UltiMate™

Capillary- and Nano HPLC Systems

Operational Qualification and Performance Qualification

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Operating Instructions

P/N 163960

LC PACKINGS A DIONEX Company

www.lcpackings.com

M1393R0

The material included in this manual is provided to assist authorized personnel in performing operation qualification (OQ) and performance qualification (PQ) on the LC Packings UltiMate Capillary and Nano HPLC system. It is assumed that the individual using this manual has sufficient training in the use of analytical instrumentation and is aware of the potential hazards including (but not limited to) electrical hazards, chemical solvent hazards, exposure to UV radiation and the exposure to pressurized solvents.

All information in this manual is subject to change without notice and does not represent a commitment on the part of LC Packings, BV.

Printed in the Netherlands.

Warnings

The Danger sign, Warning sign and the Caution sign shown below are included in various locations in this manual or in the manuals provided with the instruments which are to be tested. These signs provide the following information:

Danger: The information in a danger statement relates to a procedure, practice condition or action that if not done correctly or adhered to could lead to personal injury or loss of life.

Warning: The information in a warning statement relates to a procedure, practice, condition or action that if not done correctly or adhered to could lead to severe injury and/or damage or destruction to parts or all of the equipment.

Caution: The information in a caution statement relates to a condition that could lead to damage to equipment and/or lead to invalid analytical results.

Note: The information in a note statement relates to important information that should be read and understood before continuing.

Safety Precautions

Note: The following precautions should be followed to minimize the possibility of personal injury and/or damage to property.

Note: Make certain that you are familiar with the contents of this manual before working on the system.

The operator should follow all safety precautions, warnings, etc provided with the instruments, in addition, please note the items presented below:

1. All components of the system should be plugged into a common power line that is directly connected to a true ground.

2. Repair or replace faulty power cords and all communication cables.

3. If a leak occurs, turn off power to the instrument and remedy the situation immediately.

4. If the mobile phase includes volatile or flammable solvents, avoid open flames and sparks.

5. Many organic solvents and buffers are toxic. Make certain that you know the toxicological properties of all mobile phases that you are using.

6. The toxicological properties of many samples may not be well known. If you have any doubt about a sample, treat it as if it contained a potentially harmful substance.

7. Wear protective eye goggles when handling mobile phases or operating the instrument. An eye wash facility and a sink should be close to the unit. If any mobile phase splash on the eyes or skin, wash the affected area and seek medical attention.

8. Dispose of all waste mobile phase in an environmentally safe manner that is consistent with all local regulations. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose flammable and/or toxic solvents through the municipal sewage system

9. Wear protective eye goggles when handling fused silica tubing (i.e. installation, cutting etc.)

10. If a buffer is used as a part of the mobile phase, flush the system with several volumes of a methanol/water (50/50) solution before it is shut down. This will prevent salt buildup inside the unit.

11. Do not use the instrument in ways other than those indicated in the instructions given in this manual.

Warning: The OQ/PQ kit (P/N163932) contains a chemical or chemicals known to the State of California to cause cancer and/or birth defects or other reproductive harm. For additional information, consult the product Material Safety Data Sheet (MSDS).

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CHAPTER 1

Introduction

1.1 The Purpose of OQ/PQ

An increasing number of standards and official regulations require that the end user is able to provide evidence that the instrumentation used for analytical work is working in a satisfactory manner. In the same manner, quality management according to ISO 9000 (and similar standards) requires that the user monitor and document the ability of the equipment to obtain valid data on a periodic basis. This manual provides a detailed series of procedures to perform these Operational Qualification (OQ) and Performance Qualification (PQ) protocols on the LC Packings UltiMate system components.

The validation procedures described herein are designed to demonstrate that the instrument was working in an acceptable manner using the standards provided on the day of validation. It is likely that the instrument performance will vary over time due to small changes in the various components in the instrument and the validation protocol should be performed on a periodic basis. The frequency of validation is dependent on the level of usage of the system and the degree of tolerance that is acceptable for the system.

1.2 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:

- **Operational Qualification (OQ)** – The purpose of the Operational Qualification is to demonstrate and document that an analytical system functions according to its specifications when specific environmental conditions are taken into account. In this specification, the supplier must define exactly the conditions that must be observed for the measurement.
- **Performance Qualification (PQ)** – The purpose of the Performance Qualification is to demonstrate and document that an analytical system is capable of accurately measuring the concentration of one or more compounds in a standard sample.

To simplify the overall qualification protocol, the same procedures can be used for both OQ and PQ, but the tolerances used for Performance Qualification are less restrictive than those used for Operational Qualification.

1.3 General Notes and Recommendations

- After the validation of the different modules, a cytochrome C separation should be performed according to the LC Packings factory qualification protocol (as described in the user's manual).
- For Ultimate Dual Gradient system configurations, the gradient accuracy test (Section 4.10) must be performed on both pumps.
- Channel C & D should only be tested on customer request.
- If the customer ordered a system with two configurations (e.g. a NAN configuration and a CAP upgrade kit), the OQ/PQ should be performed for the system configuration that the customer will be using on a routine basis.
- If required, test any other configuration according to the factory qualification protocol (cytochrome C digest separation).
- After the instrument has been validated via the protocols described in this document, the analyst should perform a validation of the assay with standards of the compounds of interest.

1.4 How this Manual is structured

This manual describes the Operation and Performance Qualification for the LC Packings UltiMate Systems in its different configurations (including the FAMOS Microautosampler and the Switchos Advanced Microcolumn Switching Unit) and provides the following information:

Chapter 2: *Requirements for a Successful OQ/PQ* provides an overview of the parameters to be tested, a short description of the tests and lists the required acceptance limits for OQ and the recommended limits for PQ.

Chapter 3: *Process* lists all required materials, standards and solvents that are necessary to perform the OQ/PQ test procedures. In addition, it describes how to prepare CHROMELEON and the UltiMate system to perform the tests.

Chapter 4: *Test Procedures* provides step-by-step instructions about how to perform and evaluate the various OQ/PQ tests.

Chapter 5: *Troubleshooting* discusses how the operator can determine the cause of a difficulty in the performing of the OQ/PQ.

Chapter 6: *CHROMELEON® Listings* provides the listings of all CHROMELEON programs used to perform the QQ/PQ test procedures on an UltiMate system in NAN configuration.

1.5 For Additional Information

For more detailed information about the operation, maintenance or troubleshooting of the instruments of the UltiMate system or how to use the CHROMELEON software package, please refer to the documentation provided with these products and to the online help of CHROMELEON (F1 key).

CHAPTER 2

Requirements for a Successful OQ/PQ

2.1 Overview

The Operational Qualification (OQ) and Performance Qualification (PQ) procedures are system-specific procedures. The procedures provided with this document apply for the components of the LC Packings UltiMate Capillary HPLC system listed in TABLE 2-1:

TABLE 2-1 List of UltiMate System Components with available OQ/PQ Procedures

Instrument	Version	Option
UltiMate [Plus] Nano- and Capillary HPLC System (b)	Standard / Inert	- Flow Sensor - Without UV Detector (a) - Manual Injection Valve
UltiMate [Plus] Dual Gradient Nano- and Capillary HPLC System (a) (b)	Standard / Inert	- Flow Sensor(s)
FAMOS Well Plate Microautosampler	Standard / Inert	- Sample Cooling
FAMOS Carousel Microautosampler	Standard / Inert	- Sample Cooling
Switchos II Advanced Microcolumn Switching Unit	Standard / Inert	N/A

Notes: (a) If no UV Detector is installed, an extra detector is required to perform the OQ/PQ procedures.
(b) For test procedures regarding the MIC versions of the UltiMate system, contact LCP.

Note: The OQ/PQ procedure are identical for LC Packings UltiMate and UltiMate Plus Capillary- and Nano HPLC Systems. When the LC Packings UltiMate system is mentioned, the reader should assume that the material applies to both systems.

Note: If the system does not include an UltiMate UV Detector, a standalone UltiMate UV Detector is required to perform the OQ/PQ procedures.

The instruments should be controlled by CHROMELEON® 6.6 SP1 or higher (previous versions will have compatibility problems with the CHROMELEON report file). All necessary CHROMELEON programs and sequences are provided on the CD ROM 'IQOQPQ on UltiMate™ (Plus) Systems' (P/N 163935). If a different software package (e.g. Xcalibur™, Analyst™, HyStar™, MassLynx™, UltiChrom™, etc.) is used to control the instruments, all programs will need to be prepared manually. Some limitations may apply due to different or limited control capabilities of these software packages.

2.2 Checks and Acceptance Limits

TABLE 2-2 lists all OQ/PQ test procedures that are to be performed in the order they must be performed. In most instances, it is necessary that a test is passed before the next test in the overall is attempted. As an example, if the 'Linearity of the UV Detector' test is failed (Section 4.7), the result of the 'Gradient Accuracy' test will be questionable (Section 4.10).

TABLE 2-2 List of OQ and PQ Test Procedures to be performed

Test Procedure	Performed	Section
Lamp Intensity of the UV Detector	Manually	4.2
Wavelength Check	CM Sequence	4.3
Flow Cell Check	Manually	4.4
UltiMate Fluid Path Test	Manually	4.5
Baseline Noise and Drift Test of the UV Detector	CM Sequence	4.6
Linearity of the UV Detector	CM Sequence	4.7
Reproducibility of the Injection Volume	CM Sequence	4.8
Linearity of the injection	CM Sequence	4.9
Gradient Accuracy	CM Sequence	4.10
Switchos Fluid Path Check	Manually	4.11
Switchos Flow Rate and Pressure Stability Test	CM Sequence	4.12
Switchos Valve Position Check	Manually	4.13

Note: According to GLP, a test procedure that failed needs to be repeated and all test procedures following the one that failed must be repeated.

TABLE 2-3 presents an overview of the parameters to be tested and a short description of the tests. In addition, it presents the required acceptance limits for OQ and the recommended limits for PQ.

TABLE 2-3 Overview of the OQ and PQ Test Procedures and Limits

Instrument	Parameter	Description	Limits (a)	
			OQ	PQ
UltiMate UV Detector (Note: If the system does not include an UltiMate UV Detector, a standalone UltiMate UV Detector is required to perform the OQ/PQ test procedures).	Wavelength accuracy	External holmium filter is required.	± 2 nm	± 2 nm
	Lamp Intensity (Section 4.2)	The dummy flow cell is installed and the lamp intensity is read from the detector SIGNALS screen.	0.4 < SIG < 0.9 0.1 < REF < 0.9	0.4 < SIG < 0.9 0.1 < REF < 0.9
	Flow cell transmittance (Section 4.4) (b)	The flow cell is installed and filled with water. The transmittance of this flow cell is indicated by the 'SIG' value and is read from the detector SIGNALS screen.	> 15% of reference intensity	> 15% of reference intensity
	Baseline drift (Section 4.6)	The drift and noise are recorded for 21 minutes at 254 nm with a flow cell (filled with mobile phase A).	< 4000 µAU/hr	< 4000 µAU/hr
	Baseline noise (Section 4.6)		< 50 µAU	< 50 µAU
	Oven test (Section 4.6) (d)	An external thermometer is used to measure the oven temperature.	Accuracy +/- 1°C	Accuracy +/- 1°C

	Linearity (Section 4.7) (c)	Injections of caffeine standards covering the linear range of the UV detector are injected and the peak height is measured. The regression coefficient of the resulting calibration curve indicates the linearity.	R ≥ 99.50% peak height	R ≥ 99. 0% peak height
UltiMate System (Single and Dual Gradient Version)	Gradient accuracy, step gradient (Section 4.10) (c)	A step gradient is performed and the UV trace recorded. The step intensity indicates the gradient accuracy. Channel A: water Channel B: water with 0.8 % acetone (NAN configuration) or 0.3% acetone (CAP configuration), respectively.	The range is defined by the 0% and 100% values. A relative deviation of 3% is allowed for each step (5%, 50%, 95%).	The range is defined by the 0% and 100% values. A relative deviation of 3% is allowed for each step (5%, 50%, 95%).
	Gradient reproducibility (Section 4.10) (d)	A step gradient is programmed and measured 3 times. The reproducibility of the proportioning is evaluated.	For each step, the intensity of the signal is measured. The maximum %RSD allowed is 1%.	For each step, the intensity of the signal is measured. The maximum %RSD allowed is 1%.
FAMOS (Well Plate / Carousel Version)	Reproducibility of the injection (Section 4.8) (c) (d)	8 injections of a caffeine standard are analyzed. The relative standard deviation of the peak height is calculated.	Peak height RSD ≤ 1.5%	Peak height RSD ≤ 1.5%
	Linearity of the injection (Section 4.9) (d)	Partial loop injections of a caffeine standard are performed (from 0.05µl to 0.5µl). The regression coefficient of the resulting calibration curve indicates the linearity.	R ≥ 99.50% (calculated via peak area)	R ≥ 99.0% (calculated via peak area)
Switchos	Switchos fluid path test (Section 4.11)	The flow rate is measured for all the channels.	The flow rate is at least 0.15 mL/min.	The flow rate is at least 0.15 mL/min.
	Valve position test (Section 4.13)	The position and is switching of the valves is checked.	LED indication must correspond to the open channel on the valve.	LED indication must correspond to the open channel on the valve.
	Switchos flow rate and pressure stability (Section 4.12) (d)	The Switchos pump is programmed to deliver a flow rate of 30 µl/min. The output is connected to one of the valves with a restriction connected to port 2. During this program the valve is switched from position 1-2 to 10-1. The backpressure must be stable and reduce to zero without restriction.	Flow rate must be 30 ± 3 µL/min Pressure must be 18 ± 5 bars when restriction is in line and 0 bars otherwise.	Flow rate must be 30 ± 3 µL/min Pressure must be 18 ± 5 bars when restriction is in line and 0 bars otherwise.

