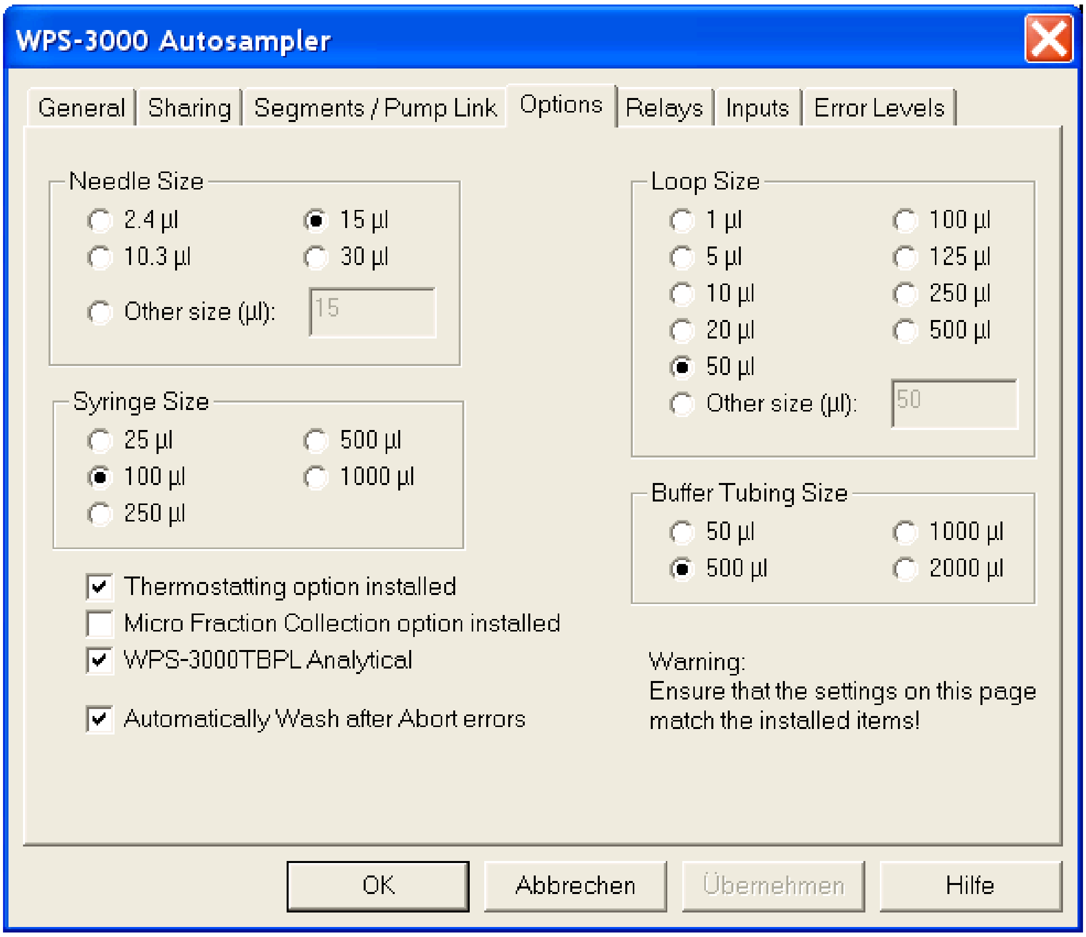


Figure 4: **ACC-3000(T) – Position of the temperature sensor**

3.3.2 Configuration

- **RF2000 fluorescence detector**
  Before you connect the RF2000 fluorescence detector with Chromeleon, set the ZWAVE parameter to 1. You can set this value only on the instrument:
  - Disable the keyboard interlock by simultaneously pressing <Shift> and <CE>.
  - Press **<func>** until the ZWAVE command appears on the display.
  - Press <**1**> on the number keypad and confirm with <**Enter**>.
  - Enable remote operation. To do so, press <**func**>, until the RS232 command appears on the display. Confirm with <**Enter**>, and then press <**func**>. On the display, the reading is CONNECT. Confirm with <**Enter**>. You can then connect the instrument with Chromeleon.
- **AD25 UV detector**
  On the **Signals** page for the AD25 in the Server Configuration program, change the unit to mAU (instead of AU) and the factor to 1000 (instead of 1.000).
- **WPS-3000(T)PL autosampler**
  For successful qualification of the Dionex WPS-3000(T)PL autosampler, make sure that the Upgrade Kit for a 250 µl-syringe is installed.
- **WPS-3000TBPL Analytical autosampler**
  In order to ensure a successful qualifcation, the Dionex WPS-3000TBPL Analytical autosampler must be equipped with the standard or large volume configuration. In addition, it must be activated in the Server Configuration on the Options page of the "WPS-3000TBPL Analytical“ driver:



- **Dionex (UltiMate 3000) autosamplers with user-defined sample loop volume**
  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	20 – 39 µl	20 µl
	40 – 124 µl	50 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
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

- **Qualifying the column compartment**

  ⇒ **Option A** Using the Column Thermostat PQ kit:

  Connect the thermometer to a free COM port on the Chromeleon server PC. Install the *Dostmann Thermometer P500/P600* in the Chromeleon Server Configuration program. On the **General** tab page, select the COM port to which the thermometer is connected. In addition, install a virtual channel (Device name: *VirtualChannels_01; s*ignal Name: *TemperatureOVEN*).

  Tip: When changing the temperature sensor, you may have to adapt the calibration values and sensor type setting of the thermometer. Otherwise, the thermometer may show the wrong temperature. This is especially important when qualifying the column compartment of the ACC-3000(T), which is qualified using a type K temperature sensor.

  ⇒ **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:

  In the Chromeleon Server Configuration program, install the *STH_manual* device. The driver is available under **Generic** on the **Manufacturers** list. Verify that the driver is in Demo Mode. In addition, install a virtual channel (device name: *VirtualChannels_01*, signal name: *TemperatureOVEN).* During the chromatographic run, you can then enter the temperature indicated on the external thermometer on the *OQ_PQ_STH_manual* control panel. Option C does not support qualification of the Agilent, Shimadzu, and Waters column compartments.

  Tip: Sequence templates created with Chromeleon < 6.50 can be used in Chromeleon 6.50 only after you have deleted the *STH_manual.connect* line from the *COLUMN_OVEN* program file. If you created new sequence templates from the Chromeleon 6.50 or later master templates, you do not need to adapt the program file manually.

  Tip: Note that option C (qualifying the column compartment with manual data acquisition) is not supported for Agilent, Shimadzu, and Waters instruments.

3.4 Preparations

3.4.1 Preparing the HPLC System

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

- **UV Detector**
  Turn on the detector lamp. Allow the lamp to be on for at least six hours before you start the check. When you use one of the following detectors, just turn on the UV lamp: DAD-3000(RS), MWD-3000(RS), PDA-100, PDA-3000, VWD-3100, VWD-3400RS, AD25, TSP UV1000, TSP UV2000, TSP UV3000, TSP UV6000, or Shimadzu SPD-10AV*vp*.
- **Refractive Index Detector**
  When you use an RI detector, turn on the instrument at least one hour before you start the check. Rinse the reference cell and the sample cell at a flow rate of 10.0 ml/min (mobile phase: water). If you check the wavelength accuracy of the UV detector using methanol (all detectors **except** the Dionex VWD-3x00 detectors and all single wavelength detectors), disconnect the fluid components of the RI detector from the HPLC system after you have rinsed the cells with water.
- **Fluorescence Detector**
  Turn on the detector lamp. Allow the lamp to be on for approximately 30 minutes before you start the check.
- **Evaporative Light Scattering Detector**

  Turn on the detector lamp. Allow the lamp to be on for approximately 30 minutes before you start the check.
- **Autosampler**
  Before you start the check, rinse the autosampler thoroughly with water. To do so, inject 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). Make sure that the fluid components and the syringe are free of air bubbles. (Note: 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.)
- **Pump**
  When qualifying an RI detector, rinse the entire HPLC system with water. If you want to check the wavelength accuracy for the UV detector, disconnect the fluid components of the RI detector from the HPLC system before you rinse channel A with methanol. When qualifying Dionex VWD-3400RS detectors and all single wavelength detectors, or if the wavelength accuracy is not checked, use water to rinse channel A. In this special case, you need not disconnect the fluid components of the RI detector from the HPLC system. For gradient pumps, use water + 0.1 % Vol. acetone to rinse channel B. For a ternary high-pressure gradient system or an M480 low-pressure gradient pump, use water to rinse channel C.

3.4.2 Checking the Fluidics

- **Injection Valve and Autosampler**

  Verify that there are no pressure fluctuations when the valve switches from **Load** to **Inject** and vice versa. Pressure fluctuations indicate system leakage or contamination. Eliminate any leaks and contamination before you start the check.

3.5 Preparing Chromeleon

3.5.1 Template Structure

To prepare Chromeleon for Operational Qualification or Performance Qualification, follow the steps below:

The Wizard generates sequence templates from the master sequence 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 (→ Figure 5, step 1). For each check that is performed on the same system, a separate copy of the sequence template is made (→ Figure 5, step 2). OQ/PQ is then performed with the sequences of the copied template (→ section 3.6) for OQ/PQ. In this way, you may need to adapt the sequence templates only once to the device configuration to be checked.

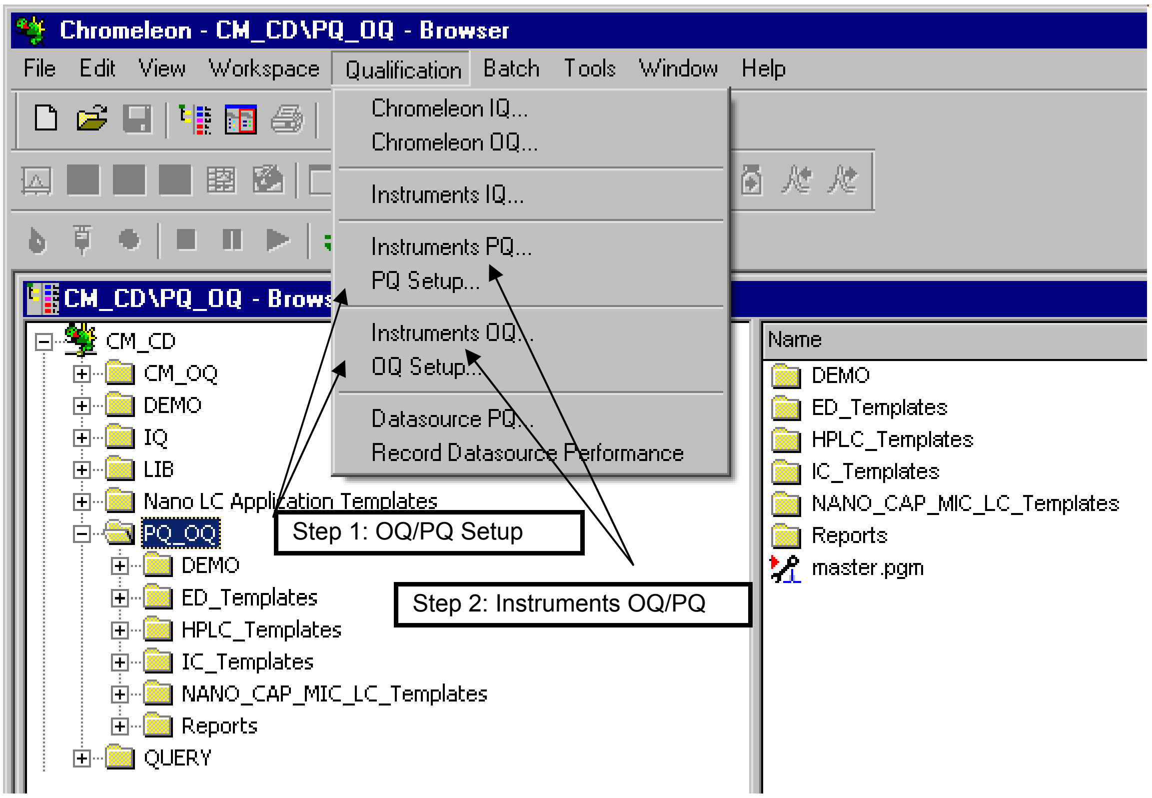


Figure 5: Performing OQ/PQ

The PQ_OQ directory on the Chromeleon CD has the following subdirectories (→ Figure 5): ED_TEMPLATES, HPLC_TEMPLATES, IC_TEMPLATES and NANO_CAP_LC_TEMPLATES, Demo, and Reports.

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 tests (→ section 4). When creating the sequence templates, the Wizard provides only those sequences that match the timebase. For IC and BioLC systems, the Wizard provides the sequences from the IC_TEMPLATES directory. For systems with electrochemical detector, the sequences from the ED_TEMPLATES directory are provided. For nano, cap, and micro systems, the Wizard provides the sequences from the NANO_CAP_LC_TEMPLATES directory, and for HPLC systems, the Wizard provides the sequences from the HPLC_TEMPLATES directory. The Dionex PDA-100 and PDA-3000 detectors are included in the master sequences of the IC_TEMPLATES and HPLC_TEMPLATES directories. These OQ/PQ operating instructions refer only to the sequences of the HPLC_TEMPLATES directory.

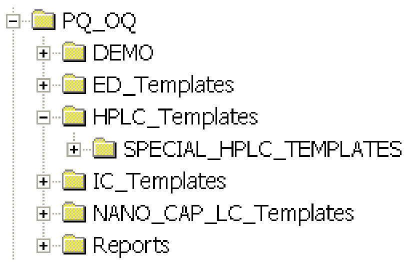


Figure 6: PQ_OQ directory structure on the CM_CD

Tip: The sequences of the HPLC_TEMPLATES directory do not support the qualification of systems that include a Dionex FLM-3x00 Flow Manager. Sequences for qualifying these systems are available in the NANO_CAP_LC_TEMPLATES directory.

3.5.2 Creating the Sequence Templates

To install the sequences required for your system, follow the steps below:

- Insert the Chromeleon CD or verify that you can access the PQ_OQ directory.
- From the Browser, open the **Qualification** menu.

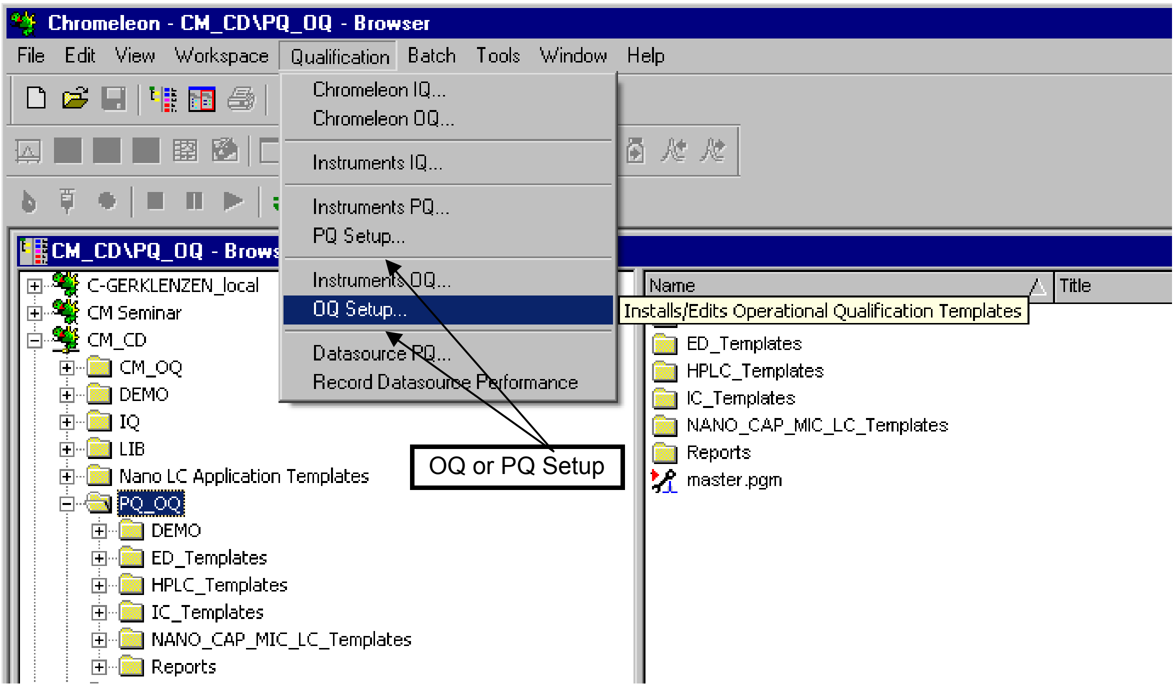


Figure 7: Selecting OQ or PQ setup

- Select **OQ Setup** or **PQ Setup**. A Wizard assists you in copying the sequences. Clicking **Next>** takes you to the next Wizard step.

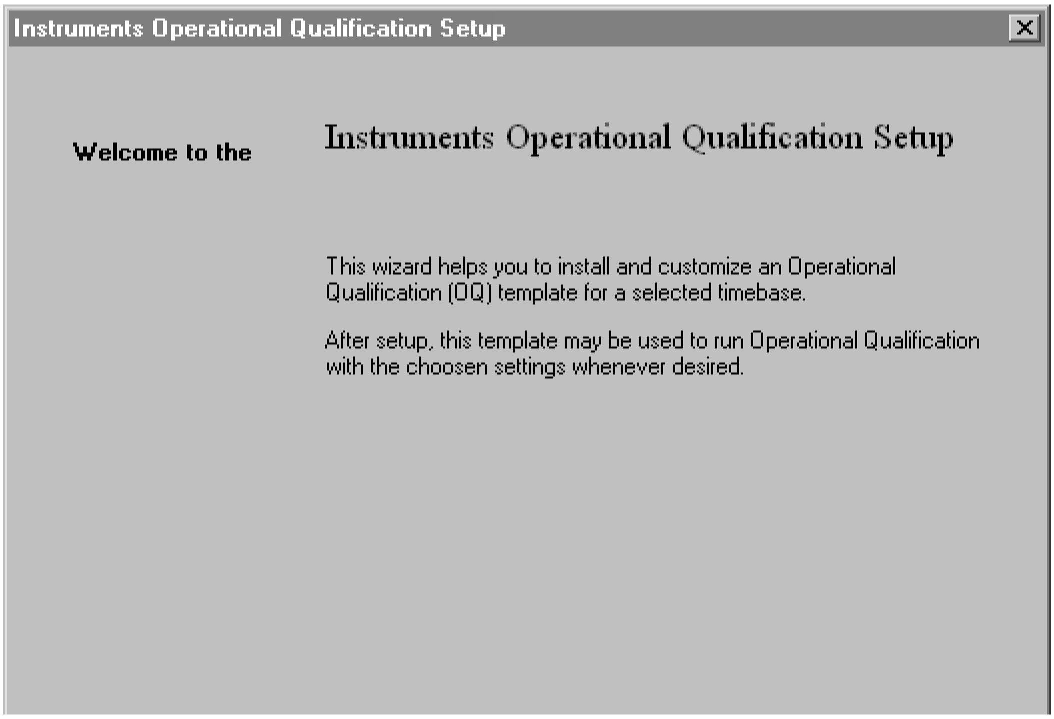


Figure 8: OQ/PQ setup wizard welcome page

- Select the timebase for which you want to perform OQ or PQ. Enter the name of the computer on which the timebase is installed.

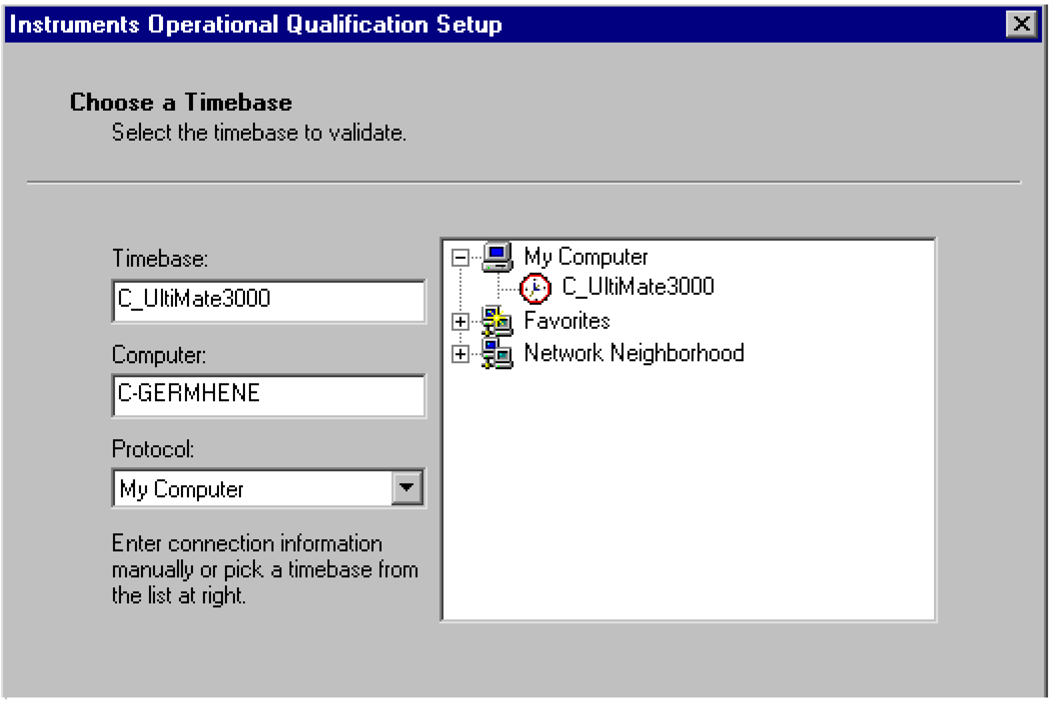


Figure 9: Selecting a timebase

- Select the source directory of the master sequences. Select **PQ_OQ**.

