

Instruments Installation Qualification for Thermo Scientific Dionex UltiMate 3000 RSLCnano Systems

Operating Instructions

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1 How to use this Manual

The material included in this manual is provided to assist authorized personnel in performing installation qualification (IQ) on the Thermo Scientific Dionex[™] UltiMate[™] 3000 RSLCnano HPLC systems configured for direct injection or preconcentration with the standard application kits. It is assumed that the individual using this manual has had 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.

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 the Installation Qualification in order to obtain a full understanding of the procedure.

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

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Warnings

The Warning sign and the Important 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:

I Important Information which must be followed for a successful IQ



Recommendation

2 Introduction

Today, analytical laboratories have to meet more and more ISO standards and FDA regulations. That is why validation is becoming an increasingly important issue. For many laboratories it is no longer sufficient to just do things right; they must also provide documented evidence to demonstrate the integrity of their data and validity of their results.

Many laboratories handle this issue using formal quality systems, which are generally implemented in accordance with one or more of the following internationally recognized quality standards:

- ⇒ISO 9001
- ⇒ISO Guide

⇒Good Laboratory Practice (21 CFR, Part 58)

⇒Good Manufacturing Practice (21 CFR, Parts 110, 210, 211, 820, 606)

However, these standards are deliberately written in broad terms so that they are as widely applicable as possible. All stipulate general requirements, such as that the instruments must be fit for purpose, properly maintained, and calibrated to national or international standards.

The Standard Operating Procedures for implementing the **Installation Qualification (IQ)** are adapted as closely as possible to the instrument parameters.

The IQ includes two parts:

1. Check lists covering all details concerning the installation of the instrument (see Section 2.1).

2. The Instrument Installation Qualification Test examines the communication between **CHROMELEON**TM or **Xcalibur**TM and the instrument (see section 2.2), as well as basic chromatographic operation. This is only possible for systems that are operated by **CHROMELEON** or **Xcalibur**.

Perform parts 1 and 2 of the IQ for the initial installation of the instruments.

• When reinstalling one or more instruments after repair or maintenance work has been carried out, only part 2 of the IQ test is required.

2.1 Installation Qualification (Check Lists)

Installation Qualification (IQ) covers all procedures relating to the installation of instruments in a specific environment. Filling in the check lists, which are shipped with the system, is required for the reporting.

The IQ check lists document the following items:

- The instrument(s) (including all modules and accessories) was (were) delivered as ordered (delivery note, order, agreed specification) and inspected for any signs of damage.
- The appropriate documentation was shipped with the instrument; for example, operating instructions, manufacturer's specification, shipping details, and all materials required for operating the instrument.
- The recommended service, maintenance, calibration, and qualification intervals were specified. A contact was named for performing service work and supplying spare parts.
- The required computer hardware and the instrument firmware and software were correctly supplied.
- Information was provided about the consumable goods required for regular operation.
- The selected environment is suitable for the system. There is sufficient space for installation, operation, and supply of the instrument. Appropriate supply and materials (electricity, special gas etc.) are available.
- Information was provided about any health, safety, and environmental issues when operating the instrument. Safety guidelines were provided about which the users were informed.
- The instrument functions perform as expected when first activated. Any deviations are recorded.

- Installation of the instrument was performed according to the manufacturer's guidelines.
- The existing peripheral equipment is correctly connected.

The installation guidelines are described in the instructions for each instrument, but can be extended or changed, as necessary.

2.2 Instrument Installation Qualification Test

The purpose of the Instrument Installation Qualification Test is to prove and document that the UltiMate 3000 functions according to a specification that is suitable for the system's routine operation. The specific conditions required for that application, e.g. solvents, column material, and temperature, must be taken into account.

The Installation Qualification must be performed when the system is installed and can be repeated at time intervals decided by the customer.

The Instrument Installation Qualification tests the communication between CHROMELEON and the connected modules as well as chromatographic function. The procedure is used to confirm that the communication and chromatographic performance meet the specifications.

2.3 For Additional Information

For more detailed information about the operation, maintenance or troubleshooting of the UltiMate 3000 system modules or how to use the CHROMELEON software package, please refer to the documentation provided with these products and to the CHROMELEON online help (F1 key).

3 Test Procedures

3.1 Supported Instruments

IQ is a system-specific procedure. The procedures described below apply to the following instruments:

Instrument	Supported Model	
UltiMate 3000 Series RSLCnano Pump ^(a)	NCS-3500RS NCP-3200RS	
Flow Meter Type:	Classic Flow Meter: Refers to flow meter with flow selectors for NAN: Nano LC (50 – 1000 nL/min) CAP: Capillary LC (0.5 - 10 µL/min) MIC: Micro LC (5 - 50 µL/min) ProFlow™ Flow Meter:	
	Refers to flow meter with thermal flow sensors for NAN: Nano LC (50 - 1500 nL/min)	
UltiMate 3000 Series UV Detector ^(b)	VWD-3X00RS	
UltiMate 3000 Series Autosampler	WPS-3000PL RS(non-cooled, RS version),WPS-3000TPL RS(cooled, RS version),WPS-3000TB(cooled, biocompatible version)	
Mass spectrometer	All	
Note: a) The NCS/NCP module has to be equipped with the flow meter (ProFlow or Classic) required for the IQ application that is being performed. If the Classic flow meter is installed, please ensure that the relevant flow selector (nano, capillary, or micro) is fitted according to the flow rate range for which the instrument is intended to be used.		
b) If the system does not include a VWD-3x00 Detector, the IQ can be performed using a mass spectrometer.		

Table 3-1 List of supported UltiMate 3000 System Components

For systems employing the classic flow meter, all instrument configurations should be controlled by CHROMELEON 6.8 SR12 or later, CHROMELEON 7.2 MUb (7.2.0.4121) or later, or by DCMSlink.