Notes:

a) OQ limits with optimum measuring conditions, recommended PQ limits.

b) The signal intensity should be ≥ 5% of the reference signal for a 30 mm UV Booster™ flow cell.

c) Up to maximum signal height of CAP = 250 mAU or NAN = 30 mAU.

d) All tests are performed by using the sequences provided with the IQ/OQ/PQ CD ROM.

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CHAPTER 3

Process

3.1 Required Materials

An OQ/PQ kit (P/N 163936) is available for performing the OQ/PQ on the UtliMate system, an OQ/PQ kit (P/N 163936) is available. In addition, parts from the standard instruments accessory kits are required. TABLE 3-1 list all accessories and standards, which are provided with the OQ/PQ kit:

TABLE 3-1 Parts included in the OQ/PQ Kit

Item Description	Part Number	Note
UltiMate System - OQ/PQ Operating Instructions	163960	
CD ROM 'IQOQPQ on UltiMate™ (Plus) Systems'	163935	
Set of 9 samples (flame sealed amber ampoule)	163932	Do NOT Freeze!
Fused silica tubing I.D. 15 μm ± 3 μm/O.D. 375 μm ± 10 μm, 3,2 meters	163933	Restriction capillary, NAN configurations only.
Fused silica tubing I.D. 30 μm ± 3 μm/O.D. 375 μm ± 10 μm, 2,5 meters	163934	Restriction capillary, CAP configurations only.
Tubing set consisting of 130 μm I.D. x 50 cm PEEK tubing	160180	
Microtight Union, includes 2 fittings and 1 gauge plug	161497	Used to connect the restriction capillary.
PEEK sleeves, precision cut and polished for connections with Microtight Union (380 μm O.D.), 10 pieces	161405	Used to connect the restriction capillary.
PEEK sleeves, precision cut and polished for connections with Microtight Union (280 μm O.D.), 10 pieces	161498	Used to connect the restriction capillary.
1/16" Valco Ferrule and Nut, stainless steel, 10 pc. (for 10-port valve)	161103	Used with stainless steel systems only.
PEEK sleeves, precision cut and polished for connections with fused silica tubing (360 μm O.D.), 5 each	160493	Used with stainless steel systems only.
PEEK 1/16" Universal Fitting for Switchos, INERT, 10 pc., long hex nut and ferrule with groove	161007	Used with INERT systems only.
Syringe adapter	160465	Flow rate (droplet) test.
Phillips-head screw M3 X 4 mm	163964	Used to install the temperature probe.
Toothed Lock Washer 3,2 mm x 6 mm	163275	Used to install the temperature probe.

TABLE 3-2 and TABLE 3-3 list accessories and standards that are necessary to perform the OQ/PQ test procedures (and which are not included in the OQ/PQ kit)

TABLE 3-2 Accessories required for the OQ/PQ

Item Description	Part Number (a)	Included in the Instrument's Accessory Kit
Accessories		
UV Detector (b)	160008 or 162346 or 163653	No
Dummy flow cell	162053	Yes
UV flow cell	160015 (NAN) 160013 (CAP)	Yes
Calibrator Cartridge	160061 (NAN) 160059 (CAP)	Yes
Dual Calibrator Cartridge	161082 (NAN/NAN) 161084 (CAP/CAP) 161083 (CAP/NAN)	Yes
P600 Precision Thermometer or equivalent	163961	No
Special Thermocouple for P600 Precision Thermometer	163962	No
Test Cell, Holmium Oxide Filter for UltiMate™ UV-Detector	163963	No
Syringe adapter for Valco valve	160259	No
Syringe of 250 μL	163241	No
Connecting tubing 30 µm ID x 15 cm	160182	Yes

Notes (a) P/N applicable depends on the system configuration.
(b) If the system does not include a UV Detector, a standalone instrument is required to perform the OQ/PQ procedures.

TABLE 3-3 Standards and Solvents for the OQ/PQ

Item Description	P/N	Included in the	
		Instrument's Accessory Kit	OQ/PQ Kit
Caffeine in water standards: 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20, 40, 60 µg/ml	163932	No	Yes
Cytochrome C Digest Test Sample	161089	Yes	No
Acetone (HPLC Grade)	N/A	No	No
Water (HPLC Gradient Grade)	N/A	No	No
Formic Acid (HPLC Grade)	N/A	No	No
Acetonitrile	N/A	No	No

3.2 Preparations

The system components that are included in the system configuration have to be prepared according to the following steps before starting the OQ/PQ procedure.

3.2.1 CHROMELEON® Setup

All CHROMELEON programs required to perform the OQ/PQ test procedures are provided as a CHROMELEON backup file on the CD ROM 'IQOQPQ on UltiMate™ (Plus) Systems' (P/N 163935). Two folders are provided, one is for the Nano LC (NAN) and capillary LC (CAP) configurations. In addition, different CHROMELEON server configuration files are available.

It is assumed that the service engineer has basic knowledge of the UltiMate system and CHROMELEON software. Please refer for more detailed information about the installation and usage of the UltiMate system and CHROMELEON to the manuals provided along with the products. A detailed description of the tests is provided in Chapter 4.

3.2.1 A Restoring the Backup Files

Refer to the CHROMELEON on-line help (F1 key) for more information on how to restore backup files.

To prepare the PC and the CHROMELEON software for the OQ/PQ test procedures:

a) Restore the CHROMELEON backup file from the CD ROM. All programs and sequences are now available for the test procedures (FIGURE 3-1).

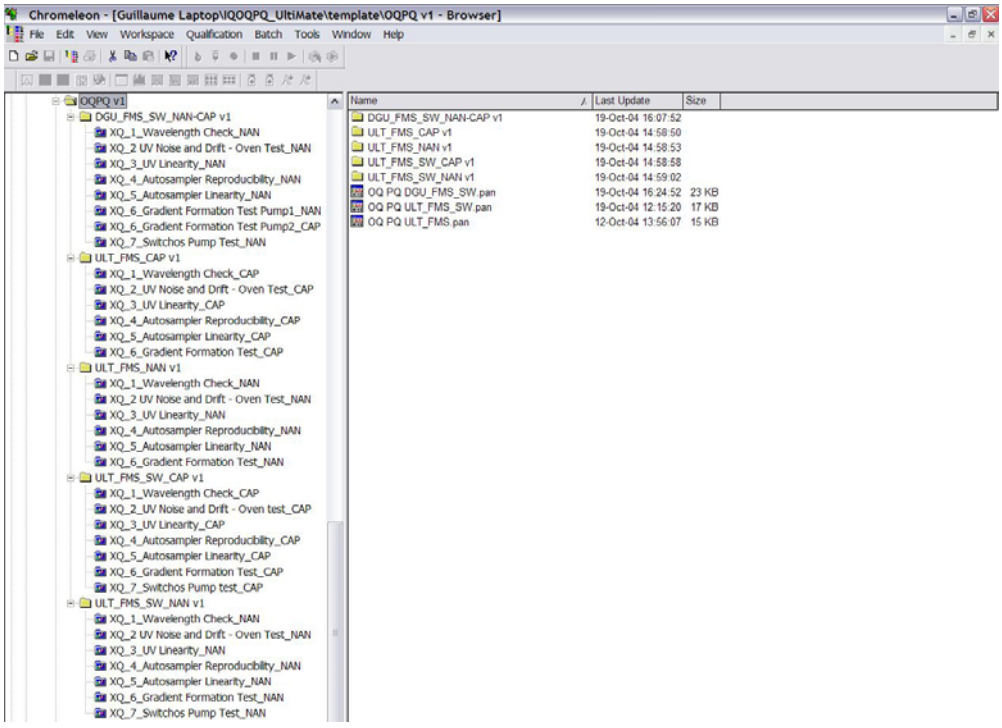


FIGURE 3-1 OQ/PQ Programs after Restoring

b) Copy the three server configuration files that are available on the same CD ROM into the **Chromel/Bin** directory of the computer.

c) Open the CHROMELEON Server Configuration. Load the server configuration file and check the COM port settings. Modify the configuration if necessary.

3.2.1 B Modifying Sequences and Programs

It is recommended that you do not modify the sequences provided on the CD ROM 'IQOQPQ on UltiMate™ (Plus) Systems' (P/N 163935) unless it is absolutely necessary. Changing them may have an impact on the links with the report. However, it may be necessary to make modifications to correspond to the available hardware. If changes are made, take care that the sample names and numbers are not modified.

3.2.1.B.1 Choosing to perform OQ or PQ

The sequences required to perform the OQ/PQ on a Nano LC system have the extension '_NAN' in their names (e.g. XQ_6_Gradient Formation Test_NAN.seq). Sequences for a capillary LC system have the extension '_CAP' (FIGURE 3-2).

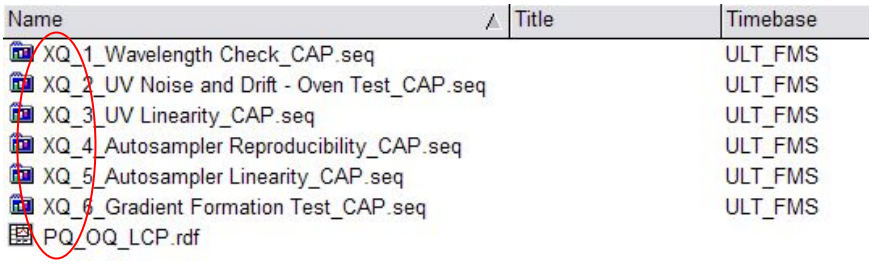


FIGURE 3-2 Modifying the Sequence Names

All sequence names start with the prefix 'XQ_'. Depending on the test that is to be performed, change 'XQ' to 'OQ' or 'PQ' (FIGURE 3-2). This will select the right limits for the report file. If you do not modify the name, the PQ limits will be selected.

3.2.1.B.2 Flow Sensor Support and CRP Value

All sequences are prepared to support the flow sensor option. If the system to be checked does not include the flow sensor, all relevant programs must be adjusted:

To change the programs for a using a fixed CRP value, identify the 'CRP' or 'CalibrateCRP' command in the program and remove the semicolon from the 'CRP' line and place it on the 'CalibrateCRP' line as presented below:

- Flow Sensor Support:

```
;CRP =    625
CalibrateCRP    When = BeforeFirstSample
```

- Using a fixed CRP Value:

```
CRP =    625
;CalibrateCRP    When = BeforeFirstSample
```

3.2.1.B.3 FAMOS Cooling Option

All sequences are prepared to support the cooling option of the FAMOS. If the system to be checked does not include the cooling option, all relevant programs must be adjusted:

To change the programs for instruments for which do not have this option installed, identify the 'Sampler_TempCtrl' command and in the programs and add a semicolon to this line and the 3 following lines as presented below:

- FAMOS with Cooling Option:

```
;Commands for FAMOS with cooling option
Sampler.TempCtrl =                  On
Sampler.Temperature.Nominal =       20.00
Sampler.Temperature.LowerLimit =    5.00
Sampler.Temperature.UpperLimit =    30.00
```

- FAMOS without Cooling Option:

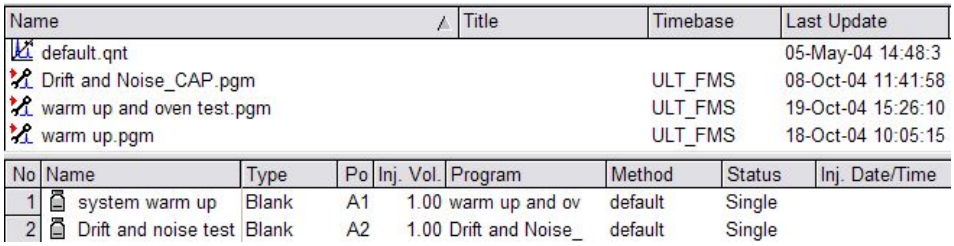
```
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                 On
;Sampler.Temperature.Nominal =      20.00
;Sampler.Temperature.LowerLimit =   5.00
;Sampler.Temperature.UpperLimit =   30.00
```

3.2.1.B.4 Oven Support

During the warm up sequence of the UV detector noise and drift test (Section 4.6) the oven accuracy is checked. If the current configuration does not include an oven, a different program must be used:

Depending on the test configuration (e.g. the Switchos is included or not),

- If the system includes an oven, run the 'warm up and oven test.pgm' (FIGURE 3-3).
- If the system does not include an oven, run the 'warm up.pgm' (FIGURE 3-3).