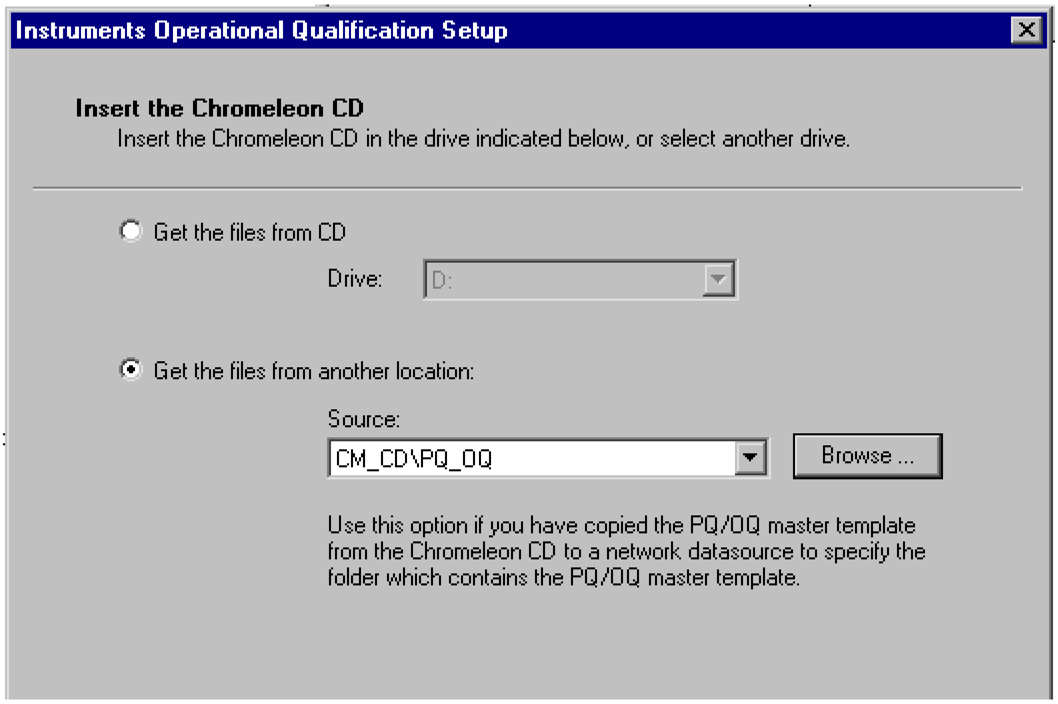


Figure 10: Selecting the source directory

- Enter a unique name for the timebase. The directory that contains all sequence templates is then saved under this name.

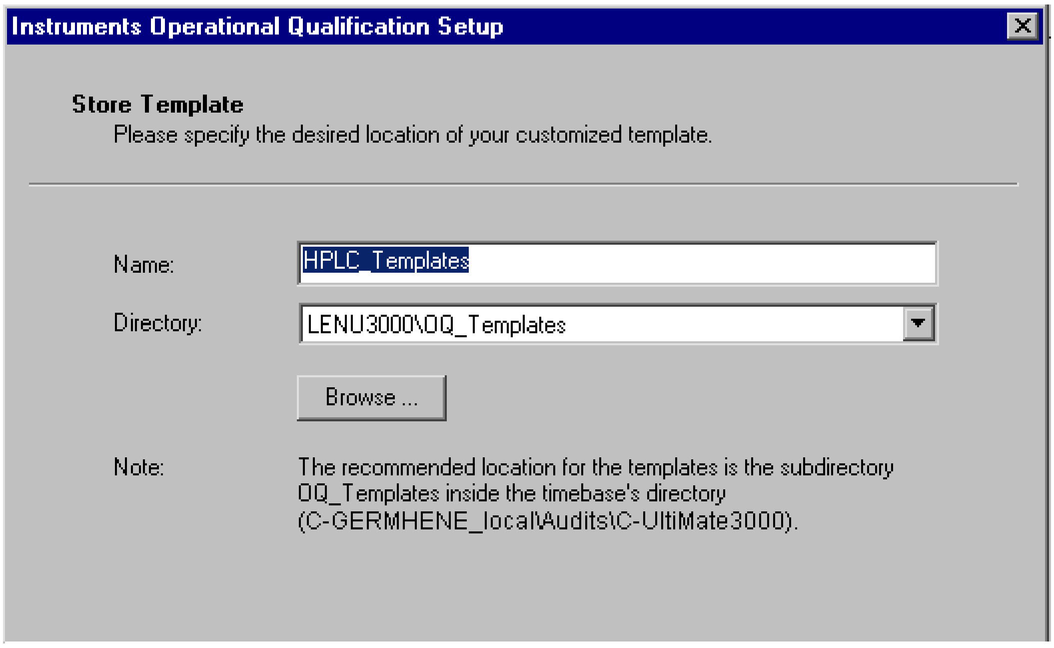


Figure 11: Entering the location for saving the template

- A list of sequences is displayed, based on the timebase configuration in the Chromeleon Server Configuration program.

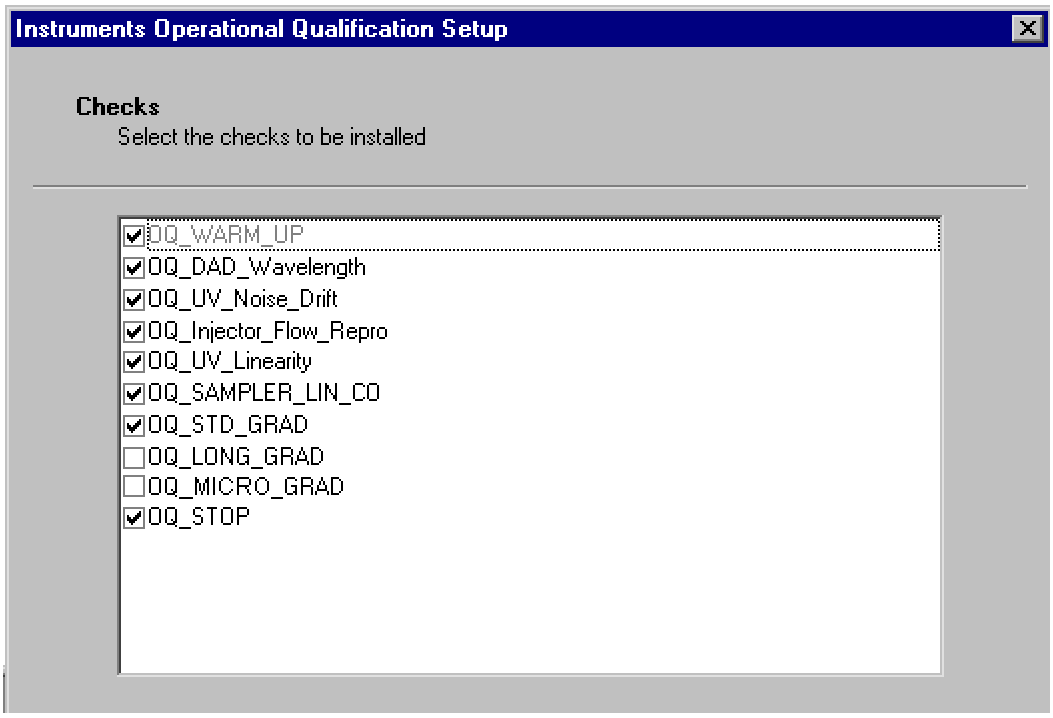


Figure 12: List of sequences for the timebase

When Chromeleon cannot automatically determine the pump type or the pump variant, you can adapt the sequences as required. This applies to the Dionex M480 pump, that is, the *HPG_M480* sequence for the M480 HPG and *LPG_M480* sequence for the M480 LPG. It also applies to the mixing chamber size of the Dionex P680 and UltiMate 3000 pumps, except for the low-pressure micro pumps - volume of the installed mixing chamber (*STD_GRAD* sequence for the standard mixing chamber, *Micro_Grad* sequence when the MicroFlow Kit is installed, and *LONG_GRAD* sequence when a mixing chamber extension is installed).
In all other cases, the selection is read-only.
Select the sequences required for the checks that you want to perform (→ section 4). The selected sequences are automatically copied to the corresponding datasource. When installation is complete, the report opens on the **Specification** page.

Tip: If you use the TSP UV1000 UV detector, you can select the sequences only if the UV lamp is turned on in the detector.

3.5.3 Adapting the Report and Method

- Disable writing protection (on the **Edit** menu, click **Layout Mode**), and then enter the
  - charge number, expiry date, and actual concentration of the standard
  - customer name and tester name
  - component that generates the backpressure [default: capillary (L: 15 m; ID: 0.18 mm)]

For all devices listed in the table below, the instrument names and limits recommended by Dionex are automatically entered into the report only when you open the report after the *Warm up* sample. The information is not yet entered when the sequence is copied. **Do not fill in the report for the supported devices** (see below). The limits are listed from line 154 on. Change the limits only if you do not want to use the limits recommended by Dionex.

Tip: When you use a Dionex UVD 170S, UVD 340S, UVD 170U, UVD 340U, VWD-3100 or VWD-3400RS detector with a non-analytical flow cell, you have to enter the specifications listed in section 2.3.2 or 4.2 manually into the report. The reason is that automatic recognition of flow cell is not supported or not implemented.

The serial number is entered automatically for the following devices: all devices of the Agilent 1100/1200 series, all devices of the Dionex UltiMate 3000 series, all P680 and P580 pumps, the Dionex ASI-100 autosampler, the Dionex PDA-100, AD25, UVD 340U, and UVD 170U detectors, and the supported Shimadzu devices. For all other devices, enter the serial number in column **K** from line 141 on. (The fields have a yellow background.) Delete the value in the related input field by clicking **Clear Values** on the **Edit** menu. This removes the Chromeleon variable from the cell and clears the **audit.xxx** entry for the cell on the status bar.

When qualifying instruments that are not listed in the table below, enter the model name in column **H** (fields with a yellow background), deleting the existing **audit.xxx** entry as before. From line 159 on, enter the limits in the column with the related model name.

- Enable writing protection (on the **Edit** menu, clicking **Layout Mode**).
- **SAVE** the report. To do so, click **Save Report Definition** on the **Workspace** menu.
- 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.

3.5.4 Device Names

The PGM files of the installed sequences apply to the following devices, device names, and channel names, as determined in the Chromeleon Server Configuration program:

Device	Supported Model	Name
Entire system	Waters Alliance 2690 Separation Module	HPLC_System
Pumps	Dionex HPG-3200RS (UltiMate 3000) Dionex HPG 3400RS (UltiMate 3000) Device Name: Pump Device Name:	 Pump Module Pump
	Dionex ISO-3100A (UltiMate 3000) Dionex LPG-3400A(B) (UltiMate 3000) Dionex HPG-3200A (UltiMate 3000) Dionex HPG-3200M (UltiMate 3000) Dionex HPG-3400A (UltiMate 3000) Dionex HPG-3400M (UltiMate 3000) Dionex P680 (all models except P680A DGP-6) Dionex P580 Dionex M480 Dionex M300 Agilent 1100/1200 series G1310A Agilent 1100/1200 series G1311A Agilent 1100/1200 series G1312A Agilent 1100/1200 series G1312B Pump module of the Waters Alliance 2690 Separation Module TSP P2000 TSP P4000 Shimadzu LC-2010 pump Shimadzu LC-10AT*vp* Shimadzu LC-10AD*vp*	Pump
	Dionex LPG-3400M(B) (UltiMate 3000)	MicroPump

Device	**Supported Model**	**Name**
Pumps *(Cont'd)*	Dionex DGP-3600A(B) (UltiMate 3000) Dionex P680 DGP-6: Left pump unit Right pump unit	 PumpLeft PumpRight
	Dionex DGP-3600M(B) (UltiMate 3000) Left pump unit Right pump unit	 LoadingPump MicroPump
Column switching valve for qualifying Dionex DGP pumps	MSV of the Dionex TCC-3000RS (UltiMate 3000) MSV of the Dionex TCC-3000SD (UltiMate 3000) MSV of the Dionex TCC-3100 (UltiMate 3000) MSV of the Dionex TCC-3200(B) (UltiMate 3000) MSV of the TCC-100	as device name: ColumnOven
	Reodyne RV/EV Valve Reodyne LabPro Valve Valco Multi Position Valve Valco Two Position Valve	Valve
UV detectors	Dionex DAD-3000 (RS) (UltiMate 3000) Dionex MWD-3000 (RS) (UltiMate 3000) Dionex PDA-3000 (UltiMate 3000) Dionex VWD-3100 (UltiMate 3000) Dionex VWD-3400RS (UltiMate 3000) Dionex PDA-100 Dionex AD25 Dionex UVD 340U Dionex UVD 170U Dionex UVD 340S Dionex UVD 170S Dionex UVD 160S Dionex UVD 320S Agilent 1100/1200 series G1315A Agilent 1100/1200 series G1315B Agilent 1100/1200 series G1315C Agilent 1100/1200 series G1315D Agilent 1100/1200 series G1314A Agilent 1100/1200 series G1314B Agilent 1100/1200 series G1314C Agilent 1100/1200 series G1365A Agilent 1100/1200 series G1365B Agilent 1100/1200 series G1365C Agilent 1100/1200 series G1365D Waters PDA996 Diode Array Detector Waters PDA2996 Diode Array Detector Waters 2487 Dual Lambda Absorbance Detector TSP UV1000 Single Lambda Detector TSP UV2000 Dual Lambda Detector TSP UV3000 (digital and analog data acquisition) TSP UV6000 PDA Shimadzu LC-2010 SPD Shimadzu SPD-10A*vp* Shimadzu SPD-10AV*vp*	UV
UV channel names		UV_VIS_1, UV_VIS_2, UV_VIS_3

Device	Supported Model	Name
Samplers	Dionex ACC-3000(T) (UltiMate 3000) Dionex WPS-3000(T)RS (UltiMate 3000) Dionex WPS-3000(T)SL (UltiMate 3000) Dionex WPS-3000(T)PL (UltiMate 3000) Dionex WPS-3000TBPL Analytical (UltiMate 3000) Dionex ASI-100 Dionex GINA 50 Dionex GINA 160 HP1100 G1313A Agilent 1100/1200 series G1329A Agilent 1100/1200 series G1329B Agilent 1100/1200 series G1367A Agilent 1100/1200 series G1367B Agilent 1100/1200 series G1367C	Sampler
Samplers *(Cont'd)*	Sampler module of the Waters Alliance 2690 Separation Module TSP AS3000/AS3500 Shimadzu LC-2010 sampler Shimadzu SIL-HTA Shimadzu SIL-HTC Shimadzu SIL-10AD*vp*	Sampler
Column thermostats	Dionex ACC-3000(T) (UltiMate 3000) Dionex TCC-3000RS (UltiMate 3000) Dionex TCC-3000SD (UltiMate 3000) Dionex TCC-3000 (UltiMate 3000) Dionex TCC-3100 (UltiMate 3000) Dionex TCC-3200(B) (UltiMate 3000) Dionex TCC-100 Dionex STH 585 Agilent 1100/1200 series G1316A Agilent 1100/1200 series G1316B Column compartment module of the Waters Alliance 2690 Separation Module Shimadzu LC-2010 column compartment Shimadzu CTO-10A*vp* Shimadzu CTO-10AC*vp* Shimadzu CTO-10A*svp*	ColumnOven
	Column oven of the TSP AS3000/AS3500 Autosampler	Sampler
Signal name of the external thermometer		TemperatureOV EN
Device name of the virtual channel		VirtualChannels _01
Fluorescence detectors	Dionex RF2000 Dionex RF1002	Emission
RI detector	Shodex RI-101 Agilent 1100/1200 series G1362A	RI
ELS detector	Polymer Laboratories ELS2100	ELSD

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.*

When qualifying a column compartment with manual data acquisition with Chromeleon 6.50 or later, you can use sequence templates that were created with Chromeleon < 6.50 only after you have deleted the *STH_manual.connect* line from the *COLUMN_OVEN* program file.

Use the above names in the Chromeleon Server Configuration program also when you use instruments other than those listed. If you do not use these names, the system cannot automatically select the checks to be performed and the appropriate sequences. It may then be necessary to adapt the program files manually.

3.6 Performing the Checks

To copy the template (→ section 3.5.2), follow the steps below:

- In the Browser, open the **Qualification** menu.
- Select **Instruments OQ** or **Instruments PQ**. A Wizard guides you through copying the sequences. Clicking **Next >** takes you to the next Wizard steps.
- Select the timebase for which you want to perform OQ or PQ. Enter the name of the computer on which the timebase is installed.
- Select the source directory of the template to be used.
- Enter a unique name for saving the copy (default: template name + date).
- A list is displayed containing all sequences of the corresponding template. Click to select the sequences required for the checks (→ section 4).
  After the sequences have been copied, the batch list of the corresponding timebase is automatically opened. Start the batch to process the sequences.

The checks are processed in the following order:

1. Fluid preparation of the system (*Warm up* sequence)
2. Temperature accuracy of the column compartment for manual data acquisition (*Column Oven* sequence)
3. Wavelength accuracy of the UV detector (*Wavelength* sequence)
4. Baseline noise, drift, and lamp intensity of the UV detector (*UV Noise Drift* sequence)
5. Precision of injection volume and flow (*Injector Flow Repro* sequence and *Injector Flow Repro_P680DGP_Left* sequence if necessary)
6. Linearity of the UV detector (*UV Linearity* sequence)
7. Linearity of the injection volume (*Sampler Lin CO* sequence)
8. Carry-over by the autosampler (*Sampler Lin CO* sequence)
9. Baseline noise, signal height, and wavelength accuracy of the fluorescence detector (*Fluorescence* sequence)
10. Baseline noise and drift of the RI detector (*RI_Noise_Drift* sequence)
11. Linearity of the RI detector (*RI_Linearity* sequence)
12. Baseline noise of the evaporative light scattering detector (*ELS_Noise* sequence)
13. Solvent composition of gradient pumps: accuracy, precision, and ripple (*LPG_M480 or HPG_M480* sequences*; STD_GRAD, MICRO_GRAD, or LONG_GRAD* sequences*, or STD_GRAD_P680DGP_Left or LONG_GRAD_P680DGP_Left* sequences)
14. Solvent composition for ternary high-pressure gradient pumps: accuracy, precision, and ripple between channels C and B (*Tern_Grad_C_B* sequence)
15. Temperature accuracy of the column compartments for automatic data acquisition (*Column Oven* sequence)
16. Resetting the solvent flow rate to 0.05 ml/min (*Stop* sequence)

Tip: When you use a manual injection valve, make sure that no air is injected with the samples. Besides, inject five times the sample loop volume, that is, inject at least 50 µl.

3.7 Check Time

If the column compartment and the RI detector are not included in the check, the entire check takes approximately 3.5 hours. Depending on the check, more time may be required:

- 2 more hours when checking Dionex P680 pumps, UltiMate 3000 pumps with mixing chamber extension, or an UltiMate 3000 LPG-3400M(B) pump (LONG_GRAD sequence instead of the STD_GRAD or MICRO_GRAD sequence)
- 2 more hours when checking Dionex P680 DGP or UltiMate 3000 DGP pumps (standard configuration)
- 4 more hours when checking Dionex P680 DGP pumps, UltiMate 3000 DGP micro pumps, or pumps with mixing chamber extension
- 3 more hours when checking the column thermostat
- 1.5 more hours when checking the RI detector
- 0.5 more hours when checking the ELS detector
- 2 more hours when checking a ternary high-pressure gradient system (channels C and B)

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 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.

Exemption: It is not necessary to change the solvent manually when qualifying systems in which the UV detector is a Dionex VWD-3400RS or a single wavelength detector.

3.8 Evaluating the Sequences

To evaluate the detector linearity, enter the actual concentrations for the used standards into the amount columns of the QNT file.

The master sequence on the Chromeleon-CD and thus, all copies made from it for OQ and PQ are linked to the corresponding report. **Do not change this report** (for exceptions, → section 3.5.3). In the report, many references link the separate data sheets. When lines or columns are inserted or deleted, the references are lost and thus, the calculations will be wrong!

To ensure that the data are correctly read and processed in the report, print the report as **Batch Report** from the Browser. Select the sequence for which you want to print the report. **Verify that no sample is selected!** On the **File** menu, select **Batch Report**, and then click **OK** to start printing.

3.9 Repeating Checks

It may be necessary to repeat one or several checks. In this case, refer to section 6. 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.

4 Special Test Procedures for Individual Modules

4.1 Introduction

This section describes test procedures that fundamentally differ from the procedures described in section 3. These procedures refer to a certain instrument; they cannot be used for any other instrument. In addition, all test sequences must be run one after the other, as the tests require different system configurations. The test procedures described in sections 3 and 5 serve as a basis; this section focuses on the differences in particular. Whenever the test steps are identical, you are referred to sections 3 and 5.