For all systems employing the ProFlow flow meter, all instrument configurations should be controlled by CHROMELEON 6.8 SR16 or later, CHROMELEON 7.2 SR4 or later or SII 1.2 or later.

It is recommended to use a Chromeleon license dongle / license file (when available) to temporarily unlock DCMSlink / SII and operate the software in Chromeleon mode.

Tip: DCMSlink can be configured to delete data automatically after 1 day. To avoid data loss, make sure to either back-up data or to change the settings. For SII setup this is not applicable.

3.2 Instrument Installation Qualification System Conditions and Limits

The sample (Cytochrome C digest, 500 fmol/ μ L for NAN, 8 pmol/ μ L for CAP and 16 pmol/ μ L for MIC) is injected in direct injection (or preconcentration mode for NAN only). For direct injection mode full-loop 1 μ l injections are used. If the nano preconcentration setup is used, 1 μ l partial loop injections are carried out with a 20 μ l sample loop. The peptides are separated on a PepMap C18 RSLC column with a standard acetonitrile gradient.

When a UV detector is used to validate the system, the relative standard deviation (RSD) of the retention times of peak #5 is calculated from 6 runs. The height and peak width at half height of peak #5 and the resolution of peaks #1-4 are determined. When the system is validated using a mass spectrometer, the retention time reproducibility of the peak with m/z 728.84 (peptide sequence TGQAPGFSYTDANK, z=2; equivalent to peak #5 on the UV IQ test) is calculated from 6 runs and the PWHH (peak width half height) from one run is determined.

The calculation of the peak resolution is described in the Chromeleon online help. This IQ procedure uses the values of the following three pairs of neighboring peaks.

Resolution of Peak	Included Peaks for Calculation
Peak 1	Peak 1 and 2
Peak 2	Peak 2 and 3
Peak 3	Peak 3 and 4

3.2.1 Instrument Installation Qualification Limits

Table 2.0	Over view of the IO Test Dress dures and Limits
Table 3-2	Overview of the IQ Test Procedures and Limits

Specification (UV)	NAN	САР	MIC
Retention time (Peak #5)	< 0.30 % RSD	< 0.30 % RSD	< 0.30 % RSD
Resolution of peaks #1 to #4	> 1.5	> 1.5	> 1.5
Peak width at half height (Peak #5)	< 12 seconds	< 12 seconds	< 20 seconds
UV absorbance at 214 nm (Peak #5)	> 3.0 mAU	> 30.0 mAU	> 15.0 mAU
Specification (MS)	NAN	САР	MIC
Retention time (m/z 728.84, 2+)	< 0.30 % RSD	< 0.30 % RSD	< 0.30 % RSD
PWHH	< 12 seconds	< 12 seconds	< 20 seconds

3.2.2 Instrument Installation Qualification System Conditions

Item	Description	P/N	Note
Eluent A	Water + 0.1% FA		
Eluent B	Water/ACN 20/80 + 0.08% FA		
Loading solvent A	Water/ACN 98/2 + 0.05% TFA		NCS-3500RS only
Loading solvent B	Water/ACN 50/50 + 0.05% TFA		NCS-3500RS only
Loading solvent C	Water/ACN 50/50 + 0.05% TFA		NCS-3500RS only
Rear seal wash solvent	Water/MeOH 90/10		
Wash Solvent WPS	Water/ACN 20/80 + 0.08% FA		Eluent B
	0.30 μl/min		NAN configuration
Flow rate	4.00 μl/min		CAP configuration
	40.00 µl/min		MIC configuration
Loading flow rate	30.00 µl/min		Preconcentration only in NAN configuration
	75 μm id x 15 cm PepMap RSLC packed with C18 2 μm	164534	NAN configuration
Column	300 μm id x 15 cm PepMap RSLC packed with C18 2 μm	164537	CAP configuration
	1.0 mm id x 15 cm PepMap RSLC packed with C18 2 μm	164711	MIC configuration
Trap column	300 μm id x 0.5 cm cartridge packed with C18 5 μm	164649/160454	Preconcentration only in NAN configuration
			500 fmol/µL for NAN
Sample	Cytochrome C digest	161089	8 pmol/µL for CAP
			16 pmol/µL for MIC
Injection volume	1 μL Full Loop	6826.2401	Direct Injection
	1 μL Partial Loop (20 μL loop)	5826.2420	Preconcentration
Column temperature	35°C (min 10°C above ambient)		NCS-3500RS only
Sample temperature	5°C		WPS-3000T(B)PL(RS) only
		6074.0270	NAN flow cell (3 nL)
UV wavelength	214 nm	6074.0280	CAP flow cell (45 nL)
		6074.0290	MIC flow cell (180 nL)

Table 3-3 IQ System Conditions

Time (min)	%B
0	4
30	55
31	90
35	90
36	4
60	4

 Table 3-4
 IQ Gradient Program in Direct Injection Configuration

Table 3-5	IQ Gradient Program in Preconcentration Configuration

Time (min)	%В	Valve Position
0	4	1-2
3	4	10-1 (or 6-1)
33	55	
34	90	
38	90	
39	4	
55	4	1-2
60	4	

3.3 Required Materials

All required consumables (e.g. column, tubing, sample) are provided with the NAN, CAP and MIC application kits (which need to be ordered with the system).

To perform the test, water, acetonitrile, TFA and FA are needed (the quality of all solvents must be LC/MS grade).

3.4 For Additional Information

For more detailed information about the operation, maintenance or troubleshooting of the instruments of the UltiMate 3000 system or how to use the CHROMELEON software package, please refer to the documentation provided with these products and to the CHROMELEON online help (F1 key).

3.5 Connecting and Configuring the System

The RSLCnano system can be configured for nano (NAN), capillary (CAP), or micro (MIC) flow rates. All references to NAN are applicable to both, the ProFlow flow meter and Classic flow meter with nano flow selector. The IQ procedures for the different configurations evaluate the same properties, but the setup must be adjusted to the actual (flow) configuration.