Name	Title	Timebase	Last Update
default.qnt			05-May-04 14:48:3
Drift and Noise_CAP.pgm		ULT_FMS	08-Oct-04 11:41:58
warm up and oven test.pgm		ULT_FMS	19-Oct-04 15:26:10
warm up.pgm		ULT_FMS	18-Oct-04 10:05:15

No	Name	Type	Po	Inj. Vol.	Program	Method	Status	Inj. Date/Time
1	system warm up	Blank	A1	1.00	warm up and ov	default	Single	
2	Drift and noise test	Blank	A2	1.00	Drift and Noise_	default	Single	

FIGURE 3-3 Selecting the proper Warm-up Program

3.2.1.B.5 Miscellaneous

The sequences for the test 4.10 and 4.12 include a stop flow method which reduces the time that the lamp is lit and minimizes solvent consumption. Select the status of these stop flow samples to 'single' (activated) or 'interrupted' (deactivated) to correspond to the test configuration (e.g. the Switchos is included or not).

3.2.1 C Report File

No changes should be made in the report file itself (PQ_OQ_LCP.rdf), except for the spreadsheets 'Specification' and 'Others tests'. These two spreadsheets allow entering of some test details (i.e. the serial numbers of the instruments) and the results of tests that were carried out manually (i.e. 4.2, 4.4, 4.5, 4.11, 4.13).

3.2.2 System Setup

The OQ/PQ procedure is similar for the different system configurations (e.g. NAN or CAP configuration). However, some parts are different and depend on the system configuration being tested (e.g. column, calibrator, connecting tubing, UV flow cell). Make sure that the system is configured properly. Please refer to the UltiMate user's manual for more information.

In addition, several test parameters used with the tests are different for the individual system configurations (e.g. flow rate, CRP value and injection volume). Refer to Section 3.2.1 and Chapter 6 for more details about how to setup CHROMELEON.

Note: Make certain that enough COM ports are available. For the Dual Gradient system with flow sensors, the FAMOS and the Switchos 9 COM ports are required (including the one required for the precision thermometer).

3.2.2 A UltiMate System

To prepare the UltiMate system for the OQ/PQ test procedures 4.4 to 4.10:

a) Switch on the UV detector at least 1 hour before starting the tests to warm-up the instrument.

b) Configure the system as required for the application (e.g. install the NAN calibrator cartridge and NAN connection tubing for a proper NAN setup).

Note: If the system does not include a UV Detector, a standalone instrument is required to perform the OQ/PQ procedures.

c) Connect the appropriate restriction tubing to the port 6 of injection valve of the FAMOS Microautosampler ('column' port). Use P/N 163933 for the NAN configuration and P/N 163934 for the CAP configuration.

d) Use the Microtight union and appropriate PEEK sleeves to connect the restriction tubing (360 μm O.D.) to the flow cell inlet (280 μm O.D.). Use P/N 161405 for the 360 μm tubing and P/N 161498 for the 280 μm tubing (e.g. for the flow cell).

e) Prepare the solvents as presented in TABLE 3-4, and start helium sparging to degas the solvents. Thoroughly purge all channels.

TABLE 3-4 Required Solvents

Channel	Solvent
A	100% Water + 0.1% FA
B	99% Water + 0.1% FA + 0.8% acetone (NAN) 99.5% Water + 0.1% FA + 0.3% acetone (CAP)
C	100% ACN + 0.1% FA (a)
D	100% Water + 0.1% FA
Note	a) The acetonitrile is used to perform a wash step during the FAMOS test procedure 4.8.

CAUTION

Caution: Older revisions of the UltiMate system may be equipped with the older type of the C and D solenoid valves (P/N160051). Due to their limited resistance against strong organic solvents, do not expose these valves to acetonitrile for a longer period than required to perform the wash cycle of test 4.8 (e.g. not longer than 4 h).

It is recommended that you perform the wash step separately and to use channel A or B on such systems. Alternatively, the system can be upgraded with the new valve type (P/N 162297). Refer to Service Information #036 for more details.

If an oven is included in the UltiMate system, install the temperature probe (P/N 163962) in the oven compartment using the supplied screw and washer (FIGURE 3-4) and connect the thermometer to a free COM port of the computer.

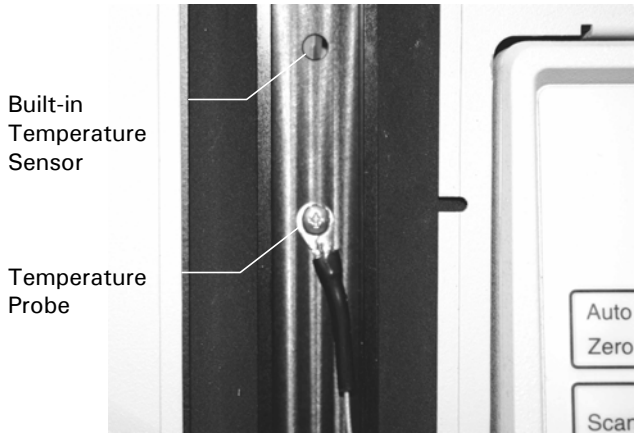


FIGURE 3-4 Installing the Temperature Probe

Note: Make certain that the low on battery indicator on the thermometer is not lit, because a weak battery may interrupt the sequence if serial communication is broken.

3.2.2 B FAMOS Microautosampler

To prepare the FAMOS Microautosampler for the OQ/PQ tests:

a) Position the caffeine standards of the OQ/PQ kit in the FAMOS sample rack as presented in TABLE 3-5.

TABLE 3-5 Sample Positions in the Autosampler Rack

Tray Position	Sample
A2	0.25 µg/ml Caffeine (CAP system only)
A3	0.5 µg/ml Caffeine (CAP system only)
A4	1.0 µg/ml Caffeine
A5	2.5 µg/ml Caffeine
A6	5.0 µg/ml Caffeine
A7	10.0 µg/ml Caffeine
A8	20.0 µg/ml Caffeine
B1	40.0 µg/ml Caffeine (NAN system only)
B2	60.0 µg/ml Caffeine (NAN system only)

b) Use mobile phase A as the wash solvent (TABLE 3-4).

c) Degas the wash solvent. Helium sparging is strongly recommended.

d) Check that there is no air in the syringe and run a wash cycle on the instrument.

3.2.2 C Switchos Advanced Microcolumn Switching Unit

To prepare the Switchos Advanced Microcolumn Switching Unit for the OQ/PQ:

a) Prepare the Switchos with the following mobile phases
   Solvent A: 0.1 % TFA in water
   Solvent B: 0.1 % TFA in water
   Solvent C: 0.1 % TFA in water
   Solvent D: 0.1 % TFA in water.

b) Connect a trap-column connecting tubing 30 µm ID x 15 cm (P/N 160182) to port 2 of switching valve A.

c) Start helium sparging to degas the solvents.

d) Thoroughly purge all channels as described in the user's manual of the instrument.

3.3 Performing the Checks

The OQ/PQ tests that need to be performed depend on the UltiMate system configuration. For a complete system (e.g. UltiMate, FAMOS and Switchos) all test procedures 4.2 to 4.13 must be performed. For different system configurations refer to TABLE 3-6.

TABLE 3-6 Tests to be performed for the different System Configurations

Test No.	Description	Duration (approx.)	System Configuration				
			UltiMate, FAMOS	UltiMate, FAMOS, Switchos	UltiMate without UV Detector (a)	UltiMate Dual Gradient (a)	UltiMate with Manual Injector (c)
	UltiMate and FAMOS tests						
4.2	Lamp Intensity of the UV Detector	10 min	•	•	•	•	•
4.3	Wavelength Check	10 min	•	•	•	•	•
4.4	Flow Cell Check	10 min	•	•	•	•	•
4.5	UltiMate Fluid Path Test	15 min	•	•	•	•	•
4.6	Baseline Noise and Drift Test of the UV Detector (d)	4 h	•	•	•	•	•
	Oven accuracy (e)		(•)	(•)	(•)	(•)	(•)
4.7	Linearity of the UV Detector	NAN: 64 min CAP: 32 min	•	•	•	•	•
4.8	Reproducibility of the Injection Volume	NAN: 72 min CAP: 36 min	•	•	•	•	
4.9	Linearity of the injection	NAN: 56 min CAP: 28 min	•	•	•	•	
4.10	Gradient Accuracy	NAN: 4.5 h CAP: 3 h	•	•	•	• • (b)	•
	Switchos Tests (if applicable)						
4.11	Switchos Fluid Path Check	15 min		•	•	•	
4.12	Switchos Flow Rate and Pressure Stability Test	15 min		•	•	•	
4.13	Switchos Valve Position Check	10 min		•	•	•	

Notes
(a) Additional UV detector required.
(b) The 'Gradient Accuracy Test' must be performed for both gradient pumps. Depending on the configuration, an additional CAP flow is required.
(c) No FAMOS Microautosampler and no Switchos Unit included in the system configuration.
(d) Includes a wash cycle of the FAMOS injection valve.
(e) If included in the configuration.

TABLE 3-6 indicates the tests for the OQ and PQ procedure for different UltiMate system configurations. The time required to complete a test procedure is also indicated. The total time required for checking an entire system (e.g. UltiMate, FAMOS and Switchos) is approximately 13 hours. UltiMate Dual Gradient systems require that the test 4.10 must be performed for both gradient pumps. For the UltiMate Dual Gradient system the entire OQ/PQ requires approximately 17 hours.

For the OQ and PQ of UltiMate system configurations which does not include an UV detector or for the UltiMate Dual Gradient system, a standalone UV detector is required and all UV Detector related tests must be performed on this unit as described in this manual.

3.4 Evaluating the Tests

The templates provided with CD ROM 'IQOQPQ on UltiMate™ (Plus) systems' and thus, all copies made from it for OQ and PQ are linked to the corresponding report. Do not change this report (except for items that are described in Section 4.14). 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! Select 'Batch Report' on the 'File' menu and start printing by clicking 'OK'.)

For the system tests 4.3, 4.6, 4.7, 4.8, 4.9, 4.10 and 4.11 the pump- and (trap) column-pressure data is saved. These traces can be opened and reviewed from the CHROMELEON program.

For the tests 4.7, 4.8 and 4.9 it is essential to check the integration carefully. Manual integration of peaks may be required.

3.4.1 Repeating Tests

It may be necessary to repeat one or several tests. In this case, refer to Chapter 5 (Troubleshooting). This chapter describes problems due to which a check may have failed. According to GLP, a test which failed and all tests following the one which failed need to be repeated. Exception: there is no need to repeat UltiMate and FAMOS related tests if a Switchos test fails. 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 detector is a basic requirement for checking the injection volume.

CHAPTER 4

Test Procedures

4.1 Overview

This section provides step-by-step instructions about how to perform and evaluate the various OQ/PQ tests. All tests, which must be performed and the order in which they must be performed are presented in TABLE 3-6. Tests which are not applicable for the current system configuration will be skipped (e.g. if the Switchos is missing in the current configuration, the tests 4.11 - 4.13 will not be performed).

The tests procedures 4.6 to 4.10 are using the same system setup as described in Section 3.2.2 A.

Testing of the UltiMate system and the FAMOS Microautosampler starts with a (manual) lamp check, followed by the automated wavelength check. After these tests are passed the condition of the flow cell and the fluid path are checked (again manually). From this point on the UltiMate and FAMOS the tests procedures 4.6 to 4.10 are automated. These automated tests are performed with the same system setup (Section 3.2.2 A).

Once all UltiMate system and FAMOS tests are performed, the Switchos unit will be tested (if included in the configuration).

4.2 Lamp Intensity of the UV Detector

The intensity of the UV lamp must be sufficient for correct detection sensitivity and baseline stability. If the lamp intensity is below the acceptance criteria, baseline instability problems may occur. For more information refer to the UltiMate service manual.

The absolute intensity of the lamp is not measured in this test, and deviations from lamp to lamp and from detector to detector are quite normal. The basic function of this test is to ensure that the output of the lamp is within an acceptable range.

4.2.1 Performing the Lamp Intensity Test

The lamp intensity values are presented in the SIGNAL screen of the SETUP menu.

To check the lamp intensity:

a) Set the wavelength to 240 nm.

b) Install the dummy cell and access the SIGNAL screen on the SETUP menu.

SIGNAL:	**sig: 0.3485**
♦ absorbtion	**ref: 0.1729**

FIGURE 4-1 UV Detector SIGNAL Screen

c) Monitor the signal value ('sig') and the reference value ('ref'). Both the signal and the reference values should be in the limits defined in TABLE 2-3.

d) Fill in the appropriate field in the 'Other Tests' page of the QO/PQ report.

Note: If the values are not within the specifications, install a new lamp and perform the test again. If the values are still not within the specifications check/adjust the 'Integration Time' setting.