The sequence templates for these tests are available in the SPECIAL_HPLC_TEMPLATES directory (see Figure 6). Start the OQ/PQ Setup from this directory as described in section 3.5.2.

4.2 Dionex VWD-3x00 Detectors: Noise and Drift with Dummy Flow Cells

For qualifying the Dionex VWD-3100 and VWD-3400 detectors with dummy flow cell, you have to add the *UV_NOISE_DRIFT_VWD3x00* sequence manually to the batch list. This sequence can be used only for the above detectors and the test procedure requires that the flow cell be changed twice.

The following table shows the drift and noise limits for dummy flow cells. The specifications have to be entered into the report manually.

Instrument	**Parameter**	**Description**	**Limits(1)**	
			OQ	PQ
VWD-3100, VWD-3400RS (dummy flow cell)	Baseline Noise	Measured with the dummy flow cell included in the shipment (without fluidics).	0.010 mAU	0.020 mAU

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

4.3 Dionex Autosamplers: Sample Temperature Accuracy

This section describes how the sample temperature accuracy is determined for the following Dionex autosamplers: WPS-3000TRS, WPS-3000TPL, WPS-3000TBPL Analytical, WPS-3000TSL, and ACC-3000T. For this test, only the autosampler is required. The other modules of the HPLC system are not required.

4.3.1 Test Procedure

The following table lists the materials required for performing the test.

Part No.	**Description**	**Quantity**
6820.0010	Type K temperature sensor for P600 thermometers	1
5705.0050A	Column Thermostat PQ Kit	1

In addition, a standard glass vial (1.8 ml) is required. Fill the vial with water (do not seal).

4.3.2 Connecting and Configuring the System

- **System connections**
  - Connect the type K temperature sensor to the thermometer and make the settings (that is, adapt the sensor type and calibration values) as described in the instructions for the thermometer.
  - Fill an open standard glass vial (1.8 ml) with water and place it at sample position RC8.
  - Insert the temperature sensor into the vial at a right angle until the tip touches the vial bottom.

Figure 13: Temperature sensor inserted into the vial

  - Rotate the carousel until the carousel cover closes completely.

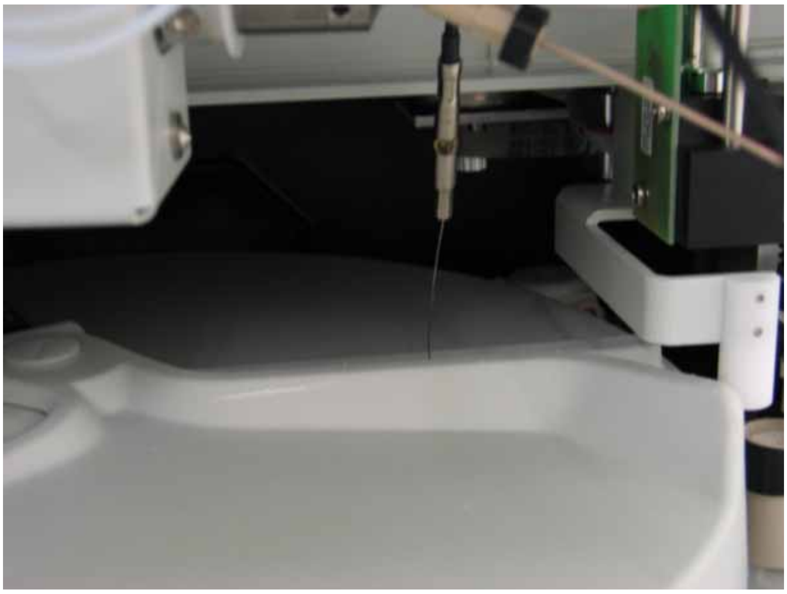


Figure 14: Cover closed

- **Configuration**

  Connect the thermometer to a free COM port on the Chromeleon Server PC. Install the *Dostmann Thermometer P500/P600* in the Chromeleon Server Configuration program On the **General** page, select the COM port to which the thermometer is connected. In addition, install a virtual channel (Device Name: *VirtualChannels_01*, Signal Name: *TemperatureOVEN*).
- **Preparing Chromeleon**

  To qualify the sample temperature accuracy, select the following sequence: SAMPLER_TEMP_ACC

4.3.3 Performing the Check

Set the sample temperature to 10°C (15°C for the ACC-3000T). When the nominal temperature is reached, the external thermometer is used to record the sample temperature over a period of 30 minutes.

Tip: Do not perform any autosampler commands during the test. Moving the needle arm or carousel may damage the thermometer or autosampler.

4.3.4 Duration

The test takes approximately 45 minutes.

5 Procedures

5.1 Baseline Noise, Drift, and Lamp Intensity of the UV Detector

5.1.1 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 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 burning for several hours. In the detector environment, avoid drafts and direct sunlight.

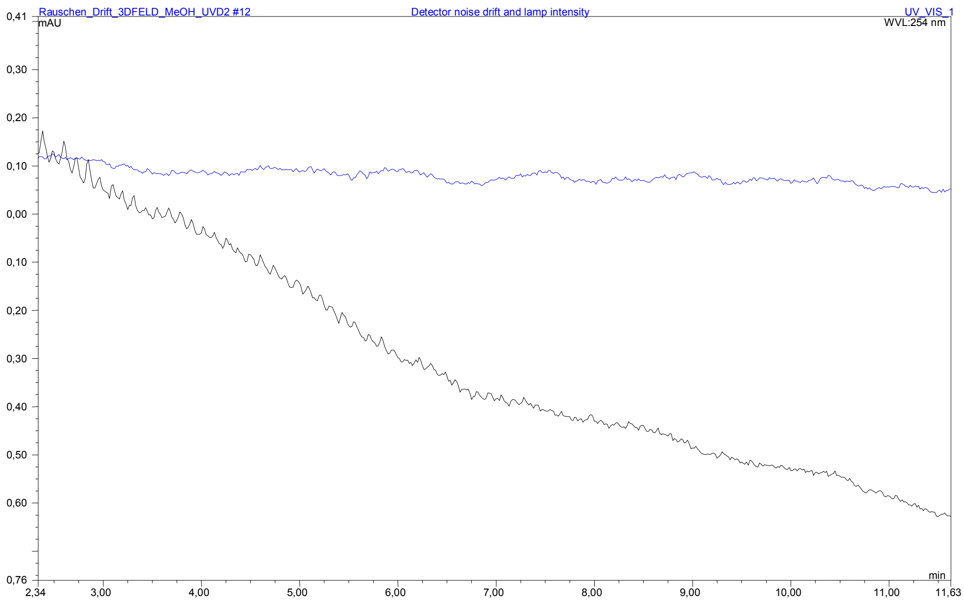


Figure 15: **Lamp drift: Directly after the lamp has been turned on (bottom chromatogram) and after it has been burning for six hours (top chromatogram)**

The lamp intensity decreases while the lamp is burning. Besides, the lamp ages when it is turned on and off very often.

5.1.2 Performing and Evaluating the Checks

The checks for noise, drift, and lamp intensity 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.

If the lamp intensity can be determined, it is read directly from Chromeleon (wavelength: λ = 254 nm). However, for the Dionex VWD-3100 and VWD-3400RS detectors, the lamp intensity is determined at a wavelength of λ = 230 nm. The results are no absolute measured physical quantities (such as, the luminous density or luminous flux). That is why deviations of 5 % from lamp to lamp and from detector to detector are quite normal. Therefore, to evaluate the data compare them to previously determined values.

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 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.

5.2 Wavelength Accuracy of the UV Detector

5.2.1 Performing the Check

For UV detectors without PDA option, the wavelength accuracy is established with the *UV_Wavelength* sequence.

For photodiode array detectors, use the *DAD_Wavelength* sequence. For single wavelength detectors and the Dionex VWD-3400RS detector, use the *Wavelength_Single* sequence. Separate sequences are available for the following detectors: Dionex UVD 160, UVD 160S, UVD 320, UVD 320S detetors, the TSP UV 2000 detector, and the Shimadzu LC-2010 SPD, SPD-10A*vp*, and SPD-10AV*vp* detectors. The Wizard uses the appropriate sequence automatically when the corresponding detector is installed.

In all cases, wavelength accuracy is determined using pyrene in methanol (c = 3 µg/ml) at a flow rate of 1 ml/min. However, for single wavelength detectors and the Dionex VWD-3400RS detector, 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.

5.2.2 Evaluating the Check for the UV Detector

The signals are recorded at 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 for pyrene (333.3 nm).

5.2.3 Evaluating the Check for the Photodiode Array Detector

The UV spectrum for pyrene is recorded between 250 nm and 350 nm. The spectral maxima between 250 nm and 290 nm and between 330 nm and 350 nm are determined by Chromeleon and compared to their theoretical values (272.1 nm and 333.3 nm).

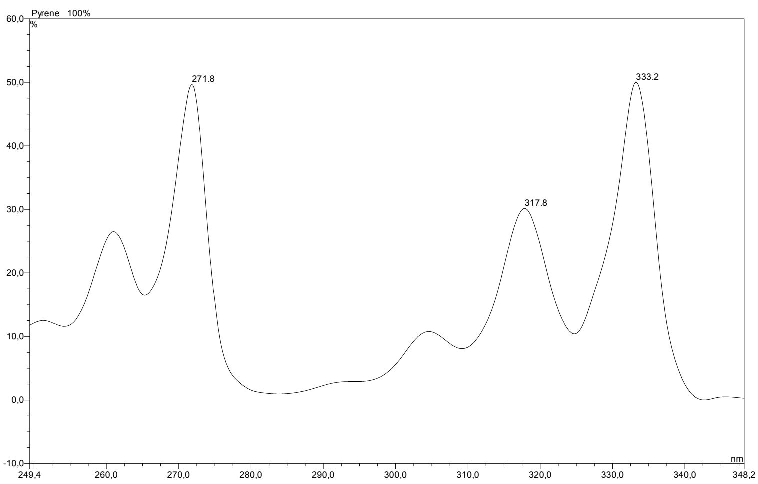


Figure 16: UV Spectrum of pyrene in methanol

5.2.4 Evaluating the Check for Two-Channel Detectors and the UVD 320S

- For the following detectors, the signals are recorded at 235 nm, 240 nm, and 245 nm: Dionex UVD 160, UVD 160S, UVD 320, UVD 320S detector, the TSP UV2000 detector, and the Waters 2487 detector. 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).
- For the Shimadzu detectors, the signals are recorded at 333 nm and 335 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 (333.3 nm).

5.2.5 Evaluating the Check for Single Wavelength and VWD-3400RS Detectors

For the following detectors, the signals are recorded at 270 nm, 272 nm and 274 nm: Dionex VWD-3100, VWD-3400, and AD25 detectors and the G1314A, G1314B, and G1314C detectors of the Agilent 1100/ 200 series. 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 for caffeine (272.5 nm).

5.3 Linearity of the UV Detector

5.3.1 Theory

The detector linearity 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, with decreasing light intensity (caused by lamp ageing or absorption of the solvent or sample), the influence of the dark current on the linearity increases. As water is the solvent for this check, the influence of the solvent is negligible. The influence of the sample is fully used to determine the detector linearity. Consider that the resulting deviations of the linear behavior are only important with high absorption rates (> 1.5 AU).

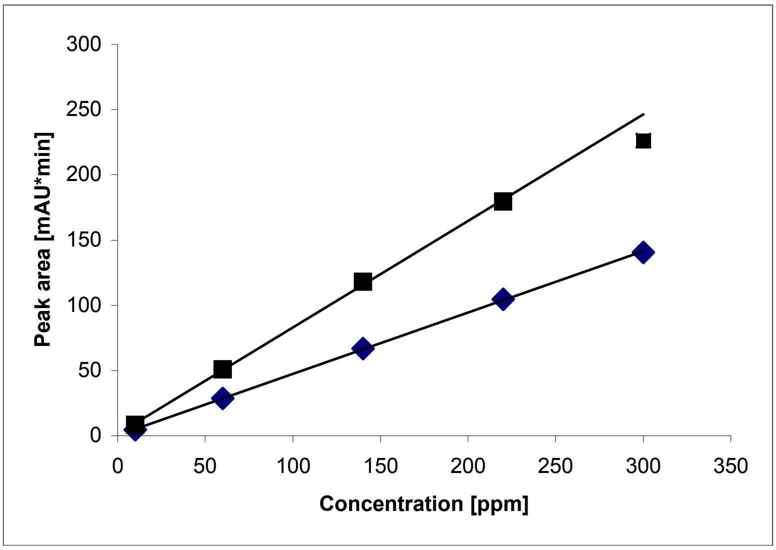


Figure 17: Linearity of the detector signal depending on the peak area

5.3.2 Performing and Evaluating the Check

The detector linearity is established with the *UV_Linearity* sequence. The detector linearity is determined at 272 nm using five 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). Water is used as solvent. The flow rate is 1 ml/min. The peak area and concentration are represented in a graph and the regression line is determined. The regression coefficient and the relative standard deviation of this line indicate the linearity.

Tip: Depending on which injection module you use, it may happen that the peak height of the sample with the highest concentration increases 1500 mAU. This is usually not within the linearity range of UV detectors. Thus, the limits for the regression coefficient and the relative standard deviation may not be met. In this case, reduce the injection volume for all samples used for the linearity check so that the peak height of the sample with the highest concentration is in the linearity range of the tested detector, i.e., usually below 1500 mAU. As an exception, a linearity range of up to 2500 mAU is specified for the supported Shimadzu detectors and the Dionex VWD-3100 and VWD-3400RS detectors with an analytical flow cell installed. The Dionex VWD-3100 and VWD-3400RS detectors with a semimicro or micro analytical flow cell installed have a specified linearity range of up to 1700 mAU.
If an autosampler is used when the Dionex PDA-100 or PDA-3000 detectors are tested, only 8 µl of sample will be injected by default. However, when qualifying the Dionex VWD-3100 and VWD-3400RS detectors using an autosampler, 13 µl of sample will be injected.

5.4 Precision of the Injection Volume

5.4.1 Theory

The precision of the injection volume is mainly influenced by the quality of the autosampler syringe and the syringe volume that has been adjusted to the injection volume. In addition, the mechanics for the syringe movement 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.

If a manual injection valve is used, inject at least five times the sample loop volume; i.e. inject at least 50 µl.

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

5.4.2 Performing the Check

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, standard, and the number of injection (see the table).

The relative standard deviation of the peak areas of the six or ten injections indicates the precision of the injection volume.

Autosampler	Standard* used	Injection volume	Number of injections
Other	Standard 4	5 µl	10
Dionex Gina 50 Dionex Gina 160	Standard 3	10 µl	6
Dionex ACC-3000(T) - Sample loop volume: 200 µl	 Standard 3	 20 µl	10
Dionex WPS-3000(T)SL - Micro - with 250 µl injection volume kit	 Standard 5 Standard 3	 2 µl 10 µl	 10 10
Dionex WPS-3000TBPL Analytical - Large Volume configuration	 Standard 3	 20 µl	 10
Dionex WPS-3000(T)RS Micro option	Standard 5	2 µl	10

*) Also see section 3.1.

5.5 Carry-over by the Autosampler

5.5.1 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.

5.5.2 Performing the Check

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 0.3 ml/min, wavelength: 272 nm). Samples 6 and 9 contain water (same vial). Sample 7 contains a solution of caffeine in water; the concentration is 10 µg/ml (standard 2 - reference sample). Sample 8 contains a solution of caffeine in water; the concentration is 2000 µg/ml (standard 7). The carry-over (CO in [%]) is calculated as follows:

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

$$= \frac{Area_{Water,CarryOver} - Area_{Water}}{Area_{Reference}} \times \frac{c_{Reference}}{c_{Conc2000mg/ml}}$$

$Area_{Water,corr}$:	Area of the caffeine peak in the water sample (sample 9 – sample 6)
$Area_{Conc:2000mg/ml}$:	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)
$c_{Conc:2000mg/ml}$:	Caffeine concentration of the carry-over solution (conc.: 2000 µg/ml)

5.6 Linearity of the Injection Volume

5.6.1 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.

5.6.2 Performing the Check

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). 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.

Autosampler	Standard* used	Injection volume
Others	Standard 2	5, 10, 20, 40, and 80 µl
Dionex Gina 50 Dionex Gina 160	Standard 2	10, 20, 40, 60, and 80 µl
Shimadzu SIL-10ADvp	Standard 2	5, 10, 20, 40, and 50 µl
Dionex ACC-3000(T) - Sample loop volume: 20 µl - Sample loop volume: 50 µl	 Standard 3 Standard 3	 1, 3, 5, 7, and 10 µl 5, 10, 15, 20, and 25 µl
Dionex WPS-3000(T)SL - Micro - with 250 µl injection volume kit	 Standard 3 Standard 2	 1, 5, 10, 15, and 20 µl 10, 20, 40, 80, and 160 µl
Dionex WPS-3000(T)PL - sample loop volume: 20 µl - sample loop volume: 21 - 99 µl	 Standard 3 Standard 3	 1, 3, 6, 9, and 12 µl 1, 5, 10, 15, and 20 µl
Dionex WPS-3000TBPL Analytical - Standard-configuration - Large Volume - configuration	 Standard 3 Standard 2	 5, 10, 15, 20 und 25 µl 20, 50, 80, 110 und 140 µ
Dionex WPS-3000(T)RS Micro Option	Standard 3	1, 5, 10, 15 und 20 µl

*) Also see section 3.1.

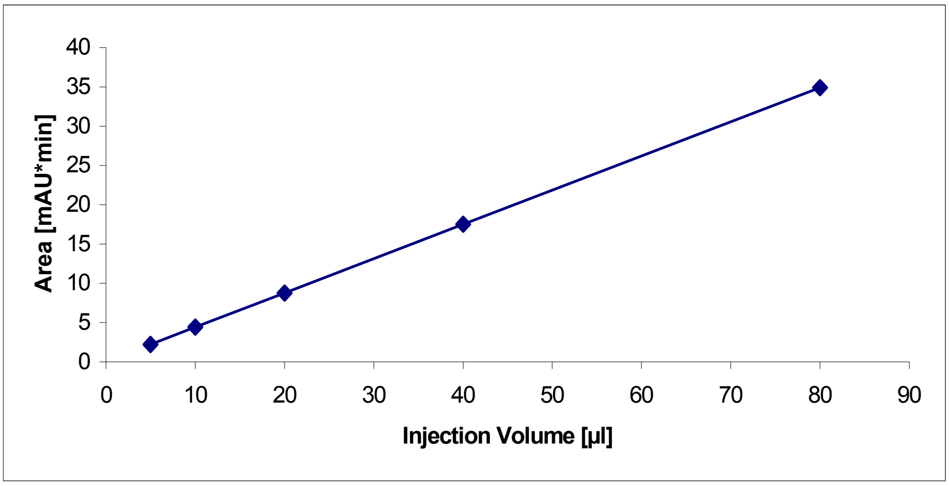


Figure 18: Linearity of the injection volume

5.7 Sample Temperature Accuracy of Autosamplers

5.7.1 Theory

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

5.7.2 Performing the Check

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 must be closed during the test. The autosampler temperature is set to a nominal temperature (e.g., 10°C), depending on the autosampler type. When the nominal temperature is reached, the sample (water) temperature is recorded over a period of 30 minutes. Within the 30 minutes, the sample temperature reaches a stable value. The temperature accuracy is the temperature difference between the sample temperature and the nominal autosampler temperature.

5.8 Flow Precision

5.8.1 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.

5.8.2 Performing the Check

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 5.4.2).

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

5.9 Solvent Composition of the Gradient Pump: Accuracy, Precision, and Ripple

5.9.1 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.). Acetone is highly absorbing in the range of λ = 265 nm. The gradient can be observed in a chromatogram. There are no injections required.

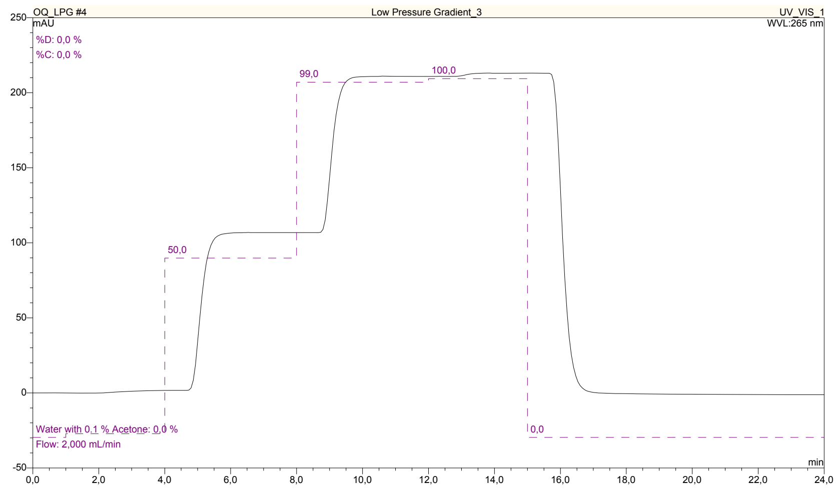


Figure 19: Theoretical (broken line) and real gradients (*STD_GRAD* standard sequence for gradient pumps)

5.9.2 Performing the Checks

Except for the TSP P2000 Pump, the following solvent compositions (in %B) are mixed: 0, 1, 50, 99, and 100. The solvent composition (in %B) for the TSP P2000 Pump is as follows: 0, 50, and 100%. This is because the pump does not support gradient programs with more than 9 steps.