Make sure that the system is configured properly. Please refer to the *Operating Instructions manual* for more information. The Chromeleon IQ wizard will automatically select the correct test sequence for the configured system (UV IQ only).

NAN/CAP/MIC: The flow selector that the customer will be using on a routine basis (or the most frequently) should be installed and the system configured before the start of the IQ procedure accordingly. If the system does not include a VWD detector, a mass spectrometer can be used instead.

3.5.1 Preparing the UltiMate 3000 RSLCnano System

While the IQ procedure is similar for the different system configurations (direct injection or preconcentration), the specific procedure depends on the system configuration being tested. Make sure that the system is configured properly. Please refer to the *UltiMate 3000 RSLCnano Standard Applications Manual* for more information.

3.5.1.1 VWD-3x00 UV Detector (when applicable)

Turn on the detector lamp. When you use a VWD-3100 or VWD-3400 detector, <u>only the UV lamp</u> <u>needs to be turned on.</u>

I Important: A VWD-3x00 detector with the appropriate flow cell (3 nL for NAN, 45 nL for CAP and 180 nL for MIC) is required for the IQ procedure if no mass spectrometer is available.

3.5.1.2 NCP/NCS-3x00 RS

3.5.1.2.1 Using the Classic Flow Meter

To prepare the NCP/NCS-3x00 RS pump for the IQ test procedure:

- a) Prepare the solvents as described in Table 3-3 and degas (e.g. ultrasonication) prior to purging.
- b) Purge the system according to the operating instructions. This means at least 30 minutes for both blocks and 30 minutes for the Classic flow meter with nano flow selector.

i Tip:

The calibration routines require the instrument to be powered on for at least one hour (total purge time) for proper thermostating of the flow meter. Full accuracy will only be reached when the module has been powered on for 24 hours.

c) After purging, use CHROMELEON diagnostics to perform the pressure sensor calibration. Then, perform the viscosity calibration for both channels A & B in the "NC Pump More Options" panel.

i Tip:

The predefined values in CHROMELEON are for ease of use. Best performance is achieved with the calibration routine through the CHROMELEON diagnostics.

- d) Connect the nanoViper[™] capillaries according to the *UltiMate 3000 RSLCnano Standard Applications* manual.
 - Direct injection with the NCP-3200RS allows connecting the nano column directly on the inject valve of the WPS-3000.
 - Direct injection with the NCS-3500RS allows also connecting the nano column directly on the inject valve, however Thermo Fisher Scientific recommends to place the column in the column oven for thermostating.
 - Preconcentration can only be performed with the NCS-3500RS using the switching valve inside the column oven.

I Important: The set-up for PRECONCENTRATION experiments needs to be exactly the same as described in Figure 2. The LEFT VALVE MUST be in the 1-2 POSITION when loading the sample onto the trap column. If the valve is in another position (or is not configured) the IQ wizard will prepare direct injection templates.

3.5.1.2.2 Using the ProFlow Flow Meter

To prepare the NCP/NCS-3x00 RS pump for the IQ test procedure

- a) Prepare the solvents as described in Table 3-3 and degas (e.g. ultrasonication) prior to purging.
- b) Set the correct Solvent Types for the left and right blocks under NC Pump More Options on the Pump Module Tab.

- c) Purge the system according to the operating instructions. This means at least 15 minutes for both blocks and at least 10 minutes for the ProFlow flow meter.
- d) After purging, run the **Adjust Zero Balance** wizard on the NC_Pump wellness sub-panel
- e) If the system has been upgraded to ProFlow flow meter: Start the Calibrate Working Pressure Transducer wizard on the **Wellness** sub-panel

3.5.1.3 Fluidic Configuration

Connect the nanoViper capillaries according to the *UltiMate 3000 RSLCnano Standard Applications Manual*.

- Direct injection with the NCP-3200RS allows connecting the nano column directly on the inject valve of the WPS-3000.
- Direct injection with the NCS-3500RS allows connecting the nano column directly on the inject valve, however Thermo Fisher Scientific recommends to place the column in the column oven for thermostating.
- Preconcentration can only be performed with the NCS-3500RS using the switching valve inside the column oven.
- **i** Important: In order to run DIRECT INJECTION experiments (Figure 3-1) with the column connected either to the autosampler valve or to (one of) the valve(s) in the column oven of the NCS-3500RS module, it is important that the LEFT VALVE (if present) is in position 6_1 or 10_1 or IS NOT CONFIGURED. Do not forget to set the valve back in the configuration after running the experiments.

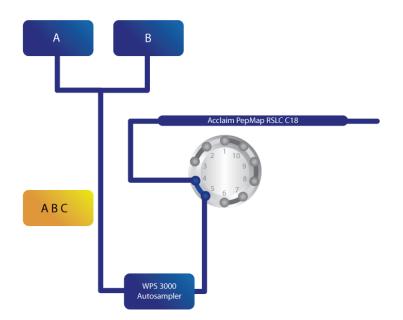


FIGURE 3-1 Fluidic layout for the IQ setup in Direct injection (NCS)

Important: The set-up for PRECONCENTRATION experiments needs to be exactly the same as described in Figure 3-2. The LEFT VALVE MUST be in the 1-2 POSITION when loading the sample onto the trap column. If the valve is in another position (or is not configured) the IQ wizard will prepare direct injection templates.