Note: This test must also be performed for <u>any</u> UV Detector that used for the OQ/PQ, even if it's not part of the UltiMate system.

4.3 Wavelength Check

The wavelength accuracy is measured by evaluating local maxima of absorbance on a holmium filter in the UV range. These results are compared with the ones provided on the certificate of the material.

4.3.1 Performing the Test

a) Install the holmium filter (FIGURE 4-2) in the UV detector.

b) Loosen the knurled screw (item 1, FIGURE 4-2) half a turn and pull the filter holder (item 2, FIGURE 4-2) out of the light path (just pull it out by 5 mm (0.2“), the filter holder remains in the filter body).

c) Perform an autozero run. Either use the F8 key of CHROMELEON or press the ‘Autozero’ button of the UV detector.

d) Place the filter back in its original position (all the way in the filter body), tighten the screw.

e) Start the sequence ‘XQ_1_Wavelength Check_NAN’ (or ‘_CAP’, respectively).

f) Check the results in the CHROMELEON report. Verify that the reference values correspond to the values listed in the certificate provided with the holmium filter.

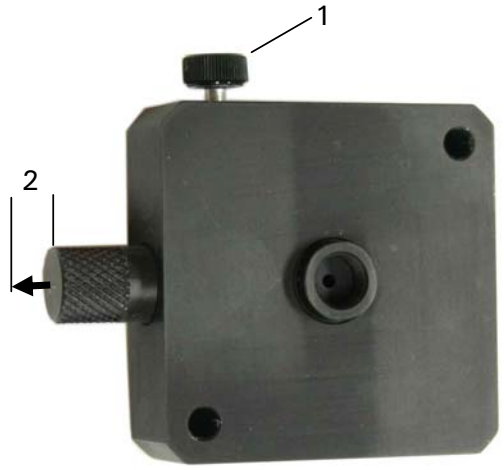


FIGURE 4-2 The Holmium Filter for the UV Detector

4.4 Flow Cell Check

The transmittance of the UV flow cell is important for proper detection sensitivity and baseline stability. The transmittance of the flow cell is read from the SIGNAL screen of the Setup menu of the UV detector (FIGURE 4-3).

SIGNAL:	**sig: 0.1795**
♦ absorbtion	**ref: 0.3429**

FIGURE 4-3 UV Detector SIGNAL Screen

4.4.1 Performing the Intensity Test of the Flow Cell

To test the flow cell:

a) Make certain that the flow cell to be tested is clean and flushed properly with mobile phase A.

b) Set the wavelength to 240 nm.

c) Access the SIGNAL screen on the SETUP menu.

d) Monitor the Reference signal (‘ref’) and the Sample signal (‘sig’).

e) Compare the result with the signal intensity limits presented TABLE 2-3 and fill in the appropriate field in the ‘Other Tests’ page of the QO/PQ report.

4.5 UltiMate Fluid Path Test

The UltiMate fluid path test is used to check the solenoids and the solvent delivery.

4.5.1 Performing the Test

a) Fill each solvent bottle until the fluid level corresponds to the top of the UltiMate housing with the solvents specified in TABLE 3-4.

b) Degas all solvents properly and purge all solvent channels.

c) Disconnect the solvent inlet line from the pump head and connect the 250 µl syringe (P/N 163241, the plunger should be removed) using an adapter (P/N 160259) as presented in FIGURE 4-4.

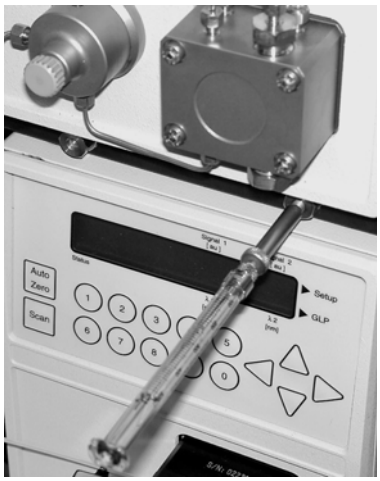


FIGURE 4-4 Placing the Syringe on the Solvent Inlet Line

d) Enter the purge screen of the micro pump, set the flow rate to 0.0 ml/min and select channel A. The LED of channel A should be illuminated

e) Measure the flow rate for one minute. The flow rate should be greater than 0.15 mL/min

f) Repeat steps d) – e) for solvent channels B, C and D.

g) Switch off the pump. The flow should stop immediately.

h) Verify the result with the limits presented in TABLE 2-3 and fill in the appropriate field in the 'Other Tests' page of the QO/PQ report.

4.6 Baseline Noise and Drift Test of the UV Detector and Oven Test

4.6.1 Drift and Noise

Note: This test must also be performed for any UV Detector that used for the OQ/PQ, even if it's not part of the UltiMate system.

Drift and baseline noise are important parameter for the UltiMate UV Detector. Increased baseline noise reduces the sensitivity considerably, as it is not possible to distinguish between low-level 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 or if the flow cell is dirty.

To minimize any effect from the flow cell, make sure that the measuring and ambient conditions are constant and that are no gas bubbles in the flow cell. In addition, it is very important that a new lamp has been burning for several hours. In the detector environment, avoid drafts and direct sunlight.

The detector baseline noise and drift test is performed at the following conditions:

- Flow cell installed (and filled with mobile phase A).
- $\lambda 1 = 254$ nm, $\lambda 2 = \lambda 3 = \lambda 4 = 0$ nm.
- Time Constant = 2 sec.
- Data Acquisition Rate = 1 Hz.
- Data Acquisition Length = 20 min.

FIGURE 4-5 shows a typical result from the noise and drift test.

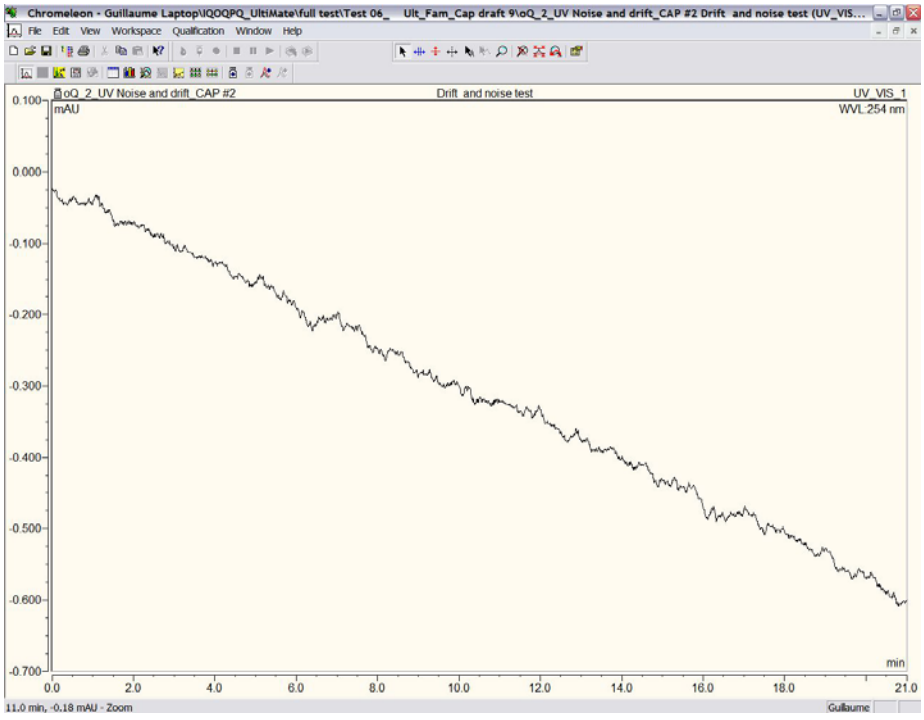


FIGURE 4-5 Typical UV Detector Noise and Drift Result (254 nm)

4.6.2 Theory of the Noise and Drift Calculation of CHROMELEON

CHROMELEON calculates the results of the Noise and Drift test as follows:

- **Signal Noise** – All data points recorded during a 60 seconds segment of a chromatogram form the basis for determining the noise value. CHROMELEON calculates a regression line using the method of least squares, then determines the maximum distance of two data points above and below the line. (When calculating the regression line, all data points are weighted with their corresponding step unless the step is equidistant.) Adding both values supplies the noise value. The noise intensity read in the report is the average noise calculated using 20 segments.
- **Drift** - To compute the drift, a regression line is drawn through all data points. The slope of the regression line is the calculated drift. Therefore, to compute the drift, always select a baseline range in which no peaks occur.

4.6.3 Oven accuracy

The temperature in the Ultimate oven is monitored by the P600 Precision Thermometer (P/N 163961). The readout of the built-in sensor is compared with the read-out of the thermometer.

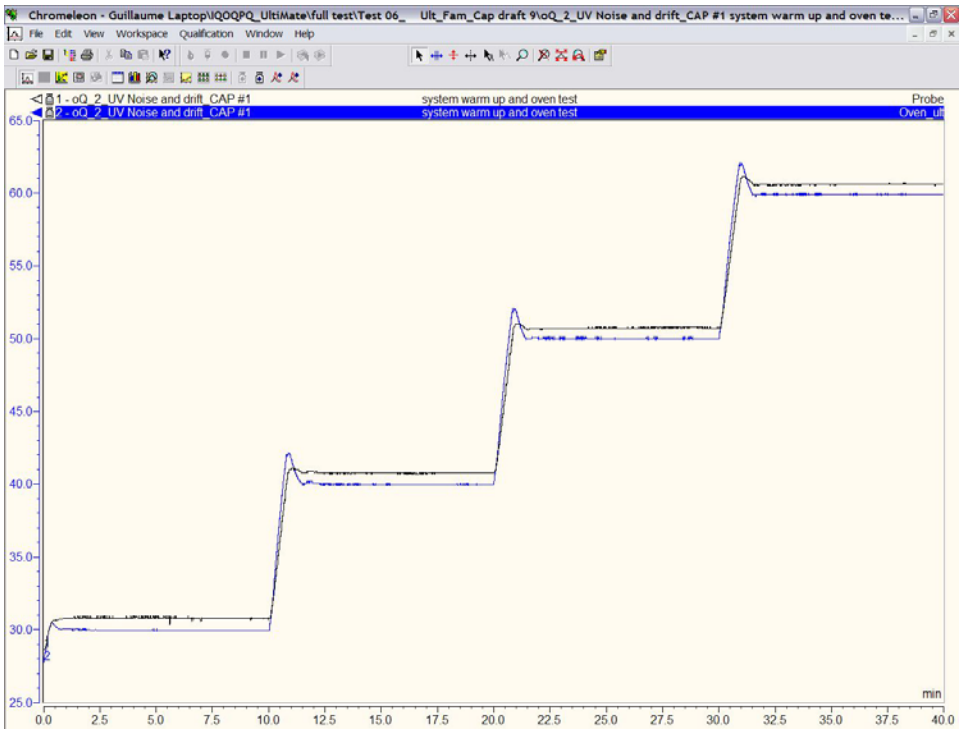


FIGURE 4-6 Typical Result of the Oven Test

4.6.4 Performing the UV Detector Drift and Noise Test and the Oven Test

To perform the Drift and Noise Test:

a) Install the flow cell and let the baseline stabilize.

b) Install the appropriate connection tubing between the Ultimate and the FAMOS injection valve and connect the restriction capillary that is provided to the outlet of the flow cell as described in Section 3.2.2 A.

c) Set the pump to the appropriate flow rate. Use 0.4 µL/min for a NAN version and 4 µL/min for CAP configuration and start the flow delivery of the UltiMate Micropump from the CHROMELEON control panel.

d) If the system includes a flow sensor, perform a manual calibration of the CRP value at this point (e.g. use the F8 key) and execute the 'CalibrateCRP' command from the 'UltiMate_System/Pump/Pump_FlowSensor' menu. The CRP value should be close to 625 for NAN system or 50 for a CAP system, respectively. The CRP value should not vary by more than ± 10 %.

e) After equilibration start the sequence 'XQ_2_UV_Noise and Drift – Oven Test_NAN' (or '_CAP', respectively).

f) Make certain to run the appropriate 'Warm up' program before (Section 3.2.1.B.3).

g) Verify that all parameters meet the acceptance criteria (TABLE 2-3). Discard data if appropriate (Section 4.14).

Note: When a new lamp is installed, optimum performance is obtained after the lamp has been used for approximately 24 hours. If this test performed on a new lamp, the noise and drift levels will be larger than the specified values.

4.7 Linearity of the UV Detector

The detector linearity is measured by injecting the different caffeine standards of the OQ/PQ kit (P/N 16395) as presented in TABLE 3-5. The resulting peak height is used for the calculation. An example of an injection (0.2 µL) of a caffeine standard of 20 µg/mL onto a Nano UltiMate system is shown in FIGURE 4-7.