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

To qualify a gradient pump (except the Dionex UltiMate 3000 pumps), the *STD_GRAD* sequence is required.

For the Dionex M480 an M300 pumps, separate sequences are available, which are automatically selected by the Wizard.

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

5.9.3 Performing the Checks for the Dionex P680 and UltiMate 3000 Pumps

Five sequences are provided for qualifying the Dionex LPG-3400A(B), DPG-3600A(B), HPG-3200A, HPG-3200M, HPG-3200RS, HPG-3400A, HPG-3400M, HPG-3400RS, and P680 pumps: *STD_GRAD*, *MICRO_GRAD*, LONG*_GRAD, STD_GRAD_P680DGP_Left*, and *LONG_GRAD_P680DGP_Left.*

The *MICRO_GRAD* sequence is used to qualify a pump with MicroFlow kit (e.g., an HPG-3200M or HPG-3400M). The gradient program corresponds to the program of the *STD_GRAD* sequence. However, the mixing volume of a pump with MicroFlow kit is lower than the volume of a pump with standard mixing chamber. That is why the detector signal is detected earlier. This fact is considered for the evaluation of the check.

The LONG*_GRAD* sequence is used if a mixing chamber extension (600 µl or 1200 µl for UltiMate 3000 pumps, or 1250 µl for the P680 pump) is installed. A higher mixing chamber volume increases the equilibration time of the gradient. The gradient program has been adapted accordingly and evaluation of the check considers this.

For DGP pumps (P680 and UltiMate 3000 DGP-3600A(B)), qualification is performed for both pump units. The sequences *STD_GRAD* and *LONG_GRAD* are used to check the right pump unit. The sequences *STD_GRAD_P680DGP_Left* and *LONG_GRAD_P680DGP_Left* are used to check the left pump unit.

For the UltiMate 3000 LPG-3400M(B) and DGP-3600M(B) micro pumps, only the *LONG_GRAD and LONG_GRAD_P680DGP_Left* sequences are available.

5.9.4 Evaluating the Check

To facilitate the comparison, the absorption values are converted and expressed as %B. To compensate the detector drift, the absorption of the pure solvent A 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 gradient precision is not determined for M480 pumps. For M480 pumps, a second gradient is measured in addition to the gradient described above. For the second gradient, channel C delivers water as solvent.

The ripple is determined for all steps. A one-minute 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 is the distance between these two lines. This is an indication for the ripple.

5.10 Temperature Accuracy of the Column Compartment

5.10.1 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.

5.10.2 Performing the Check

Four measuring points are used to check the temperature accuracy of the column compartment. The check is performed with the *Column_Oven* sequence. The achieved temperature is measured with an external, calibrated thermometer.

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

Exception:

- It is not possible to set the temperature on the column compartment module of the Waters Alliance 2690 Separation Module or on the Shimadzu column compartments (LC-2010 CTO, CTO-10A*vp*, CTO-10AC*vp* und CTO-10As*vp*) 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°C), too. 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 Dionex ACC-3000(T) is performed for three measuring points only.

Tip: With the column compartment of the Waters Alliance 2690 Separation Module, the set temperature can be changed during a sample only when the autosampler is injecting. That is why 1µl of water is injected for qualifying the column compartment of the Waters Alliance 2690 Separation Module.

5.11 Baseline Noise and Signal Height of the Fluorescence Detector

5.11.1 Theory

Drift and baseline noise are important specifications for the 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 lamp. There is a considerable increase in noise if an old lamp with poor light intensity is used. In addition, contamination in the flow cell leads to an increase noise level. Also, make sure that the measuring and ambient conditions are constant. Verify that there are no gas bubbles in the flow cell.

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.

5.11.2 Performing the Check

The *Fluorescence* sequence is used to determine the noise and the signal height. When testing the Dionex RF2000, make sure that the detector's ZWAVE parameter is set to 1 (→ 3.3). Water is pumped through the flow cell at a flow rate of 1ml/min. The excitation wavelength is 350 nm; the emission wavelength is 394 nm.

To determine the noise, the measuring signal is split into thirty intervals of thirty 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 thirty interval values are averaged.

5.12 Wavelength Accuracy of the Fluorescence Detector

5.12.1 Performing the Check

The *Fluorescence* sequence is used to determine the wavelength accuracy of the emission spectrum. When testing the Dionex RF 2000 detector, make sure that the ZWAVE parameter is set to 1 on the instrument (see section 3.4.1). Water is pumped through the flow cell at a flow rate of 1ml/min. For an excitation wavelength of 350 nm, the emission wavelength changes in 1nm increments from 380 nm to 410 nm. The relative signal maximum is compared to the theoretical maximum.

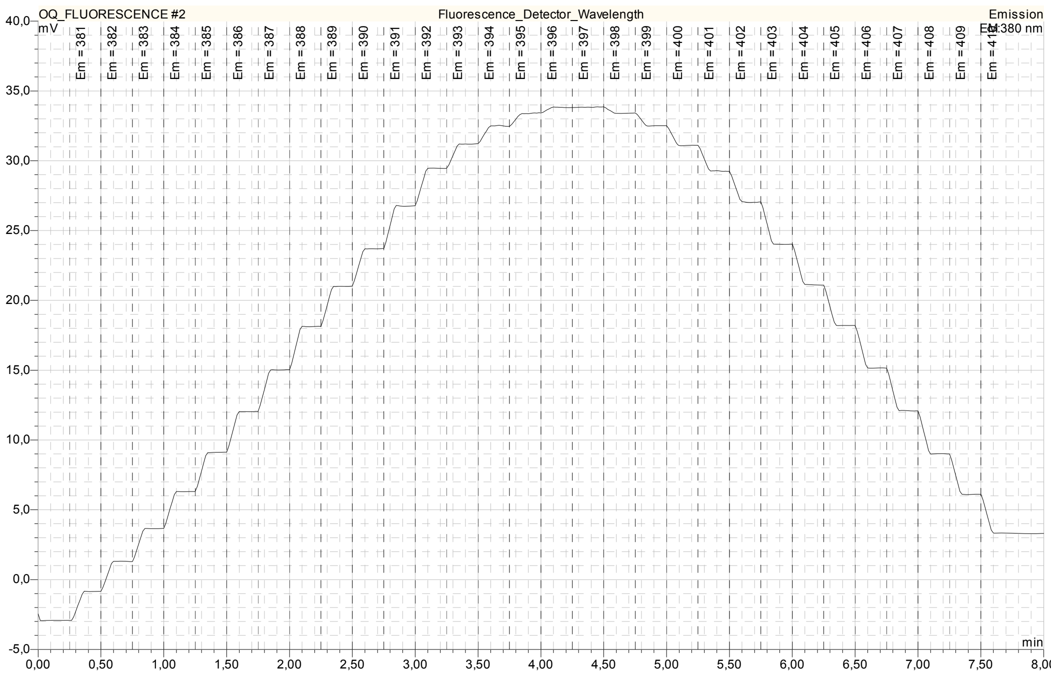


Figure 20: Chromatogram for defining the relative maximum of the emission spectra between 380 nm and 410 nm

5.12.2 Remarks 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 instrument should preferably be checked with the components used for the measurements.

5.13 Baseline Noise and Drift of the RI Detector

5.13.1 Theory

Drift and baseline noise are important specifications for a detector. Increased baseline noise considerably reduces the detection sensitivity, as it is not possible to distinguish between small 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 lamp. There is a considerable increase in noise if an old lamp with poor light intensity is used. This is also true when the reference and/or sample 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 RI detector, make sure that the measuring and ambient conditions are constant. In addition, it is very important that the lamp has been burning for several hours and that the flow cell has been rinsed sufficiently.

5.13.2 Performing and Evaluating the Check

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 the curve indicates the drift of the measured signal; the absolute value of the slope indicates the absolute value of the drift. Parallel to the regression line, two lines are drawn through the measured minimum and maximum value. The noise is the distance between these two lines. The calculated values are averaged for all 20 intervals to establish the final value.

5.14 Linearity of the RI Detector

5.14.1 Theory

The detector linearity 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, with decreasing light intensity (caused by lamp ageing or absorption of the solvent or sample), the influence of the dark current on the linearity increases. As water is the solvent in both the sample and reference cells, the influence of the solvent is negligible. The influence of the sample is fully used to determine the detector linearity. Consider that the resulting deviations of the linear behavior are only important with high sample concentrations and high absorption rates (> 600 µRIU).

5.14.2 Performing and Evaluating the Check

To establish the detector linearity, the *RI_LINEARITY* sequence is required. The detector linearity is determined using five glycerin standards (set 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). Water is used as solvent. The flow rate is 1 ml/min. The peak area and concentration are represented in a graph and the regression line is determined. The regression coefficient of this line indicates the linearity.

5.15 Baseline Noise of the ELS Detector

5.15.1 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 evaporative light scattering detector mainly depends on 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.

5.15.2 Performing and Evaluating the Check

The *ELS_NOISE* sequence is used for the noise test. Water is pumped through the detector at a flow rate of 1 ml/min. The conditions for recording the ELS signal are as follows:

- Nebuliser temperature: 50°C
- Evaporator temperature: 90°C
- Carrier gas flow: 1.6 SLM@4.1 bar

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

6 Troubleshooting

6.1 General Notes

- A system pressure that is well above 130 bar for a flow rate of 1 ml/min (solvent: water) after the pressure regulator has been connected indicates that a capillary is contaminated. Inspect and exchange the capillaries and the pressure regulator 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 instruments' manuals.

6.2 Failure of Individual Checks

6.2.1 UV Detector

Check	Reason	Remedial Action
Wavelength accuracy	Spectrum calibration was not successful during connect in Chromeleon. Increased drift	First, disconnect and then, reconnect the detector. See below.
Baseline noise	The solvent is contaminated. The lamp is too old. There are air bubbles in the flow cell.	Exchange the solvent. Exchange the lamp. Prime the flow cell.
Drift	The detector is not yet warmed. The system is not equilibrated. The lamp is defective. There are fluctuations in the ambient temperature.	Allow the detector sufficient time to warm up. Rinse the system. Exchange the lamp. If necessary, close the windows and shield the instrument from the air conditioning system.
Lamp intensity	The lamp is too old. The flow cell is incorrectly installed.	Exchange the lamp. Install the flow cell correctly.
Detector linearity	The lamp is too old. The concentration of standards is not correct. The peak height of the sample with the highest concentration is not in the linearity range specified for the detector, i.e., usually > 1500 mAU (for Shimadzu detectors and Dionex VWD-3100 and VWD-3400RS detectors > 2500 mAU).	Exchange the lamp. Use new standards. 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, i.e., usually < 1500 mAU.

6.2.2 Autosampler

Check	Reason	Remedial Action
Precision of injection volume	The 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.
	There are air bubbles in the syringe.	Prime the syringe.
	The autosampler is leaking.	→ *Autosampler Manual*
	The injection valve is leaking.	→ *Autosampler Manual*
Linearity of Injection Volume	The detector linearity check failed.	See above.
	The syringe is old.	Exchange the syringe.

6.2.3 Pump

Check	Reason	Remedial Action
Flow precision	The 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.
	There are air bubbles in the syringe.	Prime the syringe.
	The autosampler is leaking.	→ *Autosampler Manual*
	The injection valve is leaking.	→ *Autosampler Manual*
Gradient accuracy	There is air in the system.	Prime the system.
	The system is not equilibrated.	Rinse the system.
	The composition of solvent B is not correct.	Make sure that the solvent composition is correct.
Gradient precision	There is air in the system.	Prime the system.
	The system is not equilibrated.	Rinse the system.
Ripple	There is air in the system.	Prime the system.
	The system is not equilibrated.	Rinse the system.

6.2.4 RF2000 Fluorescence Detector

Check	Reason	Remedial Action
Wavelength accuracy	The Raman peak of water is not visible because the instrument performs an Autozero whenever the wavelength is changed.	On the instrument, set the **ZWAVE** parameter to 1 (→ section 3.4.1)

6.2.5 RI Detector

Check	Reason	Remedial Action
Baseline noise	There are 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	The solvent is contaminated. There are fluctuations in the ambient temperature. There are air bubbles in the flow cell.	Use new solvent. Position the detector at a location with few temperature fluctuations. Rinse the sample and reference cells with degassed water (see above).
Detector linearity	The concentration of the standard is not correct.	Use fresh standards.

6.2.6 ELS Detector

Check	Reason	Remedial Action
Baseline noise	The pump pulsation is too high.	Purge the pump and all channels if necessary.
Baseline spikes	The gas supply is contaminated.	Replace the gas supply.

7 PGM Files

As described in section 3.5, the OQ and PQ Setup Wizards adapt the program files to the instruments installed in the related timebase. The section lists the PGM files for the instruments of a Dionex HPLC system.

7.1 Wavelength Accuracy of the Photodiode Detector

```
;=================================================================
; Wavelength Accuracy for detectors UVD 340S, UVD 170S, PDA-100
; -----------------------------------------------------------------
; PGM-Version 09-02-2000
;
;
; Restriction capillary: l = 13 m, ID = 0.13 mm, PEEK
; Solvent:                Methanol, degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:         P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:         ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:         Diodearray UVD 340S
;
; Sample:                Pyrene 3µg/ml
;=================================================================
; Pump specific settings
       Log                          Pump.ModelNo
       Log                          Pump.ModelVariant
       Log                          Pump.SerialNo

; Sampler specific settings
       Log                          Sampler.ModelNo
       Log                          Sampler.SerialNo
;

; Column Oven specific settings
       Log                          ColumnOven.ModelNo
; UV Detector specific settings
       Log                          UV.ModelNo
; Fluorescence detector specific settings
       Log                          Emission.ModelNo

       Pressure.LowerLimit =        10
       Pressure.UpperLimit =        300
       %A.Equate =                  "Methanol"
; Pump specific settings
; Settings specific for the Dionex P580
       %A.Type =                    Automatic

       UV_VIS_1.Wavelength =        331
```

```
       UV_VIS_1.Bandwidth =          1
       UV_VIS_1.Step =               0.20
       UV_VIS_1.Average =            On
       UV_VIS_2.Wavelength =         333
       UV_VIS_2.Bandwidth =          1
       UV_VIS_2.Step =               0.20
       UV_VIS_2.Average =            On
       UV_VIS_3.Wavelength =         335
       UV_VIS_3.Bandwidth =          1
       UV_VIS_3.Step =               0.20
       UV_VIS_3.Average =            On
       UV_VIS_4.Wavelength =         250
       UV_VIS_4.Bandwidth =          1
       UV_VIS_4.Step =               0.20
       UV_VIS_4.Average =            On


; Detector specific settings
; Settings specific for the Dionex UVD 340S
       3DFIELD.MaxWavelength =       350.0
       3DFIELD.MinWavelength =       250.0
       3DFIELD.BunchWidth =          1.9
       3DFIELD.Step =                0.2
       3DFIELD.RefWavelength =       600.0
       3DFIELD.RefBandwidth =        1.9
       UV_VIS_1.RefWavelength =      600
       UV_VIS_1.RefBandwidth =       1
       UV_VIS_2.RefWavelength =      600
       UV_VIS_2.RefBandwidth =       1
       UV_VIS_3.RefWavelength =      600
       UV_VIS_3.RefBandwidth =       1
       UV_VIS_4.RefWavelength =      600
       UV_VIS_4.RefBandwidth =       1



; Autosampler specific settings
; Settings specific for the Dionex ASI-100
       Sampler.InjectMode =          Normal
       Sampler.UpSpeed =             10.0
       Sampler.DownSpeed =           10.0
       Sampler.SampleHeight =        0.50
       Sampler.WashSpeed =           50.00
       Sampler.DispSpeed =           50.00
       Sampler.FillSpeed =           25.00
       SyringeDelay =                5


       Flow =                        1.000

0.000  UV.Autozero
; Detector specific settings
       Inject
       3DFIELD.AcqOn
       UV_VIS_1.AcqOn
       UV_VIS_2.AcqOn
       UV_VIS_3.AcqOn
       UV_VIS_4.AcqOn

1.250  3DFIELD.AcqOff
```

```
UV_VIS_1.AcqOff
UV_VIS_2.AcqOff
UV_VIS_3.AcqOff
UV_VIS_4.AcqOff
End
```

7.2 Baseline Noise, Drift, and Lamp Intensity of the UV Detector

```
;===================================================================
; Noise and Drift
; ---------------
; PGM-Version 09-02-2000
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;         Back Pressure: 90 bar
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:         ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:         Diodearray UVD 340S
;
;===================================================================
; Pump specific settings
       Log                           Pump.ModelNo
       Log                           Pump.ModelVariant
       Log                           Pump.SerialNo

; Sampler specific settings
       Log                           Sampler.ModelNo
       Log                           Sampler.SerialNo
;

; Column Oven specific settings
       Log                           ColumnOven.ModelNo
; UV Detector specific settings
       Log                           UV.ModelNo
; Fluorescence detector specific settings
       Log                           Emission.ModelNo

       Pressure.LowerLimit =         10
       Pressure.UpperLimit =         300
       %A.Equate =                   "Water"
; Pump specific settings
       ; Settings specific for the Dionex P580
```

```
       %A.Type =                    Automatic
       Flow =                       1.000

       UV_VIS_1.Wavelength =        254
       UV_VIS_1.Step =              1.0
       UV_VIS_1.Average =           On

; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       3DFIELD.Step =               1
       UV_VIS_1.Bandwidth =         1
       UV_VIS_1.RefWavelength =     600
       UV_VIS_1.RefBandwidth =      1

; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       Sampler.InjectMode =         Normal
       Sampler.UpSpeed =            10.0
       Sampler.DownSpeed =          10.0
       Sampler.SampleHeight =       0.50
       Sampler.WashSpeed =          50.00
       Sampler.DispSpeed =          50.00
       Sampler.DrawSpeed =          25.00
       Sampler.SyringeDelay =       5


 0.000 UV.Autozero
; Detector specific settings
       Inject
       UV_VIS_1.AcqOn


 1.000 Log                          LampIntensity
       Protocol                     "The lamp intensity is given at
254 nm in [counts/s]"


20.000 UV_VIS_1.AcqOff

       End
```

7.3 Linearity of the UV Detector

```
;==================================================================
; UV Detector Linearity
; ----------------------------------------------------------------
; PGM-Version 22-05-2001
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;         Back Pressure: 80 bar
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:         ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:         Diodearray UVD 340S
;
; Samples:               Caffeine 10 µg/ml, 60 µg/ml, 140 µg/ml, 220
µg/ml, 300 µg/ml
;
;====================================================================
 ; Pump specific settings
       Log                             Pump.ModelNo
       Log                             Pump.ModelVariant
       Log                             Pump.SerialNo

; Sampler specific settings
       Log                             Sampler.ModelNo
       Log                             Sampler.SerialNo
;

; Column Oven specific settings
       Log                             ColumnOven.ModelNo
; UV Detector specific settings
       Log                             UV.ModelNo
; Fluorescence detector specific settings
       Log                             Emission.ModelNo


       Pressure.LowerLimit =           10
       Pressure.UpperLimit =           300
       %A.Equate =                     "Water"
; Pump specific settings
       ; Settings specific for the Dionex P580
       %A.Type =                       Automatic
       Flow =                          1.000
```

```
       UV_VIS_1.Wavelength =        272
       UV_VIS_1.Step =              0.20
       UV_VIS_1.Average =           On

; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       3DFIELD.Step =               0.2
       UV_VIS_1.Bandwidth =         1
       UV_VIS_1.RefWavelength =     600
       UV_VIS_1.RefBandwidth =      1

; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       Sampler.InjectMode =         Normal
       Sampler.UpSpeed =            10.0
       Sampler.DownSpeed =          10.0
       Sampler.SampleHeight =       0.50
       Sampler.WashSpeed =          50.00
       Sampler.DispSpeed =          50.00
       Sampler.DrawSpeed =          25.00
       Sampler.SyringeDelay =       5


 0.000 UV.Autozero
; Detector specific settings
       Inject
       UV_VIS_1.AcqOn


 1.250 UV_VIS_1.AcqOff
       End
```