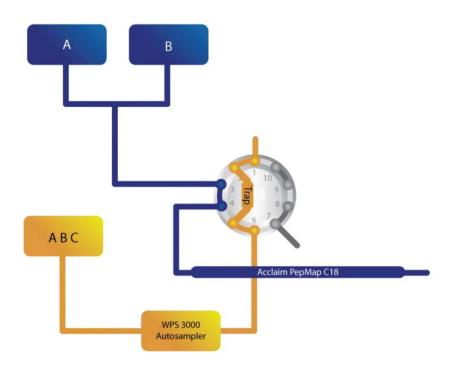


FIGURE 3-2 Fluidic layout for IQ setup in Preconcentration (back-flush configuration)

3.5.1.4 WPS-3000 Autosampler

To prepare the UltiMate WPS-3000 Autosampler for the IQ test procedure:

- a) Fill the wash bottle with Water / ACN (20/80 v/v) + 0.08% FA (Mobile phase B)
- b) Make sure the correct sample loop is installed (1 μL loop for direct injection, 20 μL loop for preconcentration)
- c) Make certain that the fluidic components and the syringe of the WPS autosampler are free of air.
- d) Place the Cytochrome C digest at the position RA1 (use a concentration of 500 fmol/µL for NAN, 8 pmol/µL for CAP & 16 pmol/µL for MIC)
- e) Place the vial with mobile phase A at the position **RA2**

4 Chromeleon 6.8

4.1 Preparation in Chromeleon

4.1.1 Template Structure

Running Instrument Qualification in Chromeleon comprises several steps:

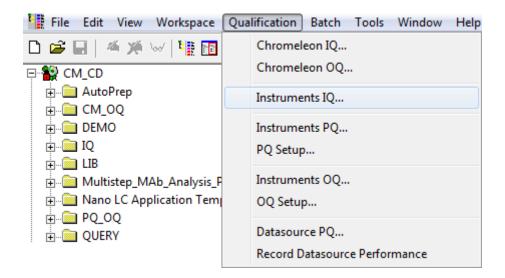
A wizard generates sequence templates from the master sequences of the Chromeleon CD, providing only sequences that match the timebase. In addition, the wizard adapts the programs automatically to the devices installed in the timebase.

I Important: Before running the IQ wizard ensure that the fluidic setup and valve configuration is as described in 3.5.1.3.

4.1.2 Creating the Sequence

To run the IQ, perform the following steps:

a) Start the Instrument IQ wizard



b) Select the Timebase

truments Installation Qualification	×
Choose a Timebase Select the timebase to validate.	
<u>T</u> imebase: RSLCnano <u>C</u> omputer: NLAMS-SDOLM <u>P</u> rotocol: My Computer ▼	My Computer RSLCnano Favorites Network Neighborhood
Enter connection information manually or pick a timebase from the list at right.	<u>Back</u> <u>N</u> ext > Cancel Help

c) Select the location where the IQ files are, in this case, the IQ folder on the CM datasource

Instruments Installation Qua	alification
Insert the Chromeleo Insert the Chromeleo	n CD on CD in the drive indicated below, or select another drive.
C Get the files from	n <u>C</u> D Drive: D:
 Get the files from 	another location: Source: NANO_CAP_MIC_LC_Templates ✓ Browse Use this option if you have copied the Instruments IQ sequence template from the Chromeleon CD to a network datasource to specify the folder which contains the Instruments IQ sequence template.
	< <u>B</u> ack <u>N</u> ext > Cancel Help

d) Select the IQ sequence that needs to be run

Instruments Insta	llation Qualification	x
Store Resu Please s	Its pecify where to store the results.	
<u>N</u> ame: <u>D</u> irectory	NANO_CAP_MIC_LC_Templates 1-23-2014 RSLCnano\IQ_Runs Browse	
	< <u>B</u> ack <u>N</u> ext > Cancel Hel	p

Instrun	nents Installation Qualification			×	
C	Thecks Select the checks to be installed				
	Q_01 DIRECT INJECTION NCx				_
				1	-
	< <u>B</u> ack	<u>N</u> ext >	Cancel	Help	

e) Click Finish in the IQ wizard dialog

f) The IQ report is opened automatically;

- Disable the write protection (on the Edit menu, click Layout Mode), and then enter the
 - Column S/N, Cytochrome C digest Lot No.
 - Names of customer and tester.

For all devices, the instrument names and limits recommended by Thermo Fisher Scientific are automatically entered into the report only when you open the report

- Enable the write protection (on the Edit menu, click Layout Mode).
- SAVE the report. To do so, click Save Report Definition on the Workspace menu.
- g) Run the sequence after the system is equilibrated and the samples are placed in RA1 (Cytochrome C digest) and RA2 (mobile phase A)

4.2 Performing the Checks

The IQ sequence consists of 16 injections in total, 13 injections of Cytochrome C digest and 3 injections of mobile phase A. The 6 first injections of Cytochrome C digest are used for column equilibration. For a new system or a new column, these injections are necessary. The last 6 injections of Cytochrome C digest (Run 10-15) are used for the determination of the system performances in the IQ report.

i Tip:

Completing the IQ sequence will take approximately 18 hours, it is recommended to run the sequence during the night.

4.3 Completing and Printing the IQ Report

Integration of the peaks #1-5 of the Cytochrome C digest is performed automatically. When integration fails, integration parameters need to be adjusted.

To that end, open the last 6 Cytochrome C digest runs and select in the tool-bar 'QNT-editor'.



QNT-Editor

In the TAB 'Peak Table', change the retention time and window for peaks #1 to 5.

lo.	Peak Name	Ret.Time	Window	Standard	Int.Type	Cal.Type	Peak Type	Group	Comment
1	Peak 1	16.000 min	1.000 A	External	Area	Lin	Auto		
2	Peak 2	17.500 min	1.000 A	External	Area	Lin	Auto		
3	Peak 3	18.500 min	1.000 A	External	Area	Lin	Auto		
	Peak 4	20.000 min	1.000 A	External	Area	Lin	Auto		
5	Peak 5	25.000 min	1.000 A	External	Area	Lin	Auto		

When the integration is correct, save the QNT file and open the report. When all peaks are correctly integrated, the result in the report tab '**Retention time_repro**' will be either '**Test passed**' or '**Test failed**'. When peaks are incorrectly integrated, the result in the report tab '**Retention time_repro**' will be '**#DIV/0**'. This also happens when the text "data evaluation" is missing in the comment field of the runs that should be used for evaluation.