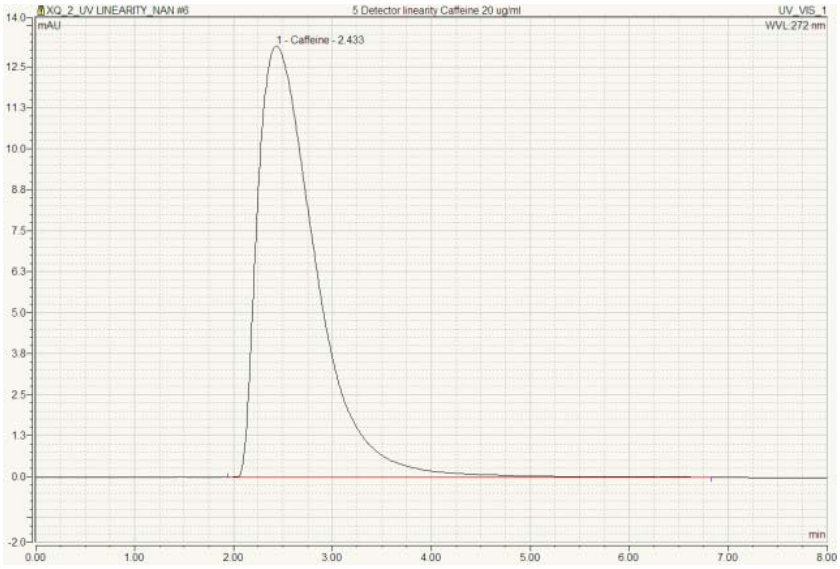


FIGURE 4-7 UV Trace of the Injection of Caffeine onto a Nano UltiMate System

An example of a calibration curve is shown in FIGURE 4-8.

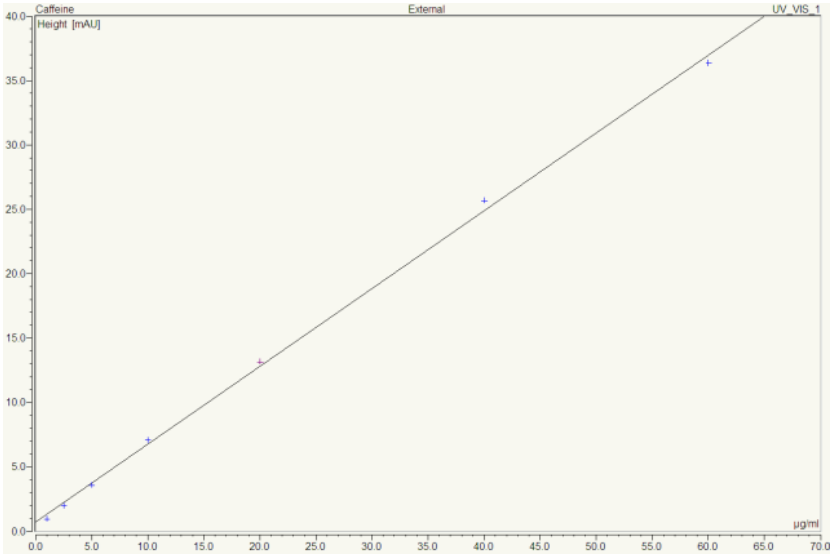


FIGURE 4-8 Example of a Calibration Curve -
Nano LC system, 99.93 % Correlation Coefficient

4.7.1 Performing the UV Detector Linearity Test

Depending on the system configuration, the '*UV Detector linearity_NAN'* or '*UV Detector linearity_CAP'* sequence is used to perform the detector linearity test. The detector linearity is determined at 272 nm using caffeine standard samples with different concentrations:

- CAP configuration: 0.25, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 µg/mL
- NAN configuration: 1.0, 2.0, 5.0, 10.0 , 20.0, 40.0 and 60.0 µg/mL

Install the samples in their proper positions in the FAMOS autosampler rack (TABLE 3-5). TABLE 2-1 presents the conditions for the linearity test for the two different system configurations.

TABLE 4-1 Experimental Conditions – UV Detector Linearity

Parameter	Nano Configuration	Capillary Configuration
Detection wavelength	272 nm	272 nm
Run time	8 min	4 min
Flow rate	0.4 µL/min	4.0 µL/min
CRP	625	50
Injection loop	1.0 µL	1.0 µL
Injection type	Partial Loop Fill, 0.2 µL	Partial Loop Fill, 0.5 µL

To perform the UV Detector linearity test:

a) Setup the UltiMate system and the FAMOS Microautosampler as discussed in Sections 3.2.2 A and 3.2.2 B.
b) Start the flow delivery of the UltiMate Micropump from the CHROMELEON control panel.
c) After equilibration, start the sequence 'XQ_3_UV_Linearity_NAN' (or '_CAP', respectively).

To evaluate the result of the 'UV Detector linearity Test':

a) Use the quantization file '*caffeine.qnt'.*
b) Check the integration and correct if necessary.
c) Verify that the correlation coefficient meets the acceptance criteria (TABLE 2-3).

4.8 Reproducibility of the Injection Volume

For the injection reproducibility 8 consecutive partial loop injections of a standard solution of caffeine (20.0 µg/ml for NAN and 5.0 µg/ml for CAP) are performed. The peak area is used for the calculation of the injection reproducibility.

4.8.1 Performing the Test

a) Setup the UltiMate system and the FAMOS Microautosampler as discussed in Sections 3.2.2 A and 3.2.2 B.

b) Start the flow delivery of the UltiMate Micropump from the CHROMELEON control panel.

c) After equilibration start the sequence 'XQ_4_Autosampler Reproducibility_NAN' (or '_CAP', respectively).

TABLE 4-2 Experimental Conditions – Repro Injection Volume

Parameter	Nano Configuration	Capillary Configuration
Detection wavelength	272 nm	272 nm
Run time	8 min	4 min
Flow rate	0.4 µL/min	4.0 µL/min
CRP	625	50
Injection loop	1.0 µL	1.0 µL
Injection type	Partial Loop Fill, 0.2 µL	Partial Loop Fill, 0.5 µL

To evaluate the result of the 'Reproducibility of the Injection Volumes Test':

a) Use the quantization file '*caffeine.qnt'.*

b) Check the integration and correct if necessary.

c) Calculate the correlation coefficient.

d) Verify that the correlation coefficient meet the acceptance criteria (TABLE 2-3).

4.9 Linearity of the injection

The injection linearity is measured by injecting different amounts of the same caffeine standard. The resulting peak area is used for the calculations.

An example of a calibration curve is presented in FIGURE 4-9.

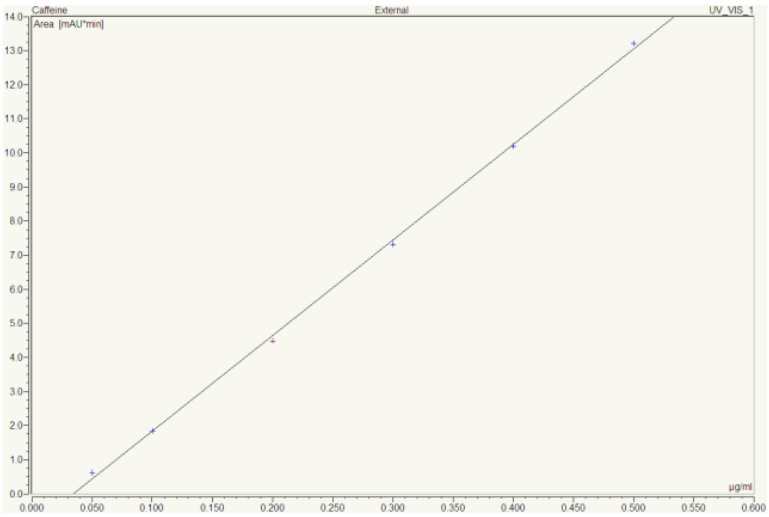


FIGURE 4-9 Example of a Calibration Curve -
Nano LC system, 99.96 % Correlation Coefficient

4.9.1 Performing the Test

a) Setup the UltiMate system and the FAMOS Microautosampler as discussed in Sections 3.2.2 A and 3.2.2 B.

b) Start the flow delivery of the UltiMate Micropump from the CHROMELEN control panel.

c) After equilibration start the sequence 'XQ_5_Autosampler Linearity_NAN' (or '_CAP', respectively).

TABLE 4-3 Experimental Conditions – Repro Injection Volume

Parameter	Nano Configuration	Capillary Configuration
Detection wavelength	272 nm	272 nm
Run time	8 min	4 min
Flow rate	0.4 µL/min	4.0 µL/min
CRP	625	50
Injection loop	1.0 µL	1.0 µL
Injection type	Partial Loop Fill, 0.2 µL	Partial Loop Fill, 0.5 µL

To evaluate the result of the 'Linearity of the Injection' Test:

a) Use the quantization file '*caffeine.qnt'.*

b) Check the integration and correct if necessary.

c) Calculate the correlation coefficient.

d) Verify that the correlation coefficient meet the acceptance criteria (TABLE 2-3).

4.10 Gradient Accuracy

The gradient accuracy is tested by performing a step gradient. Water with a trace of acetone is mixed with pure water. Detection of the acetone is performed at 254 nm. Different gradient programs are used for the NAN and the CAP configurations (the NAN program lasts 80 minutes while the CAP program is 50 minutes).

Typical examples of the step gradient profiles are presented in FIGURE 4-10 (NAN configuration) and in FIGURE 4-11 (CAP configuration).

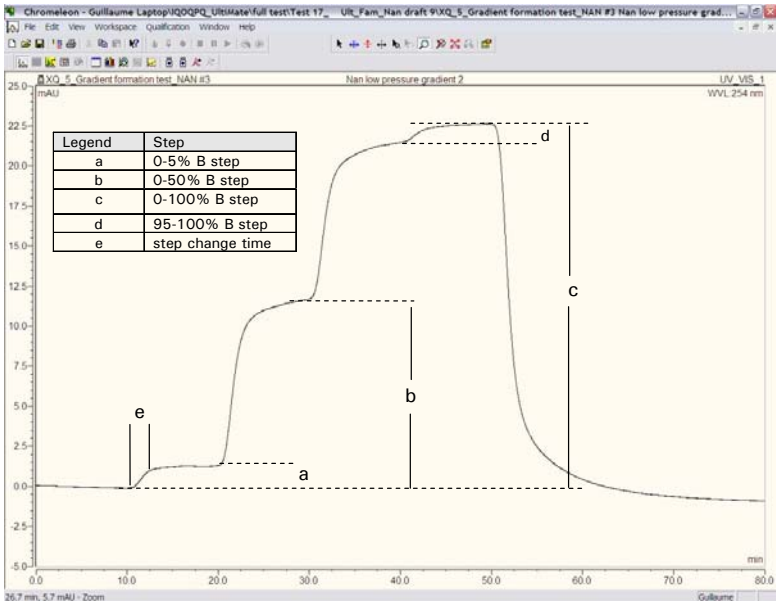


FIGURE 4-10 Typical Step Gradient Profile – NAN Configuration

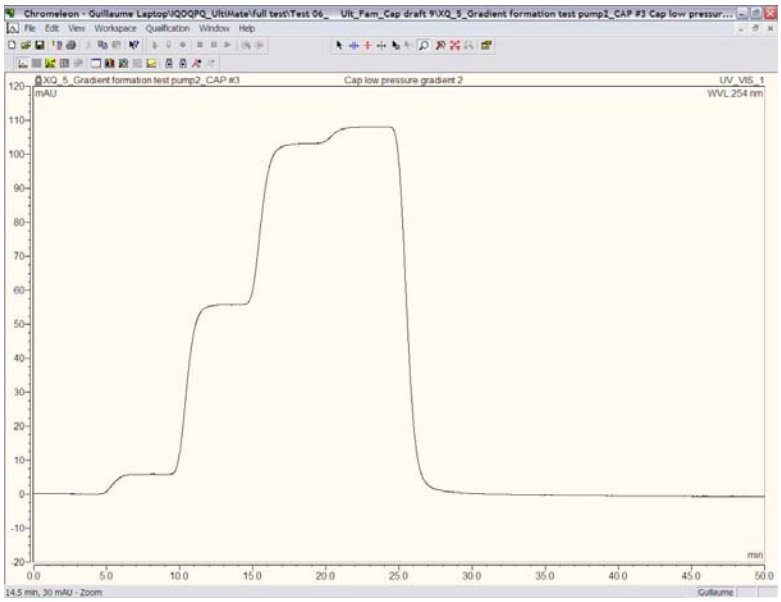


FIGURE 4-11 Typical Step Gradient Profile – CAP Configuration

The steps (items a- d, FIGURE 4-10) are quantified by measuring the UV absorbance difference. The step delay (item e, FIGURE 4-10) represents response time of the gradient formation and indicates that the volume of the tubings are correct.

The programs used for the gradient accuracy tests are listed in TABLE 4-4 and TABLE 4-5.