7.4 Precision of the Injection Volume

```
;=================================================================
; Injector and Flow Reproducibility
; ----------------------------------------
; PGM-Version 22-05-2001
;
; Restriction capillary: PEEK
;                   ID: 0.13 mm
;               Length: 13 m
;        Back Pressure: 80 bar
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:          ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:          Diodearray UVD 340S
;
; Sample:                 Caffeine 140 µg/ml
;===================================================================
; Pump specific settings
       Log                           Pump.ModelNo
       Log                           Pump.ModelVariant
       Log                           Pump.SerialNo

; Sampler specific settings
       Log                           Sampler.ModelNo
       Log                           Sampler.SerialNo
;

; Column Oven specific settings
       Log                           ColumnOven.ModelNo
; UV Detector specific settings
       Log                           UV.ModelNo
; Fluorescence detector specific settings
       Log                           Emission.ModelNo

       Pressure.LowerLimit =         2
       Pressure.UpperLimit =         300
       %A.Equate =                   "Water"
; Pump specific settings
       ; Settings specific for the Dionex P580
       %A.Type =                     Automatic
       Flow =                        0.300

       UV_VIS_1.Wavelength =         272
       UV_VIS_1.Step =               0.20
```

```
       UV_VIS_1.Average =           On

; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       3DFIELD.Step =               0.2
       UV_VIS_1.Bandwidth =         1
       UV_VIS_1.RefWavelength =     600
       UV_VIS_1.RefBandwidth =      1

; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       Sampler.InjectMode =         Normal
       Sampler.UpSpeed =            10.0
       Sampler.DownSpeed =          10.0
       Sampler.SampleHeight =       0.50
       Sampler.WashSpeed =          50.00
       Sampler.DispSpeed =          50.00
       Sampler.DrawSpeed =          25.00
       Sampler.SyringeDelay =       5


 0.000 UV.Autozero
; Detector specific settings
       Inject
       UV_VIS_1.AcqOn

 3.000 UV_VIS_1.AcqOff
       End
```

7.5 Carry-over by the Autosampler and Linearity of the Injection Volume

```
;=================================================================
; Injector Linearity and Carry over
; ----------------------------------------------------------------
; PGM-Version 22-05-2001
;
; Restriction capillary: PEEK
;                   ID: 0.13 mm
;               Length: 13 m
;        Back Pressure: 80 bars
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:         ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:         Diodearray UVD 340S
;
; Samples:                 Caffeine 10 µg/ml (Linearity)
;                          Caffeine 300 µg/ml (Carry over)
;                          Water (Carry over)
;


;==================================================================
 ; Pump specific settings
       Log                           Pump.ModelNo
       Log                           Pump.ModelVariant
       Log                           Pump.SerialNo

; Sampler specific settings
       Log                           Sampler.ModelNo
       Log                           Sampler.SerialNo
;

; Column Oven specific settings
       Log                           ColumnOven.ModelNo
; UV Detector specific settings
       Log                           UV.ModelNo
; Fluorescence detector specific settings
       Log                           Emission.ModelNo

       Pressure.LowerLimit =         10
       Pressure.UpperLimit =         300
       %A.Equate =                   "Water"
; Pump specific settings
```

```
        ; Settings specific for the Dionex P580
        %A.Type =                      Automatic
        Flow =                         1.00

        UV_VIS_1.Wavelength =          272
        UV_VIS_1.Step =                0.50
        UV_VIS_1.Average =             On

; Detector specific settings
        ; Settings specific for the Dionex UVD 340S
        3DFIELD.Step =                 0.5
        UV_VIS_1.Bandwidth =           1
        UV_VIS_1.RefWavelength =       600
        UV_VIS_1.RefBandwidth =        1

; Sampler specific settings
        ; Settings specific for the Dionex ASI-100
        Sampler.InjectMode =           Normal
        Sampler.UpSpeed =              10.0
        Sampler.DownSpeed =            10.0
        Sampler.SampleHeight =         0.50
        Sampler.WashSpeed =            50.00
        Sampler.DispSpeed =            50.00
        Sampler.DrawSpeed =            25.00
        Sampler.SyringeDelay =         5


 0.000  UV.Autozero
; Detector specific settings
        Inject
        UV_VIS_1.AcqOn


 1.250  UV_VIS_1.AcqOff
        End
```

7.6 Sample Temperature Accuracy

```
;=================================================================
; Autosampler temperature accuracy
; -------------------------------------------------------------
; PGM-Version: February 02, 2007
;
;
; HPLC-System:
; ------------
;
; Pump specific settings
; Pump:          Dionex DGP-3600A Pump (UltiMate 3000)
;
; Sampler specific settings
                ; Sampler:          Dionex WPS-3000(T)Pulledloop
Autosampler (UltiMate 3000)
;
; Detector specific settings
; Detector:         Dionex VWD-3400 UV_VIS Detector (UltiMate 3000)
;
; Sample:                 Water (Vial uncapped)
;===================================================================
; Pump specific settings
          Log                                 Pump.ModelNo

          Log                                 Pump.ModelVariant

          Log                                 Pump.SerialNo




; Sampler specific settings
          Log                                 Sampler.ModelNo

          Log                                 Sampler.SerialNo

          Log                                 Sampler.ModelVariant

          Log                                 Sampler.Temperature

          Log                                 Sampler.LoopVolume

; Column Oven specific settings
          Log                                 ColumnOven.ModelNo

          Log                                 ColumnOven.SerialNo

; UV Detector specific settings
          Log                                 UV.ModelNo

          Log                                 UV.SerialNo

; Fluorescence detector specific settings

; RI Detector specific settings

; ELS Detector specific settings
```

```
          Sampler.AcquireExclusiveAccess
          Pump.Pressure.LowerLimit =        0 [bar]
          Pump.Pressure.UpperLimit =        300 [bar]

; Virtual channel settings
          VirtualChannels_01.SamplingStep =  1.00
          TemperatureOVEN.Type =            Fixed
          TemperatureOVEN.Formula           Formula=Temperature_1

; Sampler settings
          Sampler.TempCtrl =                On
          Sampler.Temperature.Nominal =     10.0 [°C]
          Sampler.ReadyTempDelta =          0.5 [°C]

 0.000
Message"Make sure that the thermometer is located correctly in the
vial (refer to the manual for details)."
Message"Caution: To avoid damage to the system, do not perform
sampler commands while the test is running."
          Wait Sampler.Ready
          Wait Sampler.TemperatureReady
          Sampler.Inject
          TemperatureOven.AcqOn
 30.000   Message"The test is now complete. Please remove the
          thermometer."
          log Sampler.Temperature.Value
          TemperatureOven.AcqOff

          Sampler.ReleaseExclusiveAccess

          End
```

7.7 Solvent Composition of a Gradient Pump: Accuracy, Precision, and Ripple (Standard Gradient)

```
;=================================================================
; Low Pressure Gradient Pump
; --------------------------
; PGM-Version 22-05-2001
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;         Back Pressure: 80 bar
;
; Solvent A : Water (HPLC quality)
; Solvent B : Water + 0.1 % Acetone (both HPLC quality)
;
; Solvents degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       ; Sampler:          ASI-100 (T)
;
; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       ; Detector:          Diodearray UVD 340S
;
;===================================================================
; Pump specific settings
       Log                           Pump.ModelNo
       Log                           Pump.ModelVariant
       Log                           Pump.SerialNo

; Sampler specific settings
       Log                           Sampler.ModelNo
       Log                           Sampler.SerialNo
;

; Column Oven specific settings
       Log                           ColumnOven.ModelNo
; UV Detector specific settings
       Log                           UV.ModelNo
; Fluorescence detector specific settings
       Log                           Emission.ModelNo
-2.000 Pressure.LowerLimit =         10
       Pressure.UpperLimit =         300
       %A.Equate =                   "Water"
       %B.Equate =                   "Water with 0.1 % Acetone"

; Pump specific settings
       ; Settings specific for the Dionex P580
       %A.Type =                     Automatic
       %B.Type =                     Automatic
```

```
       UV_VIS_1.Step =               4.0
       UV_VIS_1.Average =            On
       UV_VIS_1.Wavelength =         265

; Detector specific settings
       ; Settings specific for the Dionex UVD 340S
       3DFIELD.Step =                4.0
       UV_VIS_1.Bandwidth =          1
       UV_VIS_1.RefWavelength =      600
       UV_VIS_1.RefBandwidth =       1

0.000  UV.Autozero
; Detector specific settings
       Inject
       UV_VIS_1.AcqOn
       Flow =                        2.000
       %B =                          0.0
       %C =                          0.0
       %D =                          0.0

 1.000 %B =                          0.0
       %B =                          1.0

 4.000 %B =                          1.0
       %B =                          50.0

 8.000 %B =                          50.0
       %B =                          99.0

12.000 %B =                          99.0
       %B =                          100.0

15.000 %B =                          100.0
       %B =                          0.0

24.000 UV_VIS_1.AcqOff
       Flow =                        2.000
       %B =                          0.0
       %C =                          0.0
       %D =                          0.0

       End
```

7.8 Temperature Accuracy of a Column Compartment (Automatic Temperature Measurement)

```
;=================================================================
; Temperature Accuracy of Column Oven
; -----------------------------------------------------------------
; PGM-Version: 09-02-2000
;
; This program file is used to determine the temperature accuracy
; of the column. The temperature is measured with an external ther-
; mometer. The values are recorded automatically with as analog
; signals.
;
;
; HPLC-System:
; ------------
;
; Settings specific for the Dionex TCC-3200
; Column Thermostat:   TCC-3200 (UltiMate 3000)
;
;===================================================================
; Pump specific settings
       Log                          Pump.ModelNo
       Log                          Pump.ModelVariant
       Log                          Pump.SerialNo

; Sampler specific settings
       Log                          Sampler.ModelNo
       Log                          Sampler.SerialNo
;

; Column Oven specific settings
       Log                          ColumnOven.ModelNo
; UV Detector specific settings
       Log                          UV.ModelNo
; Fluorescence detector specific settings
       Log                          Emission.ModelNo
-35.000ColumnOven.Temperature =     10.00

 0.000 Inject
       ColumnOven.Temperature =     10.00
       TemperatureOVEN.Step =       1.00
       TemperatureOVEN.Average =    On
       TemperatureOVEN.AcqOn
 9.900 ColumnOven.Temperature =     10.00


10.000 ColumnOven.Temperature =     20.00

49.900 ColumnOven.Temperature =     20.00

50.000 ColumnOven.Temperature =     60.00

89.900 ColumnOven.Temperature =     60.00


90.000 ColumnOven.Temperature =     80.00

130.000ColumnOven.Temperature =     80.00
```

```
130.100 TemperatureOVEN.AcqOff
        ColumnOven.Temperature =     25.00
        End
```

7.9 Baseline Noise and Signal Height of the Fluorescence Detector

```
;=================================================================
; Noisetest for fluorescence detector
; ----------------------------------------
; PGM-Version 09-02-2000
;
; Restriction capillary: PEEK
;                   ID: 0.13 mm
;               Length: 13 m
;        Back Pressure: 90 bar
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; -----------
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Fluorescence detector specific settings
       ; Settings specific for the Dionex RF2000
       ; Detector:         Fluorescence Detector RF2000
;
;===================================================================
; Pump specific settings
       Log                           Pump.ModelNo
       Log                           Pump.ModelVariant
       Log                           Pump.SerialNo

; Sampler specific settings
       Log                           Sampler.ModelNo
       Log                           Sampler.SerialNo
;

; Column Oven specific settings
       Log                           ColumnOven.ModelNo
; UV Detector specific settings
       Log                           UV.ModelNo
; Fluorescence detector specific settings
       Log                           Emission.ModelNo

       Pressure.LowerLimit =         10
       Pressure.UpperLimit =         300
       %A.Equate =                   "Water"
; Pump specific settings
       ; Settings specific for the Dionex P580
       %A.Type =                     Automatic

       Flow =                        1.000

-1.00  Emission.ExWavelength =       350
       Emission.EmWavelength =       450
       Emission.Gain =               4.0
       Emission.Response =           1.5
       Emission.Step =               1
       Emission.Average =            Off
; Detector specific settings
```

```
       ; Settings specific for the Dionex RF2000
       Emission.Sensitivity =       Med

-0.50  Emission.Autozero

0.000  Emission.AcqOn
       Inject
1.000  Emission.EmWavelength =      394

17.000 Emission.AcqOff
       End
```

7.10 Wavelength Accuracy of the Fluorescence Detector

```
;================================================================
; Wavelength accuracy for fluorescence detector
; ---------------------------------------------------------------
; PGM-Version 09-02-2000
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;         Back Pressure: 90 bar
;
; Solvent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; -----------
; Pump specific settings
       ; Settings specific for the Dionex P580
       ; Pump:          P580
;
; Fluorescence detector specific settings
       ; Settings specific for the Dionex RF2000
       ; Detector:          Fluorescence Detector RF2000
;
;==================================================================
; Pump specific settings
       Log                          Pump.ModelNo
       Log                          Pump.ModelVariant
       Log                          Pump.SerialNo

; Sampler specific settings
       Log                          Sampler.ModelNo
       Log                          Sampler.SerialNo
;

; Column Oven specific settings
       Log                          ColumnOven.ModelNo
; UV Detector specific settings
       Log                          UV.ModelNo
; Fluorescence detector specific settings
       Log                          Emission.ModelNo

       Pressure.LowerLimit =        10
       Pressure.UpperLimit =        300
       %A.Equate =                  "Water"
; Pump specific settings
       ; Settings specific for the Dionex P580
       %A.Type =                    Automatic

       Flow =                       1.000

-2.00  Emission.ExWavelength =      350
       Emission.EmWavelength =      450
       Emission.Gain =              4.0
       Emission.Response =          1.5
       Emission.Step =              1
       Emission.Average =           Off
```

```
; Detector specific settings
       ; Settings specific for the Dionex RF2000
       Emission.Sensitivity =       Med


-1.0   Emission.Autozero
-0.5   Emission.EmWavelength =      380


0.000  Inject

       Emission.AcqOn
0.250  Emission.EmWavelength =      381
0.500  Emission.EmWavelength =      382
0.750  Emission.EmWavelength =      383
1.000  Emission.EmWavelength =      384
1.250  Emission.EmWavelength =      385
1.500  Emission.EmWavelength =      386
1.750  Emission.EmWavelength =      387
2.000  Emission.EmWavelength =      388
2.250  Emission.EmWavelength =      389
2.500  Emission.EmWavelength =      390
2.750  Emission.EmWavelength =      391
3.000  Emission.EmWavelength =      392
3.250  Emission.EmWavelength =      393
3.500  Emission.EmWavelength =      394
3.750  Emission.EmWavelength =      395
4.000  Emission.EmWavelength =      396
4.250  Emission.EmWavelength =      397
4.500  Emission.EmWavelength =      398
4.750  Emission.EmWavelength =      399
5.000  Emission.EmWavelength =      400
5.250  Emission.EmWavelength =      401
5.500  Emission.EmWavelength =      402
5.750  Emission.EmWavelength =      403
6.000  Emission.EmWavelength =      404
6.250  Emission.EmWavelength =      405
6.500  Emission.EmWavelength =      406
6.750  Emission.EmWavelength =      407
7.000  Emission.EmWavelength =      408
7.250  Emission.EmWavelength =      409
7.500  Emission.EmWavelength =      410
8.000  Emission.AcqOff
       End
```

7.11 Baseline Noise and Drift of the RI Detector

```
;====================================================================
; Noise and Drift for RI Detectors
; -------------------------------------------------------------------
; PGM-Version: December 20, 2002
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;         Back Pressure: 90 bar
;
; Eluent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
;
;
; HPLC-System:
; ------------
;
; Pump specific settings
; Pump:          Dionex P580 Pump
;
; Sampler specific settings
; Sampler:          Dionex ASI-100(T) Autosampler
;
; Detector specific settings
; Detector:          Dionex UVD 340S PDA
;
; Detector specific settings
; Detector:          Shodex RI-101
;
;====================================================================
; Pump specific settings
       Log                             Pump.ModelNo

       Log                             Pump.ModelVariant

       Log                             Pump.SerialNo

; Sampler specific settings
       Log                             Sampler.ModelNo

       Log                             Sampler.SerialNo
;

; Column Oven specific settings

; RI Detector specific settings
       Log                             RI.ModelNo

; Pump specific settings
       Pressure.LowerLimit =           10[bar]
       Pressure.UpperLimit =           300[bar]
       %A.Equate =                     "Water"
       Flow =                          1.000
; Pump specific settings
; Settings specific for the Dionex P580
       %A.Type =                       Automatic
```

```
        RI_1.Step =                  1.50
        RI_1.Average =               On

; Detector specific settings
        ; Settings specific for the Shodex RI-101
        RI.Temperature.Nominal =     35
        Purge =                      off
        Rise_Time =                  0.50
        Polarity =                   Minus
        Baseline_Shift =             0

; Sampler specific settings
        ; Settings specific for the Dionex ASI-100
        Sampler.InjectMode =         Normal
        Sampler.UpSpeed =            10.0
        Sampler.DownSpeed =          10.0
        Sampler.SampleHeight =       0.50
        Sampler.WashSpeed =          50.00
        Sampler.DispSpeed =          50.00
        Sampler.DrawSpeed =          25.00
        Sampler.SyringeDelay =       5


-40.000
; Detector specific settings
        RI.Autozero
; Settings specific for complete systems
        Wait Sampler.Ready
; Column Oven specific settings
; Pump specific settings
        Wait RI.Ready
         Purge =                     on
-39.500 Purge =                      off
-39.000 Purge =                      On
-38.500 Purge =                      Off
-38.000 Purge =                      On
-20.000 Purge =                      Off
-3.000  RI.Autozero


0.000
; Pump specific settings

        Inject
        RI_1.AcqOn

20.000  RI_1.AcqOff
        End
```

7.12 Linearity of the RI Detector

```
;=================================================================
; RI Detector Linearity
; -----------------------------------------------------------------
; PGM-Version December 20, 2002
;
; Restriction capillary: PEEK
;                    ID: 0.13 mm
;                Length: 13 m
;        Back Pressure: 80 bar
;
; Eluent A : Water (HPLC quality)
;
; Solvent degassed via online degasser
;
; HPLC-System:
; ------------
;
; Pump specific settings
; Pump:         Dionex P580 Pump
;
; Sampler specific settings
; Sampler:         Dionex ASI-100(T) Autosampler
;
; Detector specific settings
; Detector:         Dionex UVD 340S PDA

; Detector specific settings
; Detector:         Shodex RI-101
;
; Samples:              Glycerine 5 mg/ml, 10 mg/ml, 15 mg/ml, 25
mg/ml, 35 mg/ml
;
;===================================================================
; Pump specific settings
       Log                             Pump.ModelNo

       Log                             Pump.ModelVariant

       Log                             Pump.SerialNo

; Sampler specific settings
       Log                             Sampler.ModelNo

       Log                             Sampler.SerialNo
;

; Column Oven specific settings

; UV Detector specific settings
       Log                             UV.ModelNo

; Fluorescence detector specific settings

; RI Detector specific settings
       Log                             RI.ModelNo
```

```
       Pressure.LowerLimit =        10[bar]
       Pressure.UpperLimit =        300[bar]
       %A.Equate =                  "Water"

; Pump specific settings
; Settings specific for the Dionex P580
       %A.Type =                    Automatic

       Flow =                       1.000

       RI_1.Step =                  0.2
       RI_1.Average =               On


; Detector specific settings
; Settings specific for the Shodex RI-101
       RI.Temperature.Nominal =     35
       Purge =                      off
       Recorder_Range =             512.00
       Integrator_Range =           500
       Rise_Time =                  0.50
       Polarity =                   plus
       Baseline_Shift =             0

; Sampler specific settings
       ; Settings specific for the Dionex ASI-100
       Sampler.InjectMode =         Normal
       Sampler.UpSpeed =            10.0
       Sampler.DownSpeed =          10.0
       Sampler.SampleHeight =       0.50
       Sampler.WashSpeed =          50.00
       Sampler.DispSpeed =          50.00
       Sampler.DrawSpeed =          25.00
       Sampler.SyringeDelay =       5

 0.000
; Detector specific settings
       RI.Autozero
; Settings specific for complete systems
; Pump specific settings
       Wait Sampler.Ready
; Column Oven specific settings
       Wait RI.Ready
       Inject
       RI_1.AcqOn


 2.000 RI_1.Acqoff
     End
```

7.13 Baseline Noise of the ELS Detector

```
;=================================================================
; Noise for ELS Detectors
;-----------------------------------------------------------------
; PGM-Version: January 08, 2007
; Pressure regulator:    Pressure: 103+/-25 bar, Flow: 0.3-2 ml/min
; Eluent A :             Water (HPLC quality)

; Solvent degassed via online degasser
; HPLC-System:
; Pump specific settings
; Pump:         Dionex P680 Pump

; Sampler specific settings
; Sampler:         Dionex ASI-100(T) Autosampler

; Detector specific settings
; Detector:         not specified

; ELS-Detector specific settings
; Detector:         Polymer Laboratories ELS 2100 Detector

;=================================================================
; Pump specific settings
          Log                                  Pump.ModelNo

          Log                                  Pump.ModelVariant

          Log                                  Pump.SerialNo



; Sampler specific settings
          Log                                  Sampler.ModelNo

          Log                                  Sampler.SerialNo

; Column Oven specific settings
; UV Detector specific settings
; Fluorescence detector specific settings
; RI Detector specific settings
; ELS Detector specific settings
          Log                                  ELSD.ModelNo


                Pump.Pressure.LowerLimit =   10 [bar]
                Pump.Pressure.UpperLimit =   300 [bar]

             Pump.%A.Equate =                "Water"

             Pump.%B =                       0.0

             Pump.%C =                       0.0

             Pump.%D =                       0.0

          ; Pump specific settings
             Pump.Flow =                     1.000
```

```
           ELS_1.Average =                   On
           ELS_1.Step =                      0.10 [s]

; Detector specific settings
           ; Settings specific for the Polymer Laboratories ELS 2100
Detector
           ELSD.Standby = NoStandby
           ELSD.LightSourceIntensity =       90 [%]
           ELSD.PMTGain =                    1.0
           ELSD.SmoothWidth =                1
           ELSD.EvaporatorTemperature =      90 [°C]
           ELSD.NebuliserTemperature =       50 [°C]
           ELSD.CarrierFlow =                1.60 [slm]

; Sampler specific settings
           ; Settings specific for the Dionex ASI-100
           Sampler.InjectMode =              Normal
           Sampler.UpSpeed =                 10.0
           Sampler.DownSpeed =               10.0
           Sampler.SampleHeight =            0.50
           Sampler.WashSpeed =               50.00
           Sampler.DispSpeed =               50.00
           Sampler.DrawSpeed =               25.00
           Sampler.SyringeDelay =            5

 0.000
; Detector specific settings
           ELSD.Autozero
; Settings specific for complete systems
; Pump specific settings
           Wait Sampler.Ready
; Column Oven specific settings
           Wait ELSD.Ready
       Sampler.Inject
           ELS_1.AcqOn
21.000     ELS_1.AcqOff

           End
```