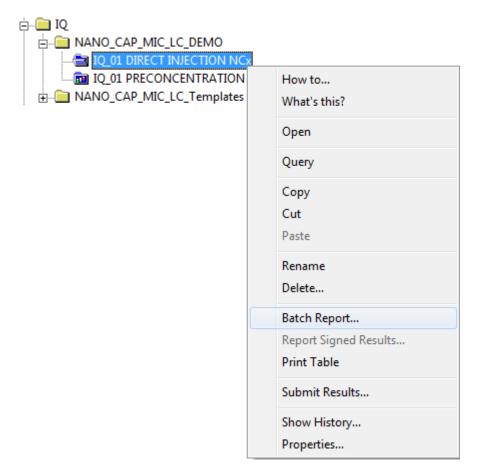
4.4 Data Evaluation (MS only)

- Data evaluation is done based on peak with *m/z* 728.84 (peptide sequence TGQAPGFSYTDANK, *z*=2; equivalent to peak #5 on the UV IQ test). Retention time reproducibility is evaluated for 6 consecutive runs and PWHH is evaluated for one run.
- Open the IQ report file (.rdf file) and enter the results on the "MS results" sheet. This will indicate if the IQ passed or not.

In the report, many references link to separate data sheets. When lines or columns are inserted or deleted, the references may get misassigned and hence the results will be false.

To ensure that the data is correctly read and processed in the report, print the report as 'Batch Report' from the Browser.

a) Right click on the IQ sequence and select "Batch Report"



b) Select the printer of choice and click OK

Use report <u>d</u> efinition

- c) Batch report will print the following sheets automatically:
 - Printing sheet 'Title Page' ...
 - Printing sheet 'Test procedures' ...
 - Printing sheet 'Retention time_repro' ...
 - Printing sheet 'RSD calculation' ...
 - Printing sheet 'MS results'...
- d) When printing the report, the report is by default automatically exported in PDF format to the following location: C:\Chromel\Export

5 Chromeleon 7.2

5.1 Chromeleon 7 Terminology

Tip: Please note that Chromeleon 7 terminology is different from the terminology used in Chromeleon 6.80. For details, refer to the 'Glossary - Chromeleon 7.0,' which is available in the Documents folder of your Chromeleon 7 installation.

5.2 Preparation in Chromeleon

5.2.1 Template Structure

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

i Important: Before running the IQ wizard ensure that the fluidic setup and valve configuration is as described in 3.5.1.3.

5.2.2 Creating the Sequence

To run the IQ, perform the following steps:

a) To start the wizard, in the "Tools" menu of the Chromeleon Console, click "Instrument Qualification"

🎁 <u>C</u> reate 👻 <u>F</u> ile	<u>E</u> dit	<u>V</u> iew	Tools Help	0
			Station Qualification	
			Instrument Qualification	
			Custom Variables Editor	
			Prefere Configure and run tests to qualify an instrument	
			Discovery Manager	

b) Select the qualification type: "Installation Qualification"

nstrument Qualification Wizard Choose Qualification Type Select the type of instrument qualification to run.		? ×	
Qualification Type			
Installation Qualification			
Checks the general functionality of your instrument.			
C Operational Qualification			
Checks the analytical operation of your instrument.			
C Performance Qualification			
Checks the analytical performance of your instrument	nt.		
		<u>N</u> ext >>	Cancel

c) Select the instrument

Instrument Qualification Wizard		8 ×
Choose an Instrument Select the instrument where the qualification will run.		
Instruments:		-
The local Instrument Controller is running idle. <u>Stop local Instrument Controller.</u>	Filter Y	
RSLCnano_NCP		
	<< <u>B</u> ack <u>N</u> ext >>	Cancel

d) Click det to connect the selected instrument to Chromeleon. The Instrument Connection dialog box shows the connection process.

Ins	trument Connection
	Connecting instrument "RSLCnano_NCS" on NLAMS-SDOLM
	Cancel

e) The IQ System test is displayed

Instrument Qualification Wizard	2	1000	8 x
Choose Tests Select the qualification tests to run.			
V IQ Systemtest			
		<< Back Next >>	Cancel

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

Instrument Qualification Wizard	S X
Define a Storage Location Specify where to store the results.	
Destination	
chrom://nlams-sdolm/ChromeleonLocal/Instrument Data/RSLCnano_NCS/Qualificat	tion
	Browse
<< <u>B</u> a	ck Finish Cancel

- g) Click Finish in the IQ wizard dialog
- h) Run the sequence after the system is equilibrated and the samples are placed in RA1 (Cytochrome C digest) and RA2 (mobile phase A)

5.3 Performing the Checks

The IQ sequence consists of 16 injections in total, 13 injections of Cytochrome C digest and 3 injections of mobile phase A. The 6 first injections of Cytochrome C digest are used for column equilibration. For a new system or a new column, these injections are necessary. The last 6 injections of Cytochrome C digest (Run 10-15) are used for the determination of the system performances in the IQ report.



Completing the IQ sequence will take approximately 18 hours, it is recommended to run the sequence overnight.

5.4 Completing and Printing the IQ Report

Integration of the peaks #1-5 of the Cytochrome C digest is performed automatically. When integration fails, integration parameters need to be adjusted.

To that end, open the last 6 Cytochrome C digest runs and select the Data Processing

T	Injection List
	Instrument Method
12	Data Processing
	Report Designer
	Electronic Report
	UV Spectral Library

In the 'Component Table', change the retention time and window for peaks #1 to 5.

Group Area Drag a co			header here to group by that column.		Run Component T	Run Component Table Wizard		
#	Name	Ret.Time 🔺	Window	Stand.Meth.	Eval.Type	Cal.Type	Level "01"	Peak Type
1	Peak 1	13.000	1.000 AG	External	Area	Lin	1.000000	Autodetect
2	Peak 2	14.000	1.000 AG	External	Area	Lin	1.000000	Autodetect
3	Peak 3	15.000	1.000 AG	External	Area	Lin	1.000000	Autodetect
4	Peak 4	18.000	1.000 AG	External	Area	Lin	1.000000	Autodetect
5	Peak 5	20.000	1.000 AG	External	Area	Lin	1.000000	Autodetect

When the integration is correct, save the Component Table and open the report.