TABLE 4-4 Program for the Gradient Accuracy Test on a NAN Configuration

Time [min]	%A	%B	Wavelength [nm]
0.00	100	0	254
10.00	100	0	"
10.01	95	5	"
20.00	95	5	"
20.01	50	50	"
30.00	50	50	"
30.01	5	95	"
40.00	5	95	"
40.01	0	100	"
50.00	0	100	"
50.01	100	0	"
80.00	100	0	"

TABLE 4-5 Program for the Gradient Accuracy Test on a CAP Configuration

Time [min]	%A	%B	Wavelength [nm]
0.00	100	0	254
5.00	100	0	"
5.01	95	5	"
10.00	95	5	"
10.01	50	50	"
15.00	50	50	"
15.01	5	95	"
20.00	5	95	"
20.01	0	100	"
25.00	0	100	"
25.01	100	0	"
50.00	100	0	"

Experimental conditions for the step gradient test for nano- and capillary LC systems are listed in TABLE 4-6.

TABLE 4-6 Experimental Conditions for the Step Gradient Test.

Parameter	Nano Configuration	Capillary Configuration
Solvents	A: HPLC – Water B: HPLC – Water with Acetone C: 100% ACN + 0.1% FA (a) D: 100% Water + 0.1% FA	
Acetone concentration of Solvent B	0.8%	0.3%
Detection wavelength	254 nm	254 nm
Run time	80 min	50 min
Flow rate	0.4 µl/min	4.0 µl/min
Note:	a) The acetonitrile is used to perform a wash step during the FAMOS test procedure 4.8.	

4.10.1 Performing the Test

a) Setup the UltiMate system and the FAMOS Microautosampler as discussed in Sections 3.2.2 A and 3.2.2 B and TABLE 4-6. Degas the solvents properly.

b) The four channels should be well flushed using the Purge function of the Micropump.

c) Start the flow delivery of channel B from the CHROMELEON panel and monitor the baseline until it is stable.

d) After equilibration start the sequence 'XQ_6_ Gradient Formation Test_NAN' (or '_CAP', respectively).

Note: If the maximum absorbance observed during this test is greater than the highest absorption monitored during the linearity test (4.7), dilute B (or D) with mobile phase A so that the signal remains on scale when this test is run.

e) Verify that the results meet the acceptance criteria (TABLE 2-3).

f) Repeat steps (a) and (h) for the Gradient Pump 2 of the UltiMate Dual Gradient system (if applicable).

4.11 Switchos Fluid Path Check

The Switchos path test is to check if the solvent selection valve close and open properly and if the resistance of the flow path is within the specifications.

4.11.1 Performing the test

To determine if all flow paths are within the specifications:

a) Prepare the Switchos with the mobile phases listed in Section 4.12.1 and degas them properly. Purge all four channels.

b) Disconnect the solvent inlet line from the pump head and connect the 250 µl syringe (P/N 163241, the plunger should be removed) using an adapter (P/N 160259) as presented in FIGURE 4-12.

FIGURE 4-12 Placing the Syringe on the Solvent Inlet Line

c) Set the flow rate to 0.00 mL/min and set the Switchos in LOCAL mode.

d) Select solvent channel A by the **SSV** button on the rear. The LED 'A' should be illuminated.

e) Measure the flow rate from the inlet tubing for one minute.

f) Repeat steps d) and (e) for each solvent line.

g) Verify that the results meet the acceptance criteria (TABLE 2-3) and fill in the appropriate field in the 'Other tests' page of the report.

4.12 Switchos Flow Rate and Pressure Stability Test

The Switchos is used for pre-concentration experiments. A stable loading flow is essential for the sample transport to the trap column. The flow rate and pressure stability are checked with a CHROMELEON program. A typical pressure profile for this test is presented in FIGURE 4-13.

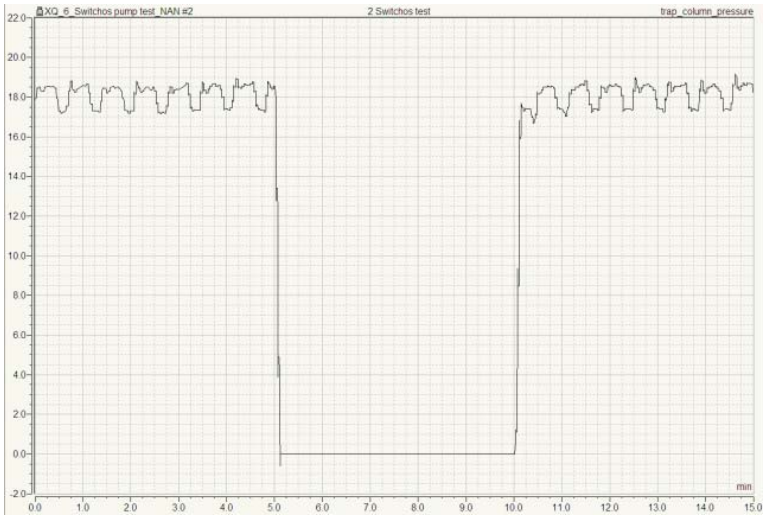


FIGURE 4-13 Typical Pressure Profile of the Switchos Loading Pump

4.12.1 Performing the test

a) Open the Helium shut-off valve on the bottle cap assembly and degas the solvent.

b) The four channels should be well flushed using the Purge function of the pump.

c) Connect a 30 µm I.D. tubing (P/N 160182) to port 2 of the Switchos valve A.

d) Connect the Switchos output to the port 1 of the Switchos valve A using a 130 µm I.D. tubing (P/N 160180).

e) Connect port 10 to waste using the same type of tubing.

f) Start the Switchos pump from the panel.

g) After equilibration start the sequence 'XQ_7_Switchos Pump Test_NAN' (or '_CAP', respectively).

h) Measure the flow rate with a syringe and chronometer watch for 5 min

i) Verify that the parameters meet the specifications presented in TABLE 2-3 and fill in the appropriate field in the 'Other Tests' page of the report. The result for the pressure stability test is on the 'Switchos' page of the report.

4.13 Switchos Valve Position Check

The proper functioning of the switching valves is checked in local control mode. The local/remote switch can be found on the back panel of the Switchos.

4.13.1 Performing the Test

a) Put the Switchos in LOCAL mode.

b) Switch valve A and valve B from position '1-2' to position '10-1' using the manual control button on the front panel.

c) Connect a syringe adapter ((P/N 160259) to port 1 of valve A.

d) Flush with a 250 µl syringe water through port 1 into port 10.

e) Verify that the valve position corresponds to the LED indicator.

4.14 Completing and Printing the OQ/PQ Report

4.14.1 Result of the Gradient Accuracy Test

The observed delay time may be slightly different than the expected delay time indicated in (TABLE 4-7) because of small differences in the tubing size due to the manufacturing process. Due to this the calculated result of the 'Gradient Accuracy' test (Section 4.10) may be wrong and the system may not pass the test. In such a case the delay times should be checked and adjusted if necessary. This applies for the 5%, 50%, 95% and 100% steps or if the result is not within the specifications only.

TABLE 4-7 Predefined Step Delay Time

Step	NAN	CAP
5 %	17.0 .. 18.0 min	8.0.. 9.0 min
50 %	27.0 .. 28.0 min	13.0 .. 14.0 min
95 %	37.0 .. 38.0 min	18.0 .. 19.0 min
100 %	47.0 .. 48.0 min	23.0 .. 24.0 min

To change the delay time:

a) Open the UV-trace obtained for the third step gradient (out of 3)

b) Measure the retention times corresponding to the middle of 5%, 50%, 95% and 100% steps.

c) Select the report layout. Open the 'Pump Gradient' sheet and go to line 137.

d) Make sure to modify the correct columns only (FIGURE 4-14):

- D, E, F, G for capillary (CAP) configurations
- J, K, L, M for nano (NAN) configurations

132										
133	Calculation of Gradientaccuracy and -reproducibility:									
134										
135										
136										
137		Observed Values for Pump in capillary mode						Observed Values for pump in nano mode		
138	Name	Signal Step	Signal Step 5	Signal Step 50	Signal Step 95	Signal Step 100	Sig	Signal Step Start	Signal Step 5	Signal Step 50
140		mAU	mAU	mAU	mAU	mAU	mA	mAU	mAU	mAU
141										
142		UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV	UV_VIS_1	UV_VIS_1	UV_VIS_1
143	Equilibration	0.006	-0.061	29.291	1.391	0.501	n.a	-0.065	29.291	1.201
144	Nan low pressure gradient	-0.088	-0.224	0.751	16.700	30.724	1	-0.243	0.751	17.003
145	Nan low pressure gradient	-0.073	-0.240	1.318	16.929	30.714	1	-0.259	1.318	17.242
146	Nan low pressure gradient	-0.086	-0.274	1.284	16.802	30.636	1	-0.286	1.284	17.128
147	stop	n.a.	n.a.	n.a.	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
148										
149										

Note: The first part is dedicated to cap LC and the other to Nano LC.

FIGURE 4-14 Section of the Report Table to be modified

e) Double click on each column to open the **Properties Report Column** box (FIGURE 4-15).

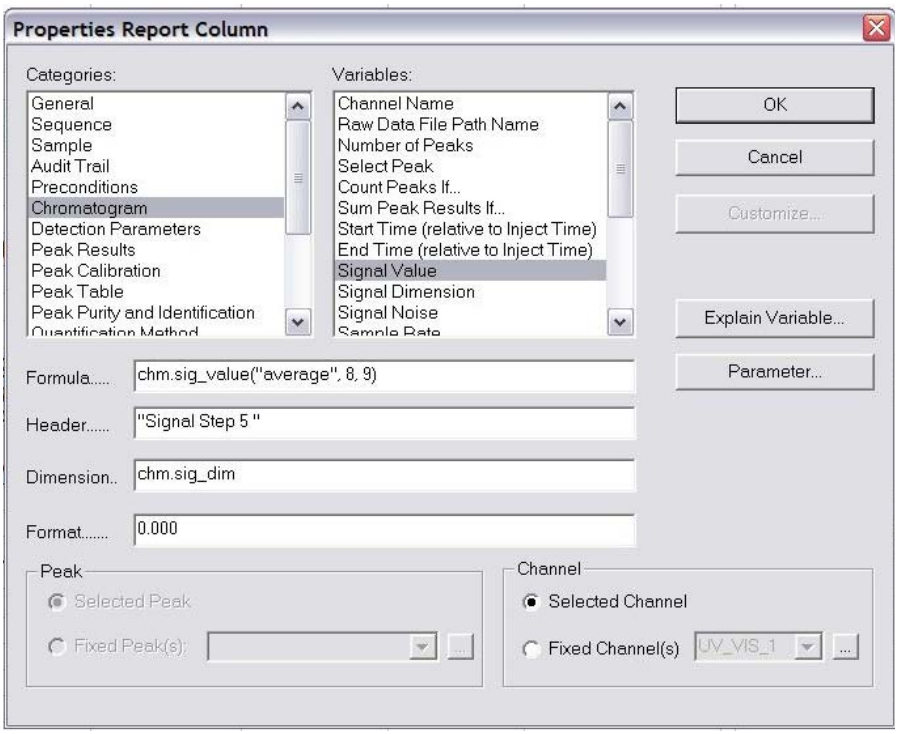


FIGURE 4-15 The Properties Report Column Box

f) Check that the segment that is used for calculation is in the middle of the step.

g) Modify the values in the 'Formula' field (FIGURE 4-15). Each segment used for the calculation is defined by a start and a stop time (e.g. in the example in FIGURE 4-15 starts at 8 minutes and ends at 9 minutes).

h) Check that the test result is correct and save the report.

4.14.2 Completing the Cover Page and Printing

Some of the instrument parameters required for the report can not be completed automatically by CHROMELEON and require manual input (e.g. customer information, S/N of the FAMOS Microautosampler).

To complete the 'Basic Tests' page of the report:

a) Open the report (PQ_OQ_LCP.rdf).

b) Choose the 'Specifications' sheet.

c) Select the 'Instruments' section and enter the missing information (e.g. S/N of the FAMOS, flow cell, thermometer, etc.).

d) Select the 'Additional Information' section and enter the customer information (e.g. name, operator, position, etc.).

e) Choose the 'Other Tests' sheet and enter the result of the manual tests (e.g. Section 4.2, 4.3, 4.4, etc.).

f) Check the result of the Gradient Accuracy Test as described in Section 4.14.1.

g) Save the report.

h) Print the report using the 'Batch Report' option form the 'File' menu.

CHAPTER 5

Troubleshooting

5.1 Overview

This section provides troubleshooting information which is related to the OQ/PQ procedures. More information about instrument specific troubleshooting (e.g. what do if an instrument performs not within its specifications) is provided in the documentation shipped with the instruments and in the service manuals available for the instruments.

5.2 Sample and Mobile Phase Considerations

To optimize performance of the system, we recommend that all samples and mobile phases are free of particulate matter. Samples and mobile phases should be filtered through a 0.22 μm membrane filter. The filter should be checked to ensure that extractable materials are not present.

Caution: It is strongly recommend that only bottled HPLC grade water and HPLC grade solvent be used for these tests. If water from water purification systems is used, polymeric contamination may seriously damage the flow cell. This is especially true if sample pre-concentration or 2D separations are performed. This polymeric contamination may also seriously damage the flow cell (e.g. coating of the capillary walls).