8 Example Report

For an OQ example report, refer to the following pages.
The report was generated for the following system configuration:

P580 LPG
ASI-100
UVD 340S
STH 585
RI-101
RF 2000

DIONEX

Operational Qualification

• *Instruments*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	ASI-100	DIONEX	1860410
Column Oven	TCC-100	DIONEX	1850409
UV Detector	UVD 340U	DIONEX	1830402
Fluorescence Detector	Other	DIONEX	not available
RI Detector	Other	Shodex	not available
ELS Detector	Other	DIONEX	not available
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

• *Additional Information*

Customer:	Customer's Name
Operator:	Operator's Name
	Operator's Jobtitle
Execution Date:	Apr-14-04
Period between two Qualifications:	6 months
Next Qualification:	Oct-04

Reviewer's signature // Date

Operator's signature // Date

• *Accessories*

Accessories	***Name***	***Lot No.***	***Exp. Date***
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)		
Sample 1	Pyrene in Methanol 3 µg/ml	LA-91960	Jul-02
Sample 2	Caffeine in Water 10 µg/ml	LA-91955	Jul-02
Sample 3	Caffeine in Water 60 µg/ml	LA-91956	Jul-02
Sample 4	Caffeine in Water 140 µg/ml	LA-91957	Jul-02
Sample 5	Caffeine in Water 220 µg/ml	LA-91958	Jul-02
Sample 6	Caffeine in Water 300 µg/ml	LA-91959	Jul-02
Sample 7	Caffeine in Water 2000 µg/ml	LA-91959	Jul-02
Sample 8	Water (HPLC-Grade)		
Sample 9	Glycerine in Water 5 mg/ml		
Sample 10	Glycerine in Water 10 mg/ml		
Sample 11	Glycerine in Water 15 mg/ml		
Sample 12	Glycerine in Water 25 mg/ml		
Sample 13	Glycerine in Water 35 mg/ml		
Sample 14	Water (Uncapped Vial)		
Solvent A	Water (HPLC-Grade)		
Solvent A for Wavelength Accuracy	Methanol (HPLC-Grade)		
Solvent B for Gradient	Water + 0.1 % Acetone		
Solvent C for Gradient	Water (HPLC-Grade)		
Thermometer	SN: 43077		
Temperature Sensor	SN: 111988		

Reviewer's signature // Date

Operator's signature // Date

• *Limits*

Criterion	***Adjusted Limits***	***Limits with Optimized Conditions***
Noise (UV)	0.030 mAU	0.030 mAU
Drift (UV)	0.80 mAU/h	0.80 mAU/h
Lamp Intensity (UV)	500000 counts/s	500000 counts/s
Detector Wavelength Accuracy	+/- 0.75 nm	+/- 0.75 nm
Detector Linearity (Corr.) (UV)	99.980 %	99.980 %
Detector Linearity	5.000 % RSD	5.000 % RSD
Injector Presicision (Area)	0.300 % RSD	0.300 % RSD
Flow Presicision (Ret. Time)	0.0100 min SD	0.0100 min SD
	0.050 % RSD	0.050 % RSD
Carry Over (Area)	0.100 %	0.100 %
Injector Linearity (Corr.)	99.99000 %	99.99000 %
Injector Linearity	0.500 % RSD	0.500 % RSD
Temperature of Injector	n.a.	n.a.
Gradient Accuracy	1.000 %	1.000 %
Gradient Presicision	0.500 % STD	0.500 % STD
Pump Ripple	0.500 %	0.500 %
Temperature of Column Oven	+/- 1.0 °C	+/- 1.0 °C
Noise (RF)	0.30 mV	0.30 mV
Signal (RF) min	40.00 mV	40.00 mV
Signal (RF) max	80.00 mV	80.00 mV
Wavelength Accuracy (RF)	+/- 10 nm	+/- 10 nm
Noise (RI)	50.0 nRIU	50.0 nRIU
Drift (RI)	500.0 nRIU/h	500.0 nRIU/h
Detector Linearity (Corr.) (RI)	99.900 %	99.900 %
Noise (ELS)	0.3 mV	0.3 mV

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Wavelength Accuracy of the DAD*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	ASI-100	DIONEX	1860410
Column Oven	TCC-100	DIONEX	1850409
UV Detector	**UVD 340U**	**DIONEX**	**1830402**
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 1	Pyrene in Methanol 3 µg/ml
Solvent A for Wavelength Accuracy	Methanol (HPLC-Grade)

Additional Information

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-13-04
Next Qualification: Oct-04

• *Limits, Values and Test Results*

	Limit	*Obs. Value*	*Result*
Wavelength Accuracy at 272,1 nm	+/- 0.75 nm	-0.22 nm	**Test passed**
Wavelength Accuracy at 333,3 nm	+/- 0.75 nm	-0.02 nm	**Test passed**

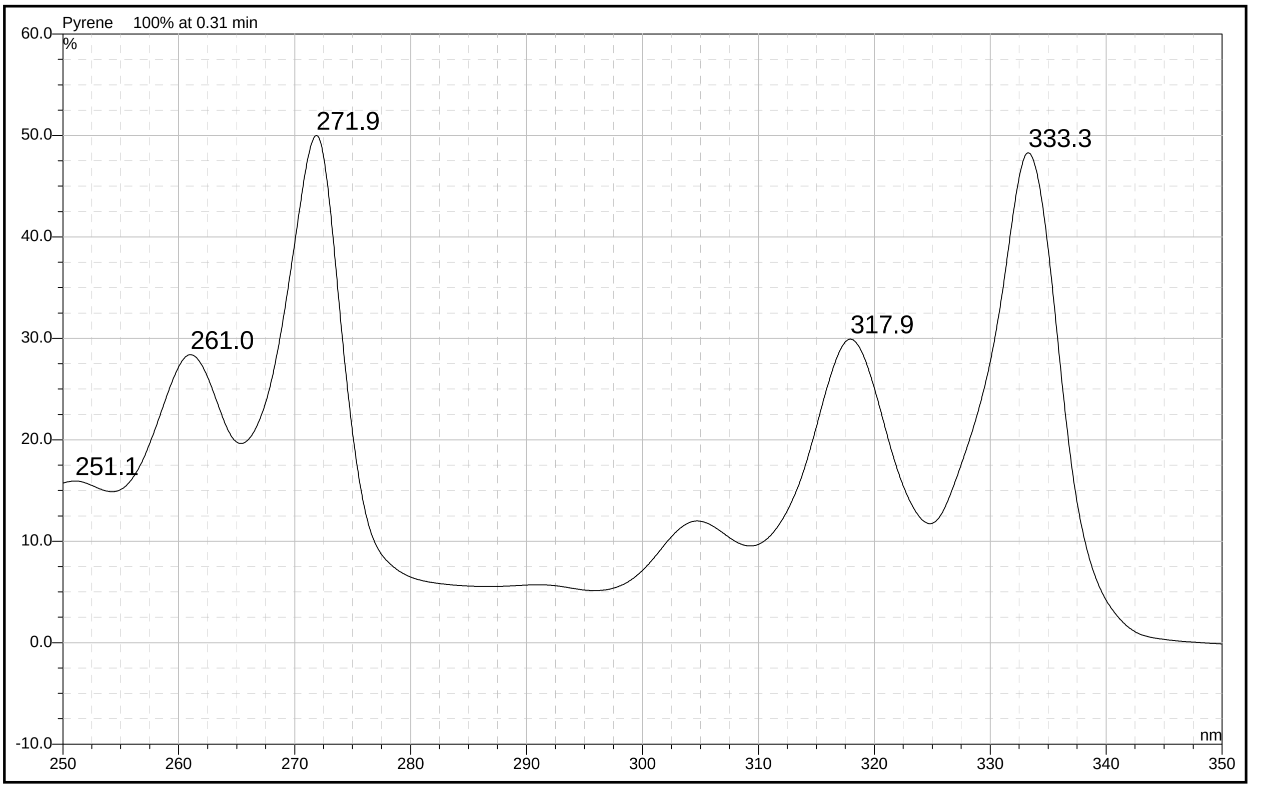
Reviewer's signature // Date

Operator's signature // Date

• *Data for the Wavelength Accuracy Test (DAD)*

Testpoint	*Observed Wavelength [nm]*	*Expected Wavelength [nm]*	*Abs.Critical Deviation[nm]*	*Calculated Deviation[nm]*	*Result*
Pyrene Maximum 1	271.88	272.10	0.75	-0.22	ok
Pyrene Maximum 2	333.28	333.30	0.75	-0.02	ok

• *Pyrene-Spectrum for the Wavelength Accuracy Test (DAD)*



Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *UV Detector Noise and Drift*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	ASI-100	DIONEX	1860410
Column Oven	TCC-100	DIONEX	1850409
UV Detector	**UVD 340U**	**DIONEX**	**1830402**
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-13-04
Next Qualification: Oct-04

• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Noise (UV)	0.030 mAU	0.021 mAU	**Test passed**
Drift (UV)	0.80 mAU/h	0.44 mAU/h	**Test passed**
Lamp Intensity (UV)	500000 counts/s	1026240 counts/s	**Test passed**

Remark: Noise and drift are measured dynamically with a floated cell. The limits are different from published specifications, because they are valid for static conditions only (empty cell).

Reviewer's signature // Date

Operator's signature // Date

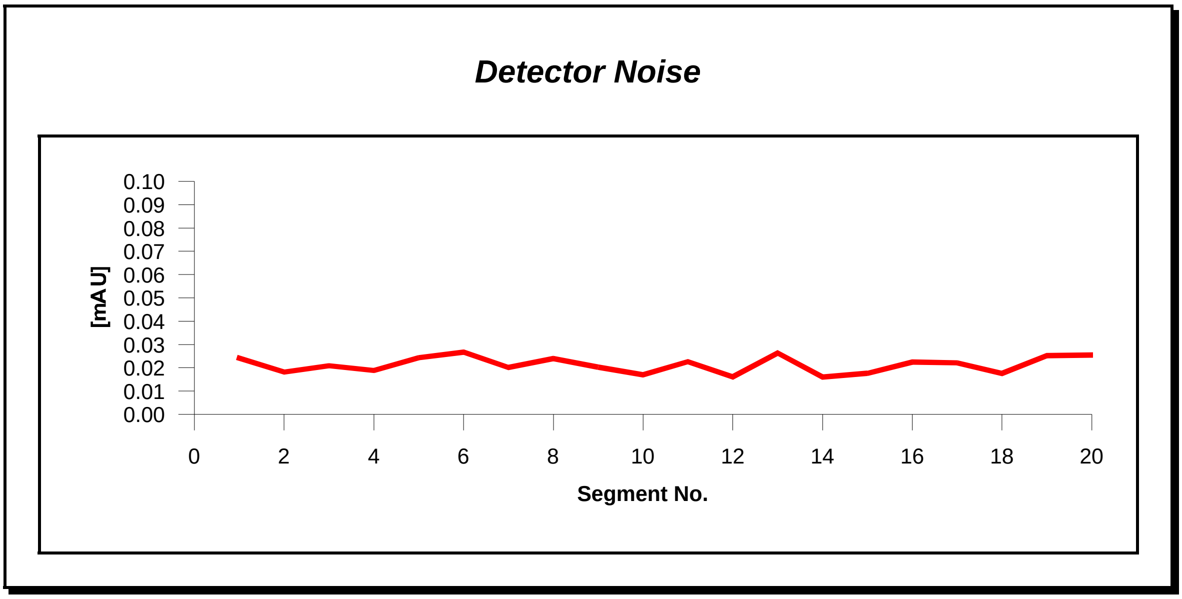
• *Data for Detector Noise Test*

Segment No.	***Noise [mAU]***
1	0.024
2	0.018
3	0.021
4	0.019
5	0.024
6	0.027
7	0.020
8	0.024
9	0.020
10	0.017
11	0.023
12	0.016
13	0.026
14	0.016
15	0.018
16	0.022
17	0.022
18	0.018
19	0.025
20	0.026
Average:	***0.021 mAU***
Limit:	***0.030 mAU***
Result:	***ok***

Reviewer's signature // Date

Operator's signature // Date

• *Chart for Noise Test*



Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Injector and Flow Reproducibility*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	**P680 LPG**	**DIONEX**	**1920401**
Autosampler	**ASI-100**	**DIONEX**	**1860410**
Column Oven	TCC-100	DIONEX	1850409
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 4	Caffeine in Water 140 µg/ml
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Injector Presicision (Area)	0.300 % RSD	0.188 % RSD	**Test passed**
Flow Presicision (Ret. Time)	0.050 % RSD 0.0100 min SD	0.104 % RSD 0.0011 min SD	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Data for Injector and Flow Reproducibility Test: Volume 5.0 µl*

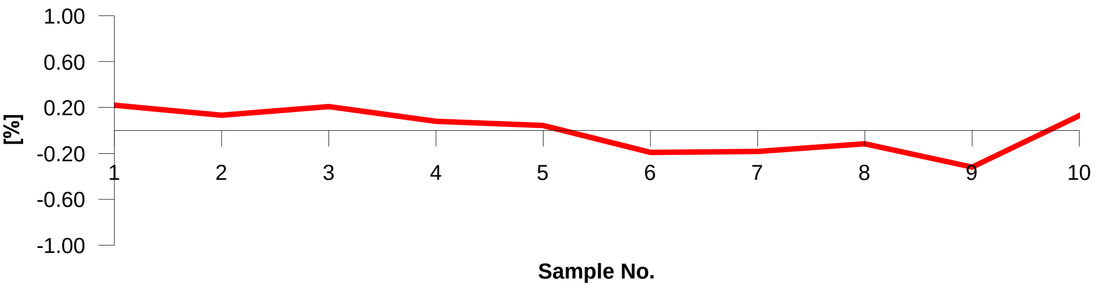
Sample Name	***Ret.Time min Caffeine UV_VIS_1***	***Area mAU*min Caffeine UV_VIS_1***
Injector and flow reproducibility_1	1.0067	93.036
Injector and flow reproducibility_2	1.0100	92.955
Injector and flow reproducibility_3	1.0100	93.025
Injector and flow reproducibility_4	1.0100	92.907
Injector and flow reproducibility_5	1.0100	92.872
Injector and flow reproducibility_6	1.0100	92.656
Injector and flow reproducibility_7	1.0100	92.663
Injector and flow reproducibility_8	1.0100	92.725
Injector and flow reproducibility_9	1.0100	92.537
Injector and flow reproducibility_10	1.0100	92.952
Average:	***1.0097***	***92.833***
RSD:	***0.104 %***	***0.188 %***
RSD Limit:	***0.050 %***	***0.300 %***
SD:	***0.0011***	
SD Limit:	***0.0100***	
Result:	***ok***	***ok***

Reviewer's signature // Date

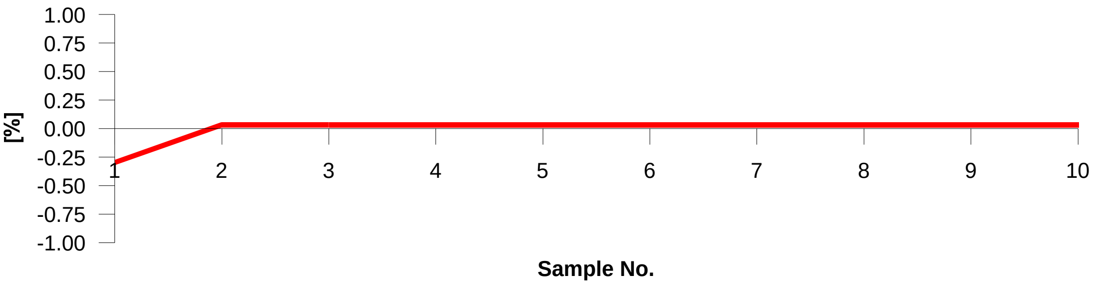
Operator's signature // Date

• *Charts for Injector and Flow Reproducibility Test*

Area % Deviation from Mean Value



Retention Time % Deviation from Mean Value



Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *UV Detector Linearity*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	ASI-100	DIONEX	1860410
Column Oven	TCC-100	DIONEX	1850409
UV Detector	**UVD 340U**	**DIONEX**	**1830402**
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 2	Caffeine in Water 10 µg/ml
Sample 3	Caffeine in Water 60 µg/ml
Sample 4	Caffeine in Water 140 µg/ml
Sample 5	Caffeine in Water 220 µg/ml
Sample 6	Caffeine in Water 300 µg/ml
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04

Next Qualification: Oct-04

• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Detector Linearity (Corr.)	99.980 %	99.999 %	**Test passed**
Detector Linearity	5.000 % RSD	0.422 % RSD	**Test passed**

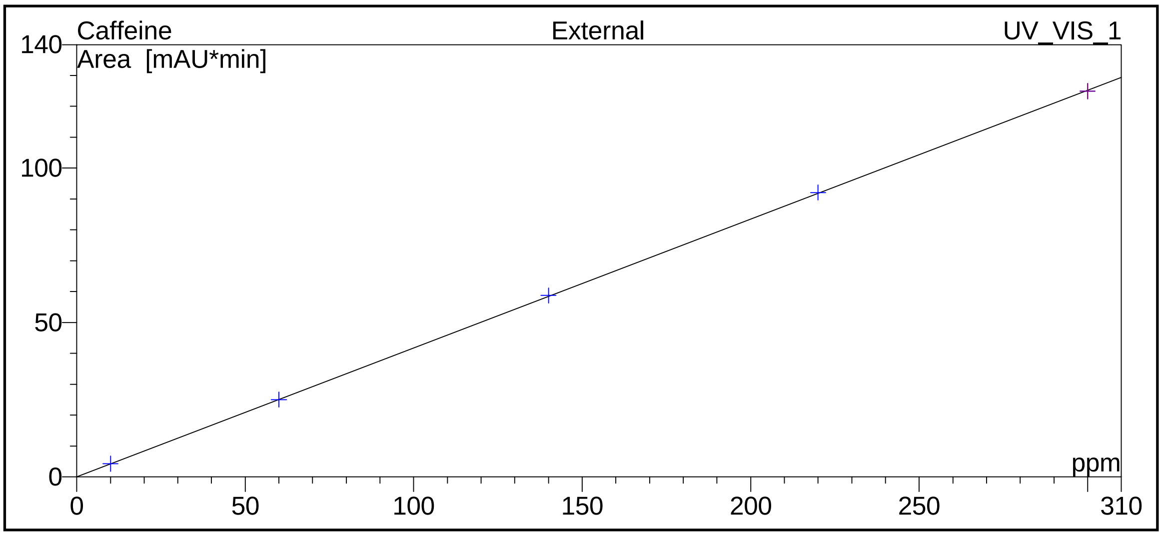
Reviewer's signature // Date

Operator's signature // Date

• *Data for Detector Linearity*

Sample Name	Amount ppm Caffeine UV_VIS_1	Area mAU*min Caffeine UV_VIS_1
Detector linearity_1	10.0	4.258
Detector linearity_2	60.0	25.036
Detector linearity_3	140.0	58.740
Detector linearity_4	220.0	92.094
Detector linearity_5	300.0	124.911

• *Calibration Curve*



Cal.Type UV_VIS_1	*Number of Points UV_VIS_1*	*Offset UV_VIS_1*	*Slope UV_VIS_1*
Lin	5	0.000	0.417

	Correlation Coefficient	*RSD*
	99.999 %	0.422 %
Limit:	***99.980 %***	***5.000 %***
Result:	***ok***	***ok***

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• RI Detector Noise and Drift

• Instruments and Fluidics

Instrument Name	***Model***	***Supplier's Name***	***Serial Number***
Pump	P680 HPG	DIONEX	1920105
Autosampler	ASI-100	DIONEX	9932012
Column Oven	TCC-100	DIONEX	Demo
RI Detector	**RI-101**	**Shodex**	**not available**
Chromeleon Datasystem	Version: 6.80 SR5 Build	DIONEX	11

Accessories	***Name***
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)

• Additional Information

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Jan-31-03
Next Qualification: Jul-03

• Limits, Values and Test Results

	Limit	***Observed Value***	***Result***
Noise (RI)	50.0 nRIU	26.6 nRIU	**Test passed**
Drift (RI)	500.0 nRIU/h	321.8 nRIU/h	**Test passed**

Remark: Noise and drift are measured dynamically with a floated cell. The limits are different from published specifications, because they are valid for static conditions only (empty cell).