T	Injection List
	Instrument Method
M	Data Processing
	Report Designer
	Electronic Report
	UV Spectral Library

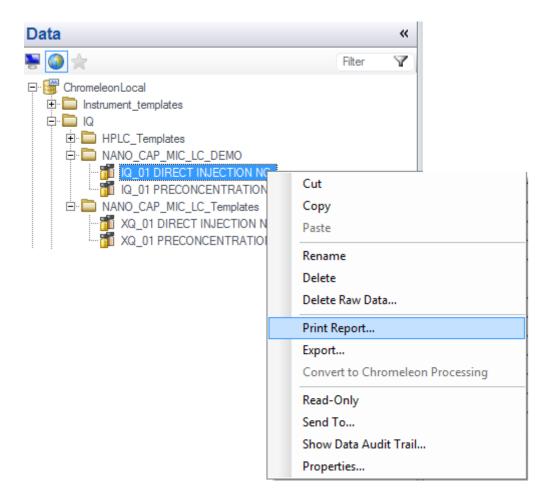
When all peaks are correctly integrated, the result in the report tab '**Retention time_repro'** will be either '**Test passed'** or '**Test failed'**. When peaks are incorrectly integrated, the result in the report tab '**Retention time_repro'** will be '**#DIV/0'**. This also happens when the text "data evaluation" is missing in the comment field of the runs that should be used for evaluation.

5.5 Data Evaluation (MS only)

- Data evaluation is done based on peak with *m/z* 728.84 (peptide sequence TGQAPGFSYTDANK, *z*=2; equivalent to peak #5 on the UV IQ test). Retention time reproducibility is evaluated for 6 consecutive runs and PWHH is evaluated for one run.
- Open the IQ report template and enter the results on the "MS results" sheet. This will indicate if the IQ passed or not.

In the report, many references link to separate data sheets. When lines or columns are inserted or deleted, the references may get misassigned and hence the results will be false.

To ensure that the data is correctly read and processed in the report, print the report as 'Batch Report' from the Browser.



i) Right click on the IQ sequence and select "Print Report..."

j) Select the printer of choice and click OK

_	IQ_NANO_CAP_MIC_NCx_DirectInject_V04						
With selected channel	UV_VIS_1						
nter							
RD_AMS (HP LaserJet P4	4015)	<u>P</u> roperties					
lect sheets to be printed ·							
-							
Sheet Name	Print	Condition					
Specification		(injection.comment contains "Print Data")					
🧊 TitlePage		(injection.comment contains "Print Data")					
Test procedures		(injection.comment contains "Print Data")					
Retention time	I	(injection.comment contains "Print Data") (injection.comment contains "Print Data")					
RSD calculation	V						
MS results	V	(injection.comment contains "Print Data")					
Results_and_headers	s 📃						
💷 Audit trail							

As an alternative, click "Print" on the Sequence Editor Toolbar to print the report.

Queued	▶ Start -		RSLCnano_NCS (Idle)
🚽 Save 🧿 Studio	🗿 Print 🧧 📩 Up 🛛 📴 Insert Row 👻 🏢 Fill Down 🔒 Lock 🛛 🍸 Filte	ing \equiv Grouping $\mid f_{\chi}$ Custom Columns \star \mid	✓ ♣ Find Next ▼
	Report		
	Injection List		

- k) Batch report will print the following sheets automatically:
 - Printing sheet 'Title Page' ...
 - Printing sheet 'Test procedures' ...
 - Printing sheet 'Retention time_repro' ...
 - Printing sheet 'RSD calculation' ...
 - Printing sheet 'MS results'...
- I) When printing the report, the report is by default automatically exported in PDF format to the following location: C:\Chromel\Export

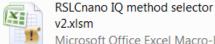
DCMSlink in combination with Xcalibur (for 6 **Classic Flow Meter ONLY)**

DCMSlink uses the Xcalibur method and sequence editor to prepare and run the IQ sequence. The level of automation available under Chromeleon is not available with DCMSlink control. The process is facilitated as much as possible by the workflow described below. A macro enabled Excel file has been prepared that contains LC methods for each supported IQ configuration. The macro part helps in selecting the correct method based on the configuration and copies it automatically to the clipboard.

🚺 Tip If macros cannot be enabled the file can still be used. The file is setup in such a way that the LC methods are also accessible even when the automation features are disabled by local macro policy. Refer to section 6.5 - Manual Selection of the IQ Method for the selection table

6.1 Using the Method Selector

Locate the RSLCnano IQ method selector excel file as shown below and open it.



v2.xlsm Microsoft Office Excel Macro-Ena...

Upon first use (and depending on the Excel version) the Macro content needs to be enabled. Click Enable Content on the warning bar on the top of the window to allow automation.

Each worksheet contains one method for one configuration and the name reflects these configurations. See section 6.5 - Manual Selection of the IQ Method for the relationship between a configuration and name.

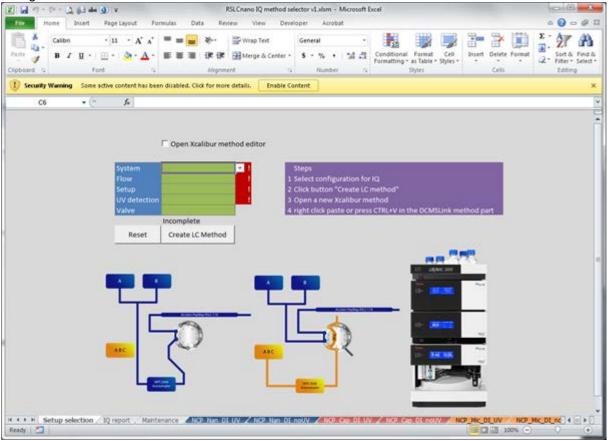


FIGURE 6-1 First sheet of method selector tool with security warning bar.