After you have finished using the system, flush the system with a water/methanol or water/acetonitrile mobile phase before shutting it down.

The solvents must be degassed via the He degassing technique described before. If other techniques are used (e.g. vacuum degassing) the performance of the system will be seriously degraded and the performance specifications will not be obtained.

Note: The pump head of the Micropump should be backflushed with iso-propanol/water (1:1). If crystalline materials are deposited in the pump head, irreversible damage to seals and or the piston may result; this will dramatically shorten the life of these components.

Caution: Older revisions of the UltiMate system may be equipped with the older type of the C and D solenoid valves (P/N160051). Due to their limited resistance against strong organic solvents, do not expose these valves to acetonitrile for a longer period than required to perform the wash cycle of test 4.8 (e.g. not longer than 4 h).

It is recommended that you perform the wash step separately and to use channel A or B on such systems. Alternatively, the system can be upgraded with the new valve type (P/N 162297). Refer to Service Information #036 for more details.

5.3 Probable Causes and Solutions

Test Procedure	Problem	Probable Cause	Solution
All	No enough COM ports available.	• -	• Control Switchos valves by event outputs.
Lamp Intensity of the UV Detector (4.2)	Intensity values too low.	• Old lamp. • Wrong wavelength. • Integration time too low.	• Replace. • Check/change. • Check/adjust.
Wavelength Check (4.3)	Failed.	• Filter not in the optical path.	• Check filter position.
Flow Cell Check (4.4)	Intensity values too low.	• See above (4.2). • Dirty flow cell	• See above (4.2). • Clean/replace.
UltiMate Fluid Path Test (4.5)	Flow rate too low.	• Solvent filter clogged.	• Check/replace.
Baseline Noise and Drift Test of the UV Detector (4.6)	Drift too high.	• Old lamp.	• Replace.
Oven accuracy (4.6)	Temperature readout not within the specifications.	• Temperature probe not properly installed.	• Install properly (e.g. use a 4 mm screw).
Linearity of the UV Detector (4.7)	Signal is out of range.	• Acetone concentration too high.	• Dilute with mobile phase A.
Reproducibility of the Injection Volume (4.8) Linearity of the injection (4.9)	'Ghost" peak before the caffeine peak.	• Contaminated injection valve	• Extend wash step. • Sonicate rotor and stator in CAN for 10 min.
	Caffeine peak shows some 'spikes', improper integration.	• Air in the syringe.	• Check syringe speed and time constant (Chapter 6).
		• Air in the syringe. • Air at the bottom of the sample vial.	• Purge syringe, • Remove air.
Gradient Accuracy (4.10)	Considerable drift of the 95% to 100% B steps.	• No/too little formic acid in mobile phase A and B.	• Check/replace solvents.
	Result not within specification	• Wrong delay time. • Hardware.	• Check/modify. • Check filter, valves, etc.
Switchos Fluid Path Check (4.11)	Flow rate too low.	• See above (4.5).	• See above (4.5).
Switchos Flow Rate and Pressure Stability Test (4.12)	Pulsation too high.	• Check valve not working properly.	• Clean/replace.
Switchos Valve Position Check (4.13)	Both position LEDs are illuminated at the same time.	• Improper initialization or faulty controller.	• See Service Information #043.

CHAPTER 6

CHROMELEON® Listings

6.1 Overview

The CD ROM 'IQOQPQ on UltiMate™ (Plus) Systems' (Version 1) provides ready-to-use CHROMELEON programs and sequences (FIGURE 6-1), which will allow easy control of the standard UltiMate system configurations. Please refer to Section 3.2.1 for modifications which may be necessary due to a different hardware configuration.

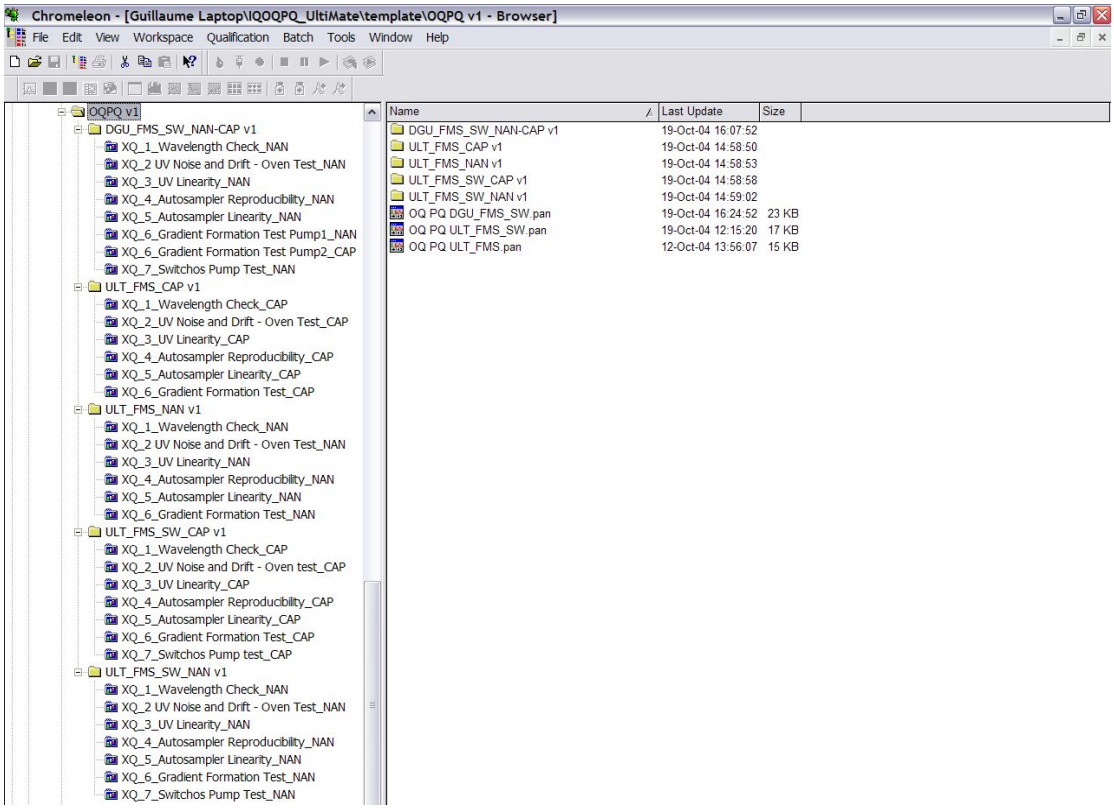


FIGURE 6-1 Available CHROMELEON Sequences for OQ/PQ

The following sections lists the CHROMELEON programs used to run a UltiMate system in NAN configuration with a flow sensor installed in conjunction with a FAMOS Well Plate Microautosampler (cooling option installed) and a Switchos Advanced Microcolumn Switching Unit.

6.2 Wavelength Check (NAN Configuration)

Sequence: XQ_1_Wavelength Check_NAN

```
;Program file for wavelength check
;System must be setup for Nano LC experiments
;Program version 08/10/2004


Sampler.TempCtrl =                  Off
Oven.TempCtrl =                     Off
pump.%A.Equate =                    "100% water + 0.1% FA"
%B.Equate =                         "Mob. phase A + 0.8% acetone"
%C.Equate =                         "%C"
%D.Equate =                         "%D"
Diameter =                          75um
Length =                            15cm
StationaryPhase =                   C18_3um_100A
; No automatic CRP calibration!
MasterPressure.LowerLimit =         0.0
MasterPressure.UpperLimit =         400.0
ColumnPressure.LowerLimit =         0.0
ColumnPressure.UpperLimit =         200.0
TrapColumnPressure.LowerLimit =     0.0
TrapColumnPressure.UpperLimit =     200.0

InjectMode =                        FullLoop
LowDispersionMode =                 Off
UseAirSegment =                     Off
UseHeadSpace =                      Off
SyringeSpeed =                      Low
SyringeSpeedFactor =                0.1
SampleHeight =                      4
FlushVolume =                       5.0
WashVolume =                        50
RinseBetweenReinjections =          Yes
Data_Collection_Rate =              2
pump.Flow =                         0.000
%B =                                0
%C =                                0
%D =                                0
;Settings for UV detector
Data_Collection_Rate =              2
UV_VIS_1.Wavelength =               254
TimeConstant =                      2.0


0.000   Wait                        UV.Ready and Pump.Ready and
                                     Pump_FlowSensor.Ready and Sampler.Ready

        Inject
        Wavelength_Check
        UV_VIS_1.AcqOn

1.00    UV_VIS_1.AcqOff
        End
```

6.3 Noise and Drift Test (NAN Configuration)

Sequence: XQ_2 UV Noise and Drift - Oven Test_NAN

```
Wait                                    Sampler.Ready
;Program file for drift and noise test
;System must be setup for Nano LC experiments
;Program version 08/10/2004

;Settings for restrictor (320cm, 15um ID)
Diameter =                              75um
Length =                                15cm
StationaryPhase =                       C18_3um_100A
;CRP =                                  625
CalibrateCRP                            When=BeforeFirstSample
;ParkPercentage =                       Disabled


;Pump limits settings
MasterPressure.LowerLimit =             0.0
MasterPressure.UpperLimit =             400.0
Pump.columnPressure.LowerLimit = 0.0
Pump.columnPressure.UpperLimit = 200.0
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         200.0


;Settings for UV detector
Data_Collection_Rate =                  2
UV_VIS_1.Wavelength =                   254
TimeConstant =                          2.0

;FAMOS settings
Wait                                    Sampler.Ready
InjectMode =                            Fullloop
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                     On
;Sampler.Temperature.Nominal =          20.00
;Sampler.Temperature.LowerLimit =       5.00
;Sampler.Temperature.UpperLimit =       30.00

;Virtual channel settings
pump_pressure.Formula                   Formula=masterpressure.value
pump_pressure.Type =                    Analog
column_pressure.Formula                 Formula=pump.columnpressure.value
column_pressure.Type =                  Analog


;Ultimate pump settings
Pump.%A.Equate =                        "100% water + 0.1% FA"
Pump.%B.Equate =                        "Mob. phase A + 0.8% acetone"
Pump.%C.Equate =                        "%C"
Pump.%D.Equate =                        "%D"
Pump.Flow =                             0.4
%B =                                    0
%C =                                    0
%D =                                    0

0.000   Wait                            UV.Ready and Pump.Ready and Sampler.Ready
                                        and pump_flowsensor.ready
        Inject
```

```
            UV_VIS_1.AcqOn
            pump_pressure.AcqOn
            column_pressure.AcqOn

21.000      UV_VIS_1.AcqOff
            pump_pressure.AcqOff
            column_pressure.AcqOff

            End
```

6.4 UV Linearity (NAN Configuration)

Sequence: XQ_3_UV Linearity_NAN

```
Wait                                Sampler.Ready
;Program file for UV linearity test
;System must be setup for Nano LC experiments
;Program version 08/10/2004


;Settings for restrictor (320cm, 15um ID)
Diameter =                          75um
Length =                            15cm
StationaryPhase =                   C18_3um_100A
;CRP =                              625
CalibrateCRP                        When = BeforeFirstSample
;ParkPercentage =                   Disabled


;Pump limits settings
MasterPressure.LowerLimit =         0.0
MasterPressure.UpperLimit =         400.0
Pump.columnPressure.LowerLimit = 0.0
Pump.columnPressure.UpperLimit = 200.0
TrapColumnPressure.LowerLimit =     0.0
TrapColumnPressure.UpperLimit =     200.0


;Settings for UV detector
Data_Collection_Rate =              2
UV_VIS_1.Wavelength =               272
TimeConstant =                      2

;FAMOS settings
Wait                                Sampler.Ready
InjectMode =                        Partial
LowDispersionMode =                 Off
UseAirSegment =                     Off
UseHeadSpace =                      Off
RinseBetweenReinjections =          Yes
SyringeSpeed =                      Low
SyringeSpeedFactor =                0.1
SampleHeight =                      9
FlushVolume =                       5.0
WashVolume =                        50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                 On
;Sampler.Temperature.Nominal =      20.00
;Sampler.Temperature.LowerLimit =   5.00
;Sampler.Temperature.UpperLimit =   30.00

;Virtual channel settings
pump_pressure.Formula               Formula = masterpressure.value
pump_pressure.Type =                Analog
column_pressure.Formula             Formula = pump.columnpressure.value
column_pressure.Type =              Analog


;Ultimate pump settings
Pump.%A.Equate =                    "100% water + 0.1% FA"
Pump.%B.Equate =                    "Mob. phase A + 0.8% acetone"
Pump.%C.Equate =                    "%C"
```

```
            Pump.%D.Equate =            "%D"
            Pump.Flow =                 0.4
            %B =                        0
            %C =                        0
            %D =                        0


    0.000   UV.Autozero
            Wait                        Sampler.Ready and UV.Ready and Pump.Ready
                                        and pump_flowsensor.ready

            Inject
            UV_VIS_1.AcqOn
            pump_pressure.AcqOn
            column_pressure.AcqOn

    8.00    UV_VIS_1.AcqOff
            pump_pressure.AcqOff
            column_pressure.AcqOff

            End
```