Reviewer's signature // Date

Operator's signature // Date

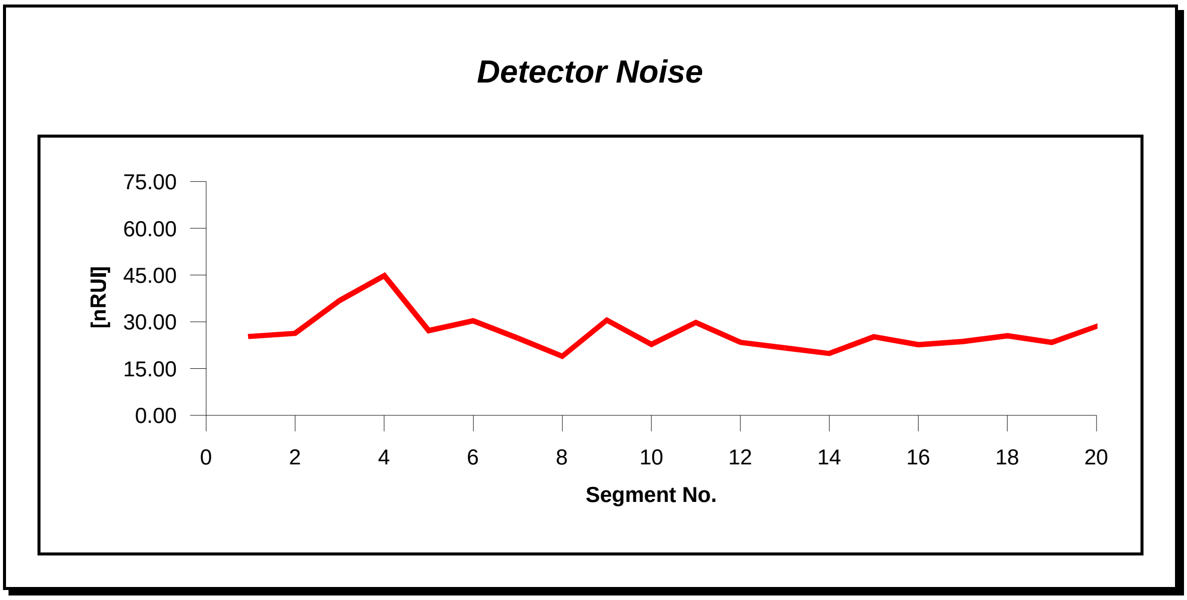
• *Data for Detector Noise and Drift Test*

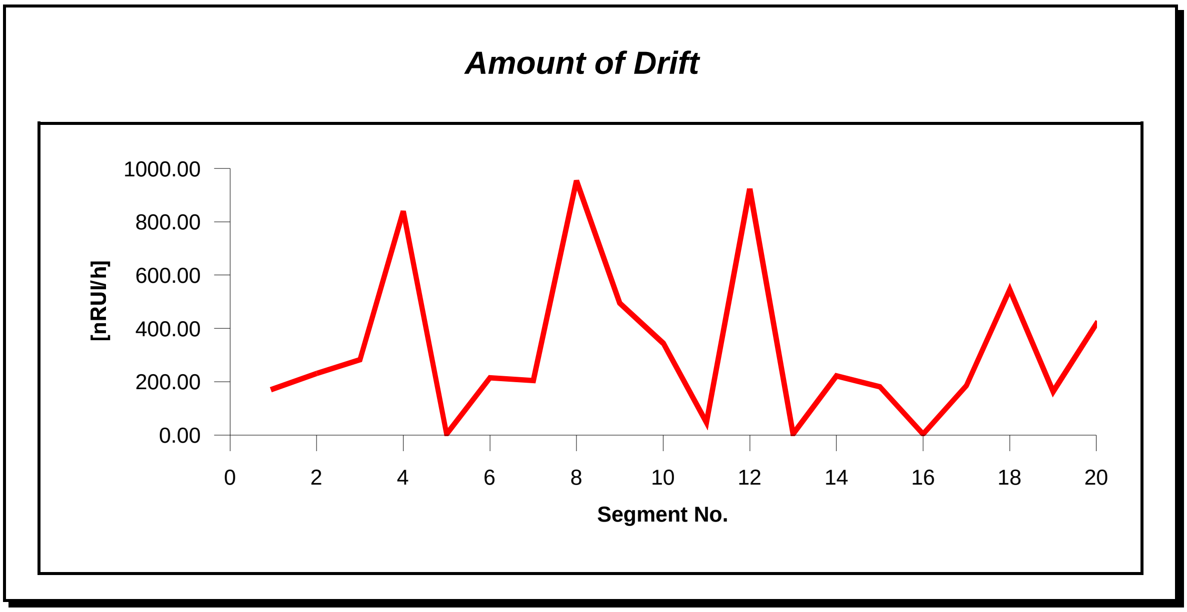
Segment No.	***Noise [nRIU]***	***Amount of Drift [nRIU/h]***
1	25.3	173.299
2	26.3	231.224
3	36.8	282.414
4	44.8	839.815
5	27.2	4.386
6	30.3	214.676
7	24.7	204.259
8	19.0	953.907
9	30.5	494.897
10	22.8	344.469
11	29.8	47.102
12	23.4	923.453
13	21.6	4.237
14	19.8	221.655
15	25.2	181.431
16	22.7	3.639
17	23.7	185.468
18	25.5	545.040
19	23.4	163.138
20	28.5	417.141
Average:	***26.6 nRIU***	***321.8 nRIU/h***
Limit:	***50.0 nRIU***	***500.0 nRIU/h***
Result:	***ok***	***ok***

Reviewer's signature // Date

Operator's signature // Date

• *Charts for Noise and Drift Test*





Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• RI Detector Linearity

• Instruments and Fluidics

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 HPG	DIONEX	1920105
Autosampler	ASI-100	DIONEX	9932012
Column Oven	TCC-100	DIONEX	Demo
RI Detector	**RI-101**	**Shodex**	**not available**
Chromeleon Datasystem	Version: 6.80 SR5 Build 24	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 9	Glycerine in Water 5 mg/ml
Sample 10	Glycerine in Water 10 mg/ml
Sample 11	Glycerine in Water 15 mg/ml
Sample 12	Glycerine in Water 25 mg/ml
Sample 13	Glycerine in Water 35 mg/ml
Solvent A	Water (HPLC-Grade)

• Additional Information

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Feb-24-03

Next Qualification: Aug-03

• Limits, Values and Test Results

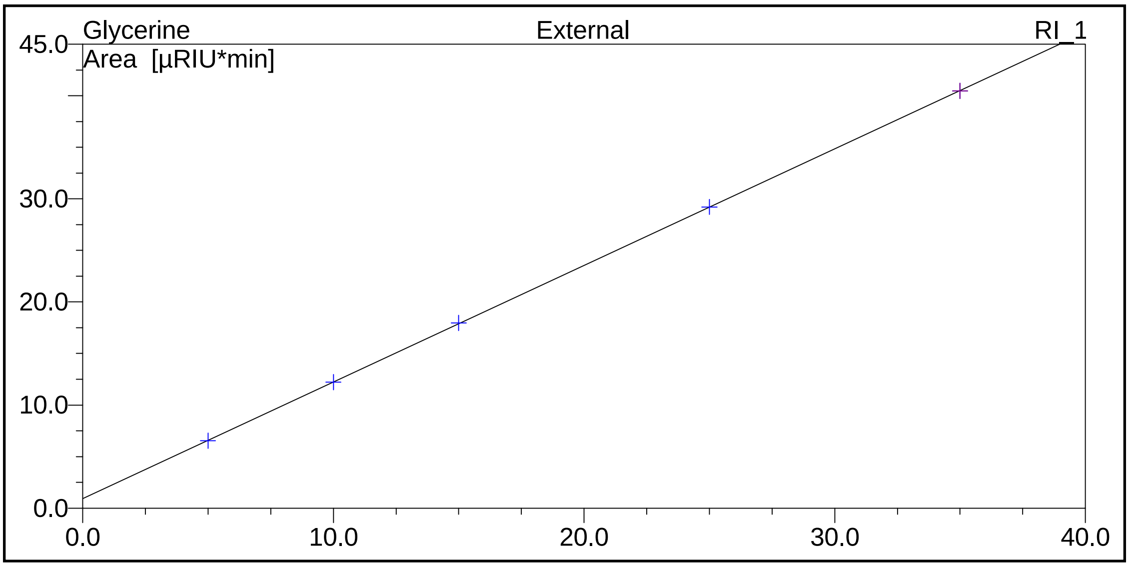
	Limit	*Observed Value*	*Result*
Detector Linearity (Corr.) (RI)	99.900 %	99.999 %	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Data for Detector Linearity*

Sample Name	Amount Glycerine RI_1	Area µRIU*min Glycerine RI_1	Height µRIU Glycerine RI_1
RI_Detector linearity_1	5.00	6.536	77.6
RI_Detector linearity_2	10.00	12.223	144.5
RI_Detector linearity_3	15.00	17.967	210.4
RI_Detector linearity_4	25.00	29.214	343.2
RI_Detector linearity_5	35.00	40.476	473.2



Cal.Type *RI*	*Number of Points* *RI*	*Offset* *RI*	*Slope* *RI*
LOff	5	0.926	1.131

	Correlation Coefficient
	99.999 %
Limit:	***99.900 %***
Result:	***ok***

Reviewer's signature // Date

Operator's signature // Date

Operational Qualification

• *Injector Linearity*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	**ASI-100**	**DIONEX**	**1860410**
Column Oven	TCC-100	DIONEX	1850409
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 2	Caffeine in Water 10 µg/ml
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

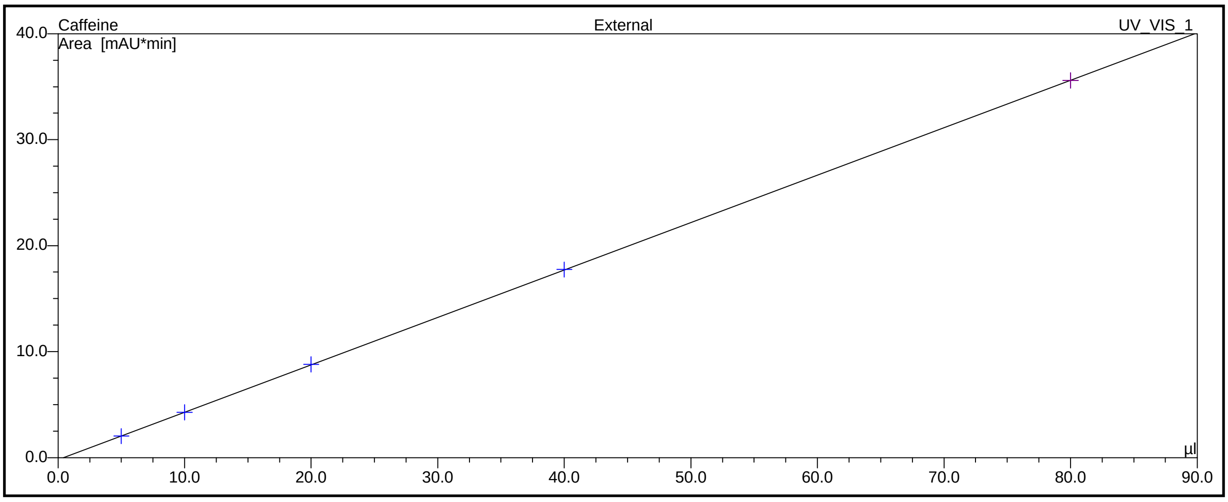
• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Injector Linearity (Corr.)	99.99000 %	99.99979 %	**Test passed**
Injector Linearity	0.500 % RSD	0.238 % RSD	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Calibration Curve*



• *Data for Injector Linearity Test*

Sample Name	***Ret.Time min Caffeine UV_VIS_1***	***Inj.Vol. µl***	***Area mAU*min Caffeine UV_VIS_1***
Injector linearity_1	0.31	5.0	2.029
Injector linearity_2	0.31	10.0	4.274
Injector linearity_3	0.32	20.0	8.795
Injector linearity_4	0.33	40.0	17.746
Injector linearity_5	0.34	80.0	35.591

Cal.Type UV_VIS_1	***Number of Points UV_VIS_1***	***Offset UV_VIS_1***	***Slope UV_VIS_1***
LOff	5	-0.184	0.447

	Correlation Coefficient	***RSD***
	99.99979 %	0.238 %
Limit:	***99.99000 %***	***0.500 %***
Result:	***ok***	***ok***

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Injector Carry Over*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	LPG-3400M	DIONEX	1340410
Autosampler	**WPS-3000PL**	**DIONEX**	**7**
Column Oven	TCC-3200	DIONEX	Demo
UV Detector	UVD 340U	DIONEX	4310409
Chromeleon Datasystem	V. 6.80 SP2 Build 2258 (Be	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Sample 2	Caffeine in Water 10 µg/ml
Sample 7	Caffeine in Water 2000 µg/ml
Sample 8	Water (HPLC-Grade)
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Jan-26-07
Next Qualification: Jul-07

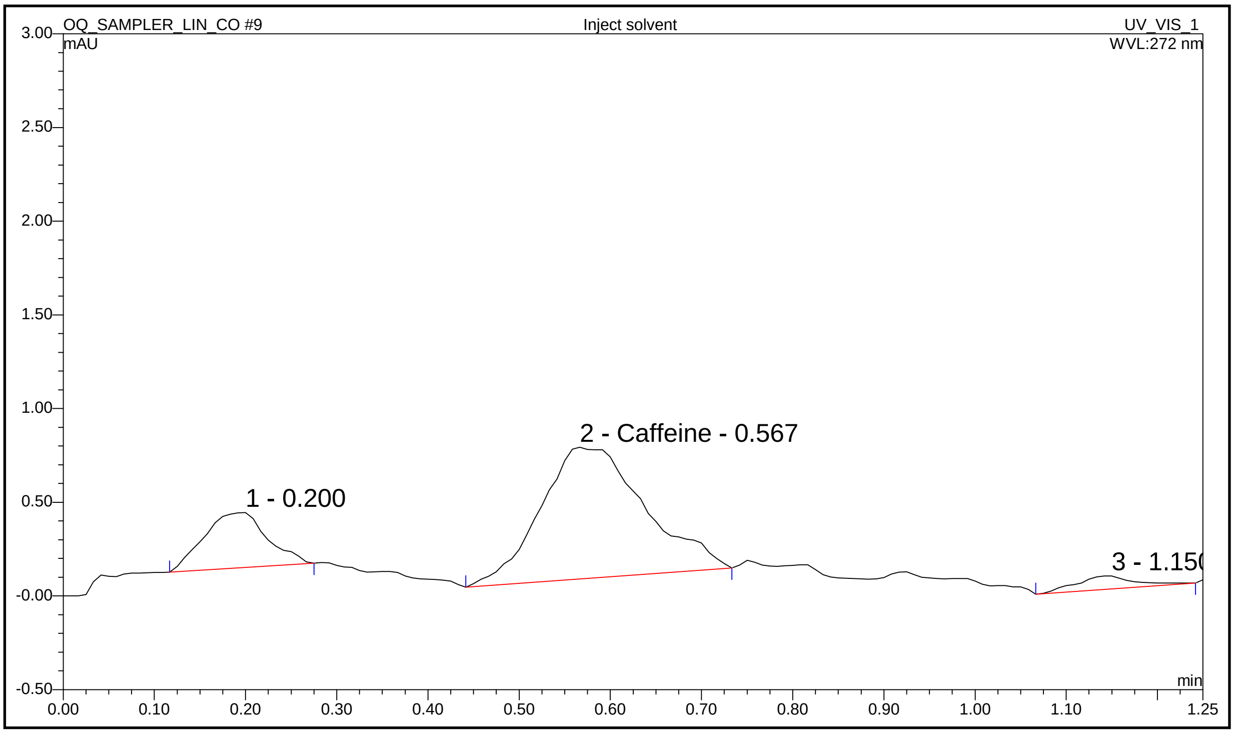
• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Carry Over (Area)	0.050 %	no data	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Chromatogram for Carry Over Test*



• *Data for Carry Over Test*

Sample Name	***Ret.Time min Caffeine UV_VIS_1***	***Area mAU*min Caffeine UV_VIS_1***
Inject solvent_Reference	0.58	0.132
Carry over_Reference	0.55	4.842
Carry over	0.53	447.221
Inject solvent	0.57	0.093
Carry over:		***no data***
Limit:		***0.050 %***
Result:		***ok***

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Fluorescence Detector Noise*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P580 LPG	DIONEX	9730 028
Autosampler	ASI-100	DIONEX	9932012
Column Oven	STH 585	DIONEX	not available
Fluorescence Detector	**RF2000**	**DIONEX**	**not available**
Chromeleon Datasystem	Version: 6.80 SR5 Build 2	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: May-30-01
Next Qualification: Nov-01

• *Limits, Values and Test Results*

	Limit	*Observed Value*	*Result*
Noise (RF)	0.30 mV	0.10 mV	**Test passed**
Signal (RF) min	40.00 mV	42.97 mV	**Test passed**
Signal (RF) max	80.00 mV	43.10 mV	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

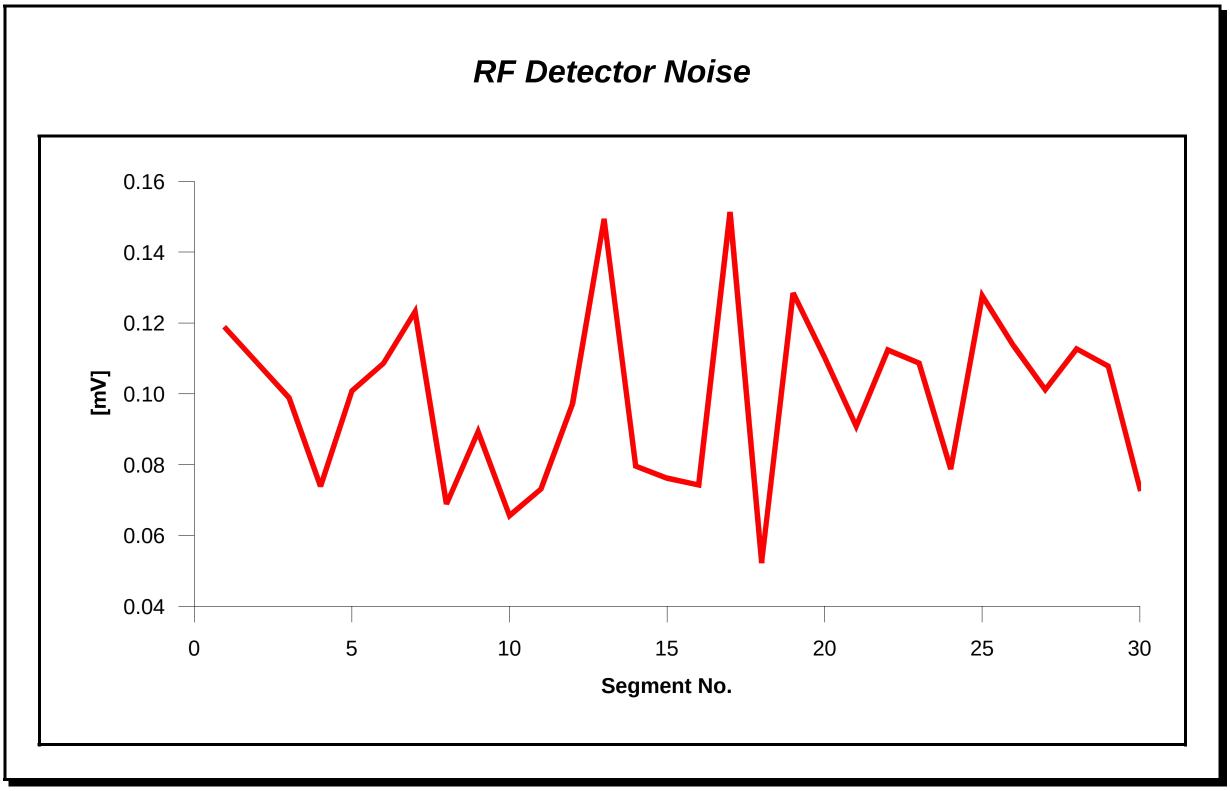
• *Data for RF Detector Noise*

Segment No.	***Noise [mV]***
1	0.118
2	0.109
3	0.099
4	0.074
5	0.101
6	0.109
7	0.123
8	0.069
9	0.089
10	0.066
11	0.073
12	0.097
13	0.149
14	0.080
15	0.076
16	0.074
17	0.151
18	0.052
19	0.129
20	0.110
21	0.091
22	0.112
23	0.109
24	0.079
25	0.128
26	0.113
27	0.101
28	0.113
29	0.108
30	0.073
Average:	***0.10 mV***
Limit:	***0.30 mV***
Result:	***ok***

Reviewer's signature // Date

Operator's signature // Date

• *Charts for RF Detector Noise Test*



Reviewer's signature // Date

Operator's signature // Date

Operational Qualification

• *Fluorescence Detector Wavelength Accuracy*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P580 LPG	DIONEX	9730 028
Autosampler	ASI-100	DIONEX	9932012
Column Oven	STH 585	DIONEX	not available
Fluorescence Detector	**RF2000**	**DIONEX**	**not available**
Chromeleon Datasystem	Version: 6.80 SR5 Build 2	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: May-30-01
Next Qualification: Nov-01

• *Limits, Values and Test Results*

	Limit	*Obs. Deviation*	*Result*
Wavelength Accuracy (RF)	+/- 10 nm	0 nm	**Test passed**

Reviewer's signature // Date

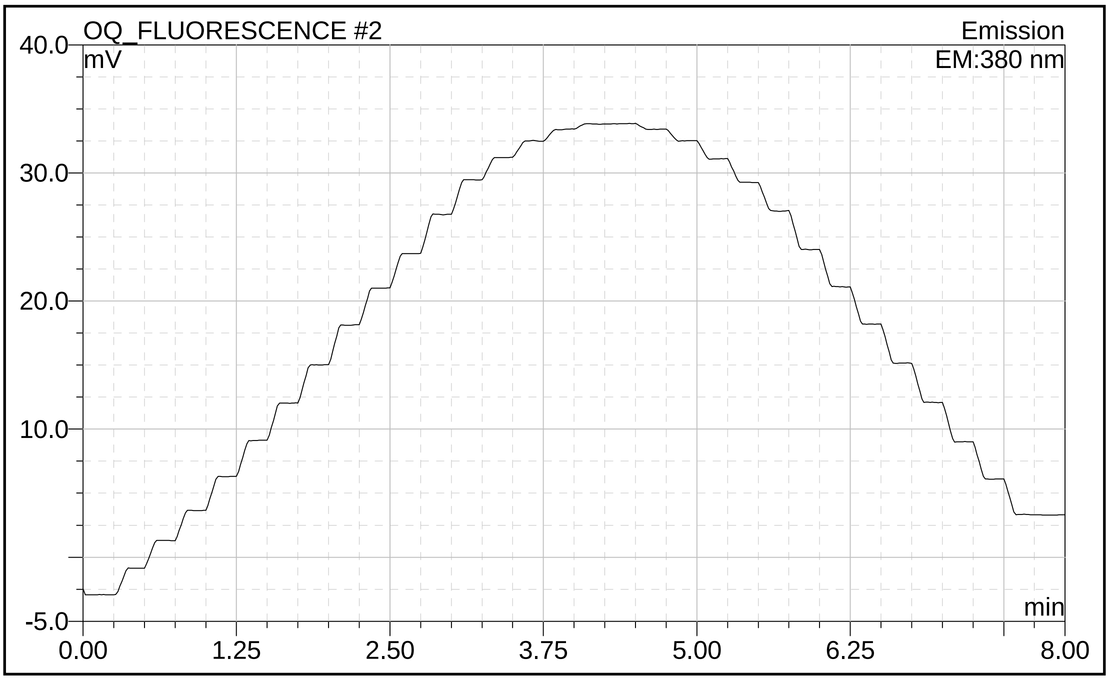
Operator's signature // Date

• *Data for the Wavelength Accuracy Test (Fluorescence Detector)*

Obs. Wavelength	*Exp. Wavelength*	*Deviation*	*Limit*
397 nm	397 nm	0 nm	+/- 10 nm

• *Chromatogram of Wavelength Accuracy Test (Fluorescence Detector)*

The emission wavelength is changed from 380 nm to 410 nm in steps of 1 nm per 15 sec. The maximum of the emission spectrum is determinated as maximum of the signal of this chromatogram.



Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Step Accuracy of the STD Gradient_1*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	**P680 LPG**	**DIONEX**	**1920401**
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)
Solvent B for Gradient	Water + 0.1 % Acetone

• *Additional Information*

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

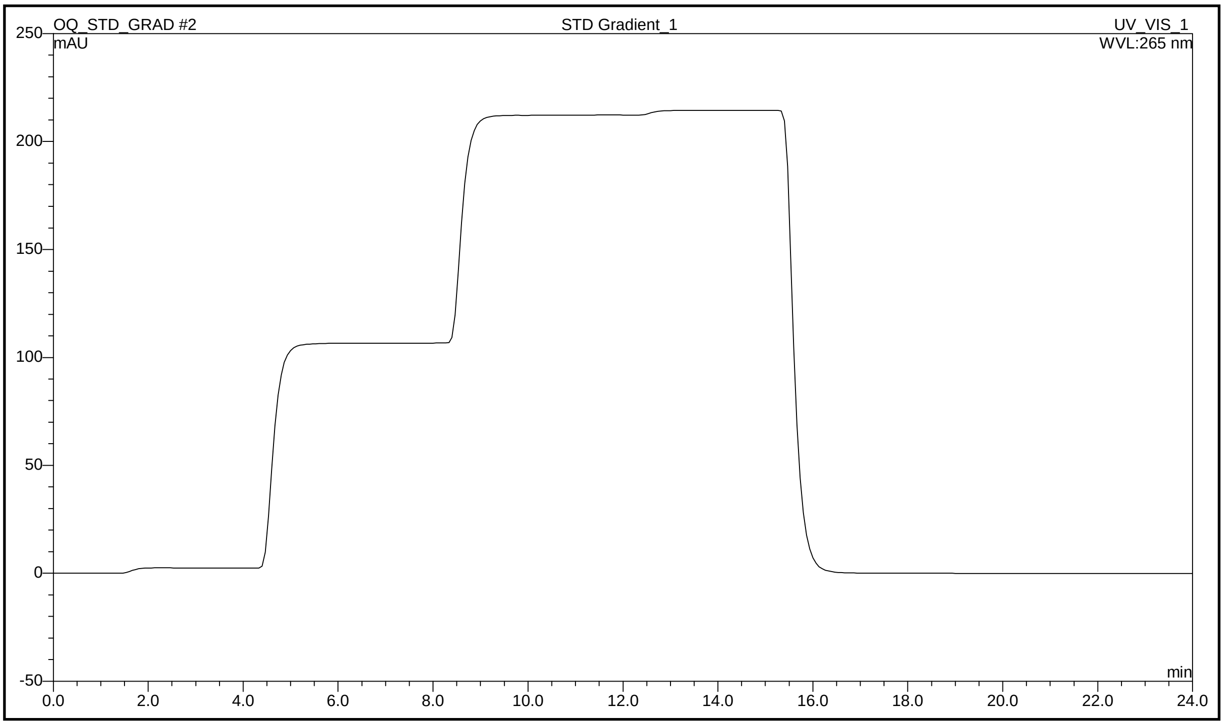
• *Limits and Test Results*

	Limit	*Observed max. Deviation*	*Result of all Steps*
Step Accuracy	1.000 %	0.275 %	**Test passed**
Step Ripple	0.500 %	0.025 %	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Chromatogram of STD Gradient_1*



Flow [ml/min]: **2.000**

Reviewer's signature // Date

Operator's signature // Date

• Data of STD Gradient_1

Observed Value [mAU]	Expected Value [%]	Calculated Value [%]	Abs. Critical Deviation [%]	Calculated Deviation [%]	Result
0.00	0.00	0.000	1.000	0.000	ok
2.40	1.00	1.118	1.000	0.118	ok
106.65	50.00	49.725	1.000	-0.275	ok
212.37	99.00	99.017	1.000	0.017	ok
214.48	100.00	100.000	1.000	0.000	ok

• Ripple of STD Gradient_1

Step [%]	Ripple [mAU]	Calculated Ripple [%]	Critical Ripple [%]	Result
1.00	0.007	0.003	0.500	ok
50.00	0.018	0.008	0.500	ok
99.00	0.053	0.025	0.500	ok

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Step Accuracy of the STD Gradient_2*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	**P680 LPG**	**DIONEX**	**1920401**
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)
Solvent B for Gradient	Water + 0.1 % Acetone

• *Additional Information*

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

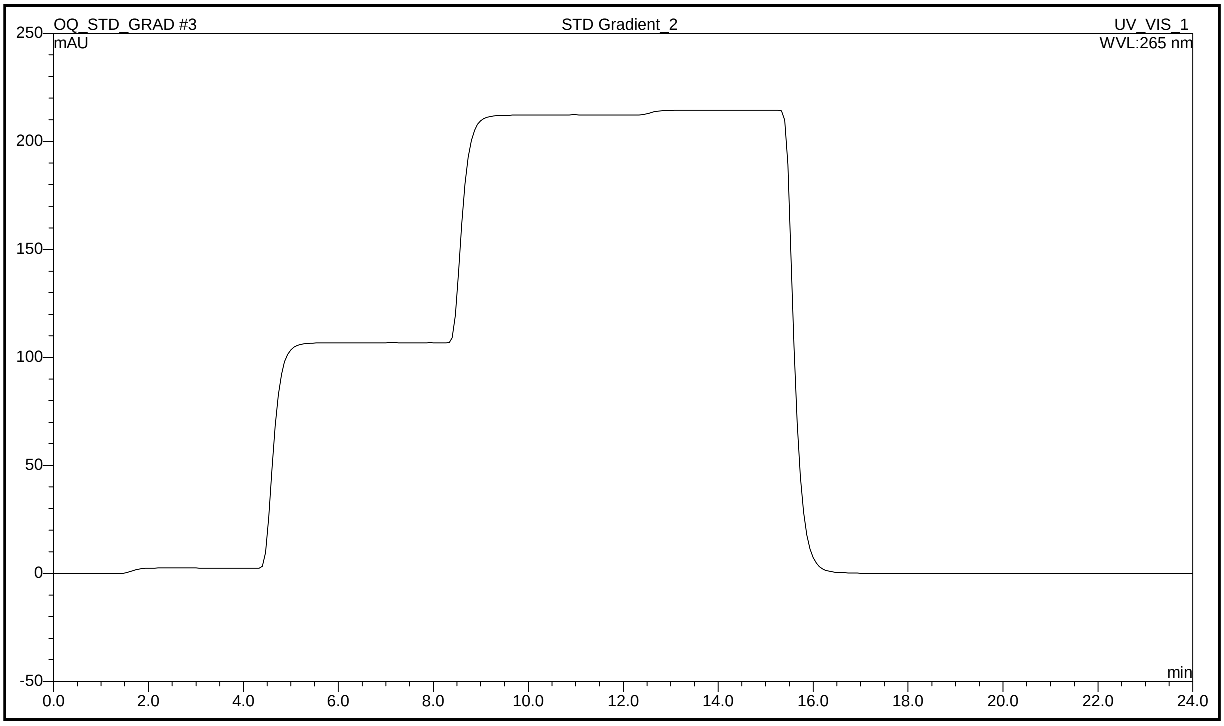
• *Limits and Test Results*

	Limit	*Observed max. Deviation*	*Result of all Steps*
Step Accuracy	1.000 %	0.212 %	**Test passed**
Step Ripple	0.500 %	0.017 %	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Chromatogram of STD Gradient_2*



Flow [ml/min]: **2.000**

Reviewer's signature // Date

Operator's signature // Date

• Data of STD Gradient_2

Observed Value [mAU]	Expected Value [%]	Calculated Value [%]	Abs. Critical Deviation [%]	Calculated Deviation [%]	Result
0.00	0.00	0.000	1.000	0.000	ok
2.47	1.00	1.150	1.000	0.150	ok
106.78	50.00	49.788	1.000	-0.212	ok
212.30	99.00	98.991	1.000	-0.009	ok
214.46	100.00	100.000	1.000	0.000	ok

• Ripple of STD Gradient_2

Step [%]	Ripple [mAU]	Calculated Ripple [%]	Critical Ripple [%]	Result
1.00	0.033	0.015	0.500	ok
50.00	0.036	0.017	0.500	ok
99.00	0.012	0.006	0.500	ok

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Step Accuracy of the STD Gradient_3*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	**P680 LPG**	**DIONEX**	**1920401**
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)
Solvent B for Gradient	Water + 0.1 % Acetone

• *Additional Information*

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

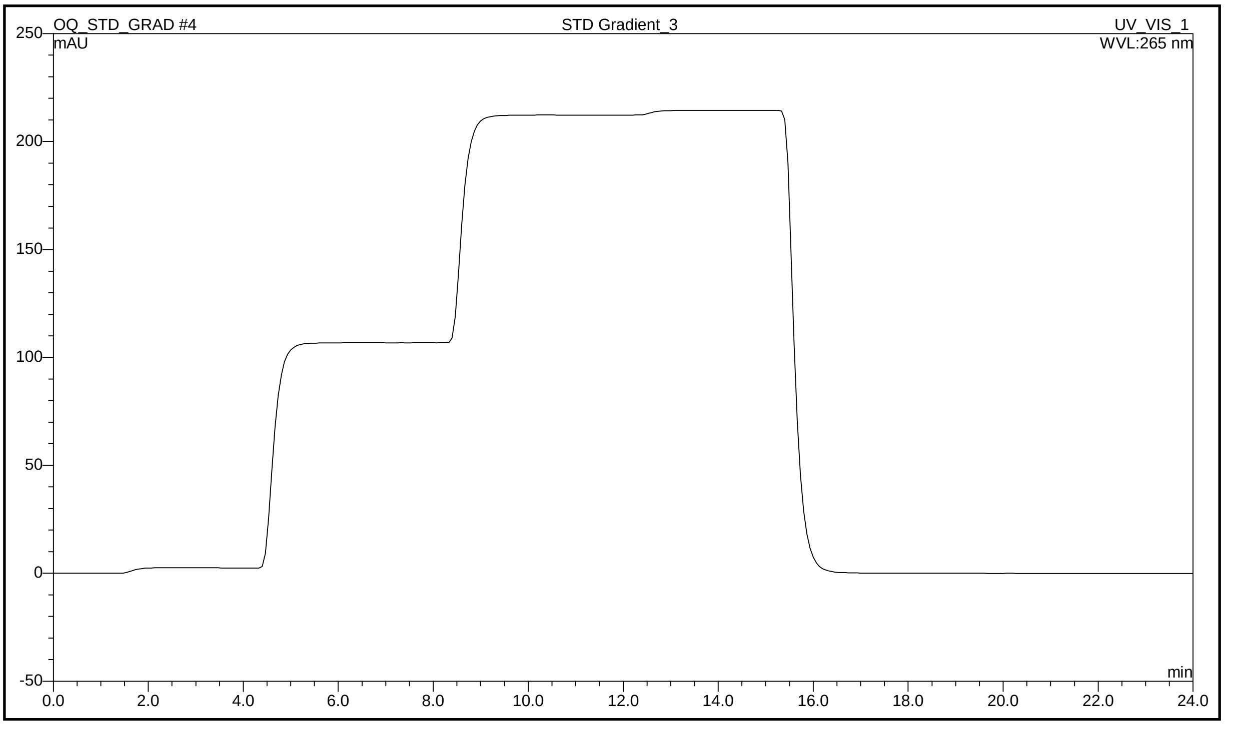
• *Limits and Test Results*

	Limit	*Observed max. Deviation*	*Result of all Steps*
Step Accuracy	1.000 %	0.174 %	**Test passed**
Step Ripple	0.500 %	0.011 %	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Chromatogram of STD Gradient_3*



Flow [ml/min]: **2.000**

Reviewer's signature // Date

Operator's signature // Date

• Data of STD Gradient_3

Observed Value [mAU]	Expected Value [%]	Calculated Value [%]	Abs. Critical Deviation [%]	Calculated Deviation [%]	Result
0.00	0.00	0.000	1.000	0.000	ok
2.50	1.00	1.163	1.000	0.163	ok
106.87	50.00	49.826	1.000	-0.174	ok
212.29	99.00	98.976	1.000	-0.024	ok
214.48	100.00	100.000	1.000	0.000	ok

• Ripple of STD Gradient_3

Step [%]	Ripple [mAU]	Calculated Ripple [%]	Critical Ripple [%]	Result
1.00	0.012	0.006	0.500	ok
50.00	0.023	0.011	0.500	ok
99.00	0.017	0.008	0.500	ok

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Reproducibility of the Stand. Gradient*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	**P680 LPG**	**DIONEX**	**1920401**
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	*Name*
Back Pressure Device	Capillary (L:15 m; ID:0,18 mm)
Solvent A	Water (HPLC-Grade)
Solvent B for Gradient	Water + 0.1 % Acetone

• *Additional Information*

Customer: Customer's Name

Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

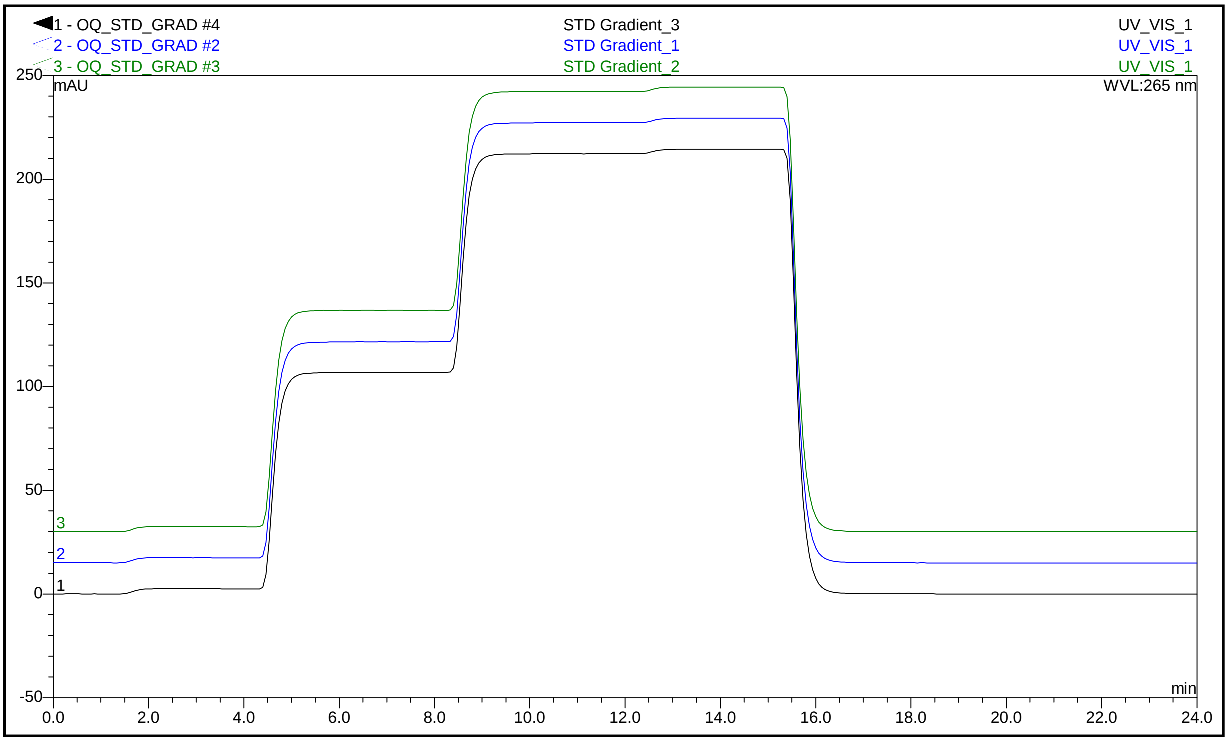
• *Limits and Test Results*

	Limit	*Observed max. STD*	*Result of all Steps*
Gradient Presicision	0.500 %	0.051 %	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Overlay of three Gradients*



• *Data of three Gradients*

Expected Value [%]	***Calculated Value Gradient_1 [%]***	***Calculated Value Gradient_2 [%]***	***Calculated Value Gradient_3 [%]***	***Critical STD [%]***	***Calculated STD [%]***	***Result***
0.00	0.00	0.00	0.00	0.50	0.000	ok
1.00	1.12	1.15	1.16	0.50	0.023	ok
50.00	49.72	49.79	49.83	0.50	0.051	ok
99.00	99.02	98.99	98.98	0.50	0.020	ok
100.00	100.00	100.00	100.00	0.50	0.000	ok

Reviewer's signature // Date

Operator's signature // Date

DIONEX

Operational Qualification

• *Temperature Accuracy of the Column Oven*

• *Instruments and Fluidics*

Instrument Name	*Model*	*Supplier's Name*	*Serial Number*
Pump	P680 LPG	DIONEX	1920401
Autosampler	ASI-100	DIONEX	1860410
Column Oven	**TCC-100**	**DIONEX**	**1850409**
UV Detector	UVD 340U	DIONEX	1830402
Chromeleon Datasystem	V. 6.60 Build 1428	DIONEX	11

Accessories	
Thermometer	SN: 43077
Temperature Sensor	SN: 111988

• *Additional Information*

Customer: Customer's Name
Operator: Operator's Name
Operator's Jobtitle

Execution Date: Apr-14-04
Next Qualification: Oct-04

• *Limits, Values and Test Results*

	Limit	*Obs. max. Deviation*	*Result*
Temperature of Column Oven	+/- 1.0 °C	0.4 °C	**Test passed**

Reviewer's signature // Date

Operator's signature // Date

• *Data for Temperature Accuracy*

Setpoint Temperature [°C]	***Measured Temperature [°C]***	***Deviation [°C]***	***Result***
80	80.4	0.4	**ok**
60	60.2	0.2	**ok**
30	30.3	0.3	**ok**
10	10.1	0.1	**ok**
Obs. max. Deviation		***0.4***	***ok***
Limit:		***+/- 1.0***	

If the setpoint temperature of 10°C cannot be reached, the reason can be that the ambient temperature is too high. Nevertheless, the test is passed, if the measured temperature is at least 15°C below ambient.

Reviewer's signature // Date

Operator's signature // Date