FIGURE 6-2 Zoom in on the worksheet names, where each worksheet contains one method for one configuration.

6.2 Creating the LC Method

There are five drop down menus which determine the IQ program that is required. The following figure shows this part of the method selector and what the various elements do.

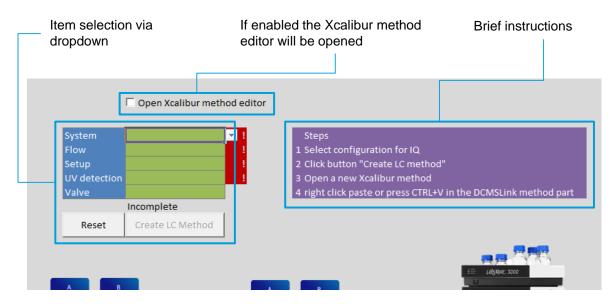


FIGURE 6-3 Interaction area on the opening screen. The method is selected via 5 drop down menus

- The selection criteria feature inherent checks making unsupported configurations not possible.
- Missing information is indicated by the red exclamation sign and the label "Incomplete". As long as the parameters are incomplete the **Create LC Method** button remains inactive.
- After making the final selection, click on an empty cell.

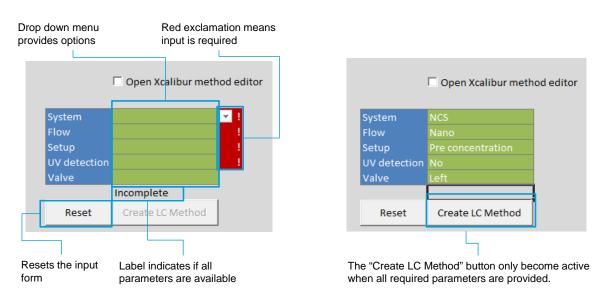


FIGURE 6-4 Before and after the parameters are provided

• When the configuration is selected, click **Create LC Method**. A confirmation dialog will appear describing the selected configuration

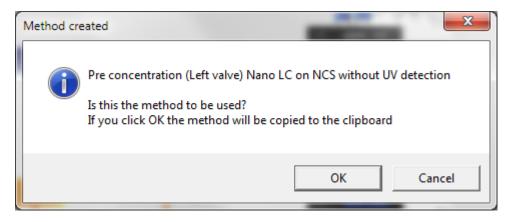


FIGURE 6-5 Confirmation dialog for selected configuration

- Click OK to accept this configuration or Cancel to make changes
- After clicking OK, the automation features will
 - Select the corresponding worksheet where the method conditions are stated
 - o Select the method
 - Copy it to the Windows clipboard

ile	Home Insert Page Layout Formulas	Data Review View Devel	oper Acrobat		-			-	4		2P
	Courier New - 11 - A A ==	😑 🗞 - 🚟 Wrap Text	General	٠	1			3	Σ-	27 6	R
ste	- 	🔳 🗊 🗊 🚮 Merge & Center •	s · % · .	86. 80	Conditional	Format Cell s Table - Styles	Insert	Delete Forma	t 0-	Sort & Fir	nd
board	rd a Font a	Alignment	Number	14		tyles		Cells		Editing	ICI
	A3 • (
	A B	c		D	E	F	G	н	1	J	
	selection		140	Last ro	w						
			Construction of the								
;==											
; 0	Cytochrome-C digest separation for	r Instrument Qualificat	ion								
1 -											
; P	PGM-Version December 2013										
; 3	Sample: Cytochrome-C digest			1							
				1							
- ===											
				1							
	Settings for NC Pump			1							
	NC_Pump.MaximumFlowRampDown =	998 [µl/min ²]									
	NC Pump.MaximumFlowRampUp =	998 [µl/min ²]									
	NC_Pump.Pressure.LowerLimit =	0 [bar]									
	NC_Pump.Pressure.UpperLimit =	800 [bar]		1							
		100%water + 0.1%FA									
{	NC_Pump.%A.Equate = NC_Pump.%B.Equate =	20%water + 80% ACN + 0.08%FA		1							
	NC_Pump.%B.Equate =	20%water + 80% ACN + 0.08%FA	•								
				-							
; 8	Settings for LoadingPump			1							
	LoadingPump.Pressure.LowerLimit =	0 [bar]									
	LoadingPump.Pressure.UpperLimit =	500 [bar]									
	LoadingPump.%A.Equate =	98%water + 2% ACN + 0.05%FA									
	LoadingPump.%B.Equate =	%B									
	LoadingPump.%C.Equate =	%C									
	LoadingPump.Flow =	0.030 [m]/min]									

FIGURE 6-6 Method selected and copied to clipboard

- The selection hyperlink will take the user back to the selection screen, without scrolling through all the worksheets.
 - The number in cell C1 indicates until which row the script should be copied.

i Important

Do not edit the content on these sheets!

- If the **Open Xcalibur method editor** checkbox is enabled, the Xcalibur method editor should open automatically. Otherwise open manually.
- Right click and paste or press control + V directly to paste the method.