6.5 Autosampler Reproducibility (NAN Configuration)

Sequence: XQ_4_Autosampler Reproducibility_NAN

```
Wait                                    Sampler.Ready
;Program file for injection reproducibility test
;System must be setup for Nano LC experiments
;For this test time constant is 0.5 instead of 2.0
;Program version 08/10/2004


;Settings for restrictor (320cm, 15um ID)
Diameter =                              75um
Length =                                15cm
StationaryPhase =                       C18_3um_100A
;CRP =                                  625
CalibrateCRP                            When=BeforeFirstSample
;ParkPercentage =                       Disabled


;Pump limits settings
MasterPressure.LowerLimit =             0.0
MasterPressure.UpperLimit =             400.0
Pump.columnPressure.LowerLimit = 0.0
Pump.columnPressure.UpperLimit = 200.0
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         200.0


;Settings for UV detector
Data_Collection_Rate =                  2
UV_VIS_1.Wavelength =                   272
TimeConstant =                          0.5

;FAMOS settings
Wait                                    Sampler.Ready
InjectMode =                            Partial
LowDispersionMode =                     Off
UseAirSegment =                         Off
UseHeadSpace =                          Off
RinseBetweenReinjections =              Yes
SyringeSpeed =                          Low
SyringeSpeedFactor =                    0.1
SampleHeight =                          9
FlushVolume =                           5.0
WashVolume =                            50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                     On
;Sampler.Temperature.Nominal =          20.00
;Sampler.Temperature.LowerLimit =       5.00
;Sampler.Temperature.UpperLimit =       30.00


;Virtual channel settings
pump_pressure.Formula                   Formula=masterpressure.value
pump_pressure.Type =                    Analog
column_pressure.Formula                 Formula=pump.columnpressure.value
column_pressure.Type =                  Analog


;Ultimate pump settings
Pump.%A.Equate =                        "100% water + 0.1% FA"
```

```
            Pump.%B.Equate =                "Mob. phase A + 0.8% acetone"
            Pump.%C.Equate =                "%C"
            Pump.%D.Equate =                "%D"
            Pump.Flow =                     0.4
            %B =                            0
            %C =                            0
            %D =                            0


     0.000  UV.Autozero
            Wait                            Sampler.Ready and UV.Ready and Pump.Ready
                                            and pump_flowsensor.ready

            Inject
            UV_VIS_1.AcqOn
            Pump_pressure.AcqOn
            Column_pressure.AcqOn

      8.00  UV_VIS_1.AcqOff
            Pump_pressure.AcqOff
            Column_pressure.AcqOff

            End
```

6.6 Autosampler Injection Linearity (NAN Configuration)

Sequence: XQ_5_Autosampler Linearity_NAN

```
Wait                                    Sampler.Ready
;Program file for injection linearity test
;System must be setup for Nano LC experiments
;For this test time constant is 0.5 instead of 2.0
;Program version 08/10/2004


;Settings for restrictor (320cm, 15um ID)
Diameter =                              75um
Length =                                15cm
StationaryPhase =                       C18_3um_100A
;CRP =                                  625
CalibrateCRP                            When=BeforeFirstSample
;ParkPercentage =                       Disabled


;Pump limits settings
MasterPressure.LowerLimit =             0.0
MasterPressure.UpperLimit =             400.0
Pump.columnPressure.LowerLimit = 0.0
Pump.columnPressure.UpperLimit = 200.0
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         200.0


;Settings for UV detector
Data_Collection_Rate =                  2
UV_VIS_1.Wavelength =                   272
TimeConstant =                          0.5

;FAMOS settings
Wait                                    Sampler.Ready
InjectMode =                            Partial
LowDispersionMode =                     Off
UseAirSegment =                         Off
UseHeadSpace =                          Off
RinseBetweenReinjections =              Yes
SyringeSpeed =                          Low
SyringeSpeedFactor =                    0.1
SampleHeight =                          9
FlushVolume =                           5.0
WashVolume =                            50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                     On
;Sampler.Temperature.Nominal =          20.00
;Sampler.Temperature.LowerLimit =       5.00
;Sampler.Temperature.UpperLimit =       30.00

;Virtual channel settings
pump_pressure.Formula                   Formula=masterpressure.value
pump_pressure.Type =                    Analog
column_pressure.Formula                 Formula=pump.columnpressure.value
column_pressure.Type =                  Analog


;Ultimate pump settings
Pump.%A.Equate =                        "100% water + 0.1% FA"
Pump.%B.Equate =                        "Mob. phase A + 0.8% acetone"
```

```
            Pump.%C.Equate =                "%C"
            Pump.%D.Equate =                "%D"
            Pump.Flow =                     0.4
            %B =                            0
            %C =                            0
            %D =                            0


    0.000   UV.Autozero
            Wait                            Sampler.Ready and UV.Ready and Pump.Ready
            Inject
            UV_VIS_1.AcqOn
            pump_pressure.AcqOn
            column_pressure.AcqOn

   10.000   UV_VIS_1.AcqOff
            pump_pressure.AcqOff
            column_pressure.AcqOff

            End
```

6.7 Gradient Accuracy Test (NAN Configuration)

Sequence: XQ_6_Gradient Formation Test_NAN

```
Wait                                    Sampler.Ready
;Program file for gradient formation test
;System must be setup for Nano LC experiments
;The time constant must be set to 0.5 instead of 2.0
;Program version 08/10/2004

;Settings for restrictor (320cm, 15um ID)
Diameter =                              75um
Length =                                15cm
StationaryPhase =                       C18_3um_100A
;CRP =                                  625
CalibrateCRP                            When = BeforeFirstSample
;ParkPercentage =                       Disabled


;Pump limits settings
MasterPressure.LowerLimit =             0.0
MasterPressure.UpperLimit =             400.0
ColumnPressure.LowerLimit =             0.0
ColumnPressure.UpperLimit =             200.0
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         200.0


;Settings for UV detector
Data_Collection_Rate =                  2
UV_VIS_1.Wavelength =                   254
TimeConstant =                          0.5

;FAMOS settings
InjectMode =                            Fullloop
LowDispersionMode =                     Off
UseAirSegment =                         Off
UseHeadSpace =                          Off
RinseBetweenReinjections =              Yes
SyringeSpeed =                          Low
SyringeSpeedFactor =                    0.1
SampleHeight =                          4
FlushVolume =                           5.0
WashVolume =                            50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                     On
;Sampler.Temperature.Nominal =          20.00
;Sampler.Temperature.LowerLimit =       5.00
;Sampler.Temperature.UpperLimit =       30.00


;Virtual channel settings
pump_pressure.Formula                   Formula = masterpressure.value
pump_pressure.Type =                    Analog
column_pressure.Formula                 Formula = pump.columnpressure.value
column_pressure.Type =                  Analog


;Ultimate pump settings
pump.%A.Equate =                        "100% water + 0.1% FA"
%B.Equate =                             "Mob. phase A + 0.8% acetone"
%C.Equate =                             "%C"
```

```
            %D.Equate =                     "%D"


    0.000   UV.Autozero
            pump.Flow =                     0.400
            %B =                            100
            %C =                            0
            %D =                            0
            Wait                            UV.Ready and Pump.Ready and
                                            Pump_FlowSensor.Ready and Sampler.Ready
            Sampler.Inject
            UV_VIS_1.AcqOn
            Pump_pressure.AcqOn
            Column_pressure.AcqOn
            pump.Flow =                     0.400
            %B =                            100
            %C =                            0
            %D =                            0

    7.000   %B =                            100

    7.010   %B =                            0

   30.000   UV_VIS_1.AcqOff
            Pump_pressure.AcqOff
            Column_pressure.AcqOff
            pump.Flow =                     0.400
            %B =                            0
            %C =                            0
            %D =                            0

            End
```

6.8 Switchos Pump Test (NAN Configuration)

Sequence: XQ_7_Switchos Pump Test_NAN

Program:

```
;Program file for switchos pump stability test
;Program version 08/10/2004


;Settings for restrictor (320cm, 15um ID)
Diameter =                          75um
Length =                            15cm
StationaryPhase =                   C18_3um_100A
;CRP =                              625
CalibrateCRP                        When = BeforeFirstSample
;ParkPercentage =                   Disabled


;Pump limits settings
MasterPressure.LowerLimit =         0.0
MasterPressure.UpperLimit =         400.0
ColumnPressure.LowerLimit =         0.0
ColumnPressure.UpperLimit =         200.0
TrapColumnPressure.LowerLimit =     0.0
TrapColumnPressure.UpperLimit =     200.0

;Settings for UV detector
Data_Collection_Rate =              2
UV_VIS_1.Wavelength =               254
TimeConstant =                      2

;FAMOS settings
InjectMode =                        Fullloop
LowDispersionMode =                 Off
UseAirSegment =                     Off
UseHeadSpace =                      Off
RinseBetweenReinjections =          Yes
SyringeSpeed =                      Low
SyringeSpeedFactor =                0.1
SampleHeight =                      4
FlushVolume =                       5.0
WashVolume =                        50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                 On
;Sampler.Temperature.Nominal =      20.00
;Sampler.Temperature.LowerLimit =   5.00
;Sampler.Temperature.UpperLimit =   30.00


;Virtual channel settings
pump_pressure.Formula               Formula = masterpressure.value
pump_pressure.Type =                Analog
column_pressure.Formula             Formula = pump.columnpressure.value
column_pressure.Type =              Analog
Loading_Pump.TrapColumnPressure.LowerLimit =  0.0
Loading_Pump.TrapColumnPressure.UpperLimit =  400.0
Trap_Column_Pressure.Formula
Formula = Loading_Pump.TrapColumnPressure.value
Trap_Column_Pressure.Type =         Analog
```

```
            ;Switchos pump settings
            Loading_Pump.%A.Equate =        "Pure water + 0.1% FA"


0.000       Loading_Pump.Flow =             0.030
            Wait                            Loading_Pump.Ready
            inject
            Trap_Column_Pressure.AcqOn
            Loading_Pump.Flow =             0.030

0.000       Valve_A.Position =              1_2

5.000       Valve_A.Position =              10_1

10.000      Valve_A.Position =              1_2

15.000      Trap_Column_Pressure.AcqOff
            Loading_Pump.Flow =             0.030

            End
```

6.9 Stop Flow

Program:

```
Wait                                    Sampler.Ready
;Program file to stop the flow and lamp at the end of experiments
;Disable the "stop" sample when further experiments have to be carried out
;System must be setup for Nano LC experiments
;Program version 08/10/2004


;Settings for restrictor (320cm, 15um ID)
Diameter =                              75um
Length =                                15cm
StationaryPhase =                       C18_3um_100A
CalibrateCRP                            When = BeforeFirstSample
;CRP =                                  625
;ParkPercentage =                       Disabled


;Pump limits settings
MasterPressure.LowerLimit =             0.0
MasterPressure.UpperLimit =             400.0
ColumnPressure.LowerLimit =             0.0
ColumnPressure.UpperLimit =             200.0
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         200.0

;Settings for UV detector
Data_Collection_Rate =                  2
UV_VIS_1.Wavelength =                   254
TimeConstant =                          2

;FAMOS settings
InjectMode =                            Fullloop
LowDispersionMode =                     Off
UseAirSegment =                         Off
UseHeadSpace =                          Off
RinseBetweenReinjections =              Yes
SyringeSpeed =                          Low
SyringeSpeedFactor =                    0.1
SampleHeight =                          4
FlushVolume =                           5.0
WashVolume =                            50
;Commands for FAMOS with cooling option
;Sampler.TempCtrl =                     On
;Sampler.Temperature.Nominal =          20.00
;Sampler.Temperature.LowerLimit =       5.00
;Sampler.Temperature.UpperLimit =       30.00


;Virtual channel settings
pump_pressure.Formula                   Formula = masterpressure.value
pump_pressure.Type =                    Analog
column_pressure.Formula                 Formula = pump.columnpressure.value
column_pressure.Type =                  Analog

;loading pump settings
TrapColumnPressure.LowerLimit =         0.0
TrapColumnPressure.UpperLimit =         400.0

;Ultimate pump settings
```

```
          pump.%A.Equate =              "100% water + 0.1% FA"
          pump.%B.Equate =              "Mob. phase A + 0.8% acetone"
          pump.%C.Equate =              "%C"
          pump.%D.Equate =              "%D"

  0.000   UV.Autozero
          %B =                          0
          %C =                          0
          %D =                          0
          Wait                          UV.Ready and Pump.Ready and Sampler.Ready
          Inject
          pump.Flow =                   0.4
          %B =                          0
          %C =                          0
          %D =                          0

  4.000   pump.Flow =                   0.000
          Loading_Pump.Flow =           0.000
          %B =                          0
          %C =                          0
          %D =                          0
  5.000   Lamp =                        Off

          End
```