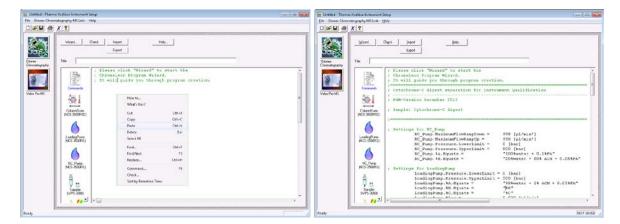


FIGURE 6-7 Pasting into the Xcalibur LC method part editor

```
The standard text can be deleted
; Please click "Wizard" to start the
; Chromeleon Program Wizard.
; It will guide you through program creation.
```

- The last thing to do is enable the synchronization between the LC and the MS
- The methods have a placeholder for the trigger (Relay) between the LC and the MS

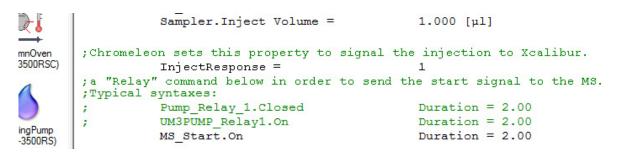


FIGURE 6-8 Trigger settings in the LC method part editor to synchronize LC and MS acquisition start

- Depending on how this is configured, these commands should be adapted. A common way is to use Relay_1 on the autosampler, renamed to MS_Start. This syntax must be activated in the methods. Manually correct for situations that are different.
- Finish the wizard for the LC method
- Complete the MS part of the method (make sure the acquisition times are adjusted accordingly). It is recommended to acquire data with Full MS1 scan.
- Save the method

6.3 Creating the Sequence

- Once the method has been created, generate a sequence in the standard way in Xcalibur.
- The sequence consists of 2 sets of 8 Cytochrome C digest runs and 1 mobile phase A injection after each set.

Sample Ty 1 Unknown 2 Unknown	Cytochrome C digest 500 fmol	File Name Cyt_CO1	Path D:\RD\	Inst Meth	Position BA1	Inj Vol Comm
		Cyt_C01	D:\RD\	D-MDL IQ	DA1	
2 Unknown						1.000
	Cytochrome C digest 500 fmol	Cyt_C02	D:\RD\	D:\DI_IQ	RA2	1.000
3 Unknown	Cytochrome C digest 500 fmol	Cyt_C03	D:\RD\	D:\DI_IQ	RA3	1.000
· orienterin				D:\DI_IQ		1.000
						1.000
- oneorm				D:\DI_IQ		1.000
· OTIKITOWIT				D:\DI_IQ		1.000
- ornererini				D:\DI_IQ		1.000
9 Unknown	Mobile Phase A	Mob_A_1	D:\RD\	D:\DI_IQ	RA8	1.000
10 Unknown	Cytochrome C digest 500 fmol	Cyt_C09	D:\RD\	D:\DI_IQ	RB1	1.000
	Cytochrome C digest 500 fmol	Cyt_C10		D:\DI_IQ		1.000
···· ormenentiti	Cytochrome C digest 500 fmol	Cyt_C11		D:\DI_IQ		1.000
- oneonn	Cytochrome C digest 500 fmol	Cyt_C12	D:\RD\	D:\DI_IQ		1.000
	Cytochrome C digest 500 fmol	Cyt_C13	D:\RD\	D:\DI_IQ	RB5	1.000
	Cytochrome C digest 500 fmol		D:\RD\	D:\DI_IQ	RB6	1.000
16 Unknown	Cytochrome C digest 500 fmol	Cyt_C15	D:\RD\	D:\DI_IQ	RB7	1.000
17 Unknown	Cytochrome C digest 500 fmol	Cyt_C16	D:\RD\	D:\DI_IQ	RB8	1.000
18 Unknown	Mobile Phase A	Mob_A_2	D:\RD\	D:\DI_IQ	RB8	1.000
*						0.001
	4 Unknown 5 Unknown 6 Unknown 7 Unknown 9 Unknown 10 Unknown 11 Unknown 13 Unknown 13 Unknown 14 Unknown 15 Unknown 16 Unknown 17 Unknown	Unknown Cytochrome C diget 500 lmol Unknown Cytochrome C diget 500 lmol	4 Unknown Cytochrome C diget 500 fmol Cyt.C04 5 Unknown Cytochrome C diget 500 fmol Cyt.C05 6 Unknown Cytochrome C diget 500 fmol Cyt.C05 7 Unknown Cytochrome C diget 500 fmol Cyt.C05 8 Unknown Cytochrome C diget 500 fmol Cyt.C07 9 Unknown Cytochrome C diget 500 fmol Cyt.C08 9 Unknown Cytochrome C diget 500 fmol Cyt.C03 10 Unknown Cytochrome C diget 500 fmol Cyt.C03 11 Unknown Cytochrome C diget 500 fmol Cyt.C10 12 Unknown Cytochrome C diget 500 fmol Cyt.C11 13 Unknown Cytochrome C diget 500 fmol Cyt.C12 14 Unknown Cytochrome C diget 500 fmol Cyt.C13 15 Unknown Cytochrome C diget 500 fmol Cyt.C14 16 Unknown Cytochrome C diget 500 fmol Cyt.C15 17 Unknown Cytochrome C diget 500 fmol Cyt.C15 17	4 Unknown Cytochrome C digest 500 fmol Cyt_ C04 D:VRD\ 5 Unknown Cytochrome C digest 500 fmol Cyt_ C05 D:VRD\ 6 Unknown Cytochrome C digest 500 fmol Cyt_ C05 D:VRD\ 7 Unknown Cytochrome C digest 500 fmol Cyt_ C06 D:VRD\ 8 Unknown Cytochrome C digest 500 fmol Cyt_ C07 D:VRD\ 9 Unknown Cytochrome C digest 500 fmol Cyt_ C08 D:VRD\ 10 Unknown Cytochrome C digest 500 fmol Cyt_ C10 D:VRD\ 10 Unknown Cytochrome C digest 500 fmol Cyt_ C10 D:VRD\ 11 Unknown Cytochrome C digest 500 fmol Cyt_ C11 D:VRD\ 13 Unknown Cytochrome C digest 500 fmol Cyt_ C13 D:VRD\ 14 Unknown Cytochrome C digest 500 fmol Cyt_ C13 D:VRD\ 14 Unknown Cytochrome C digest 500 fmol Cyt_ C14 D:VRD\ 15 Unknown Cytochrome C digest 500 fmol Cyt_ C15	4 Unknown Cytochrome C diget 500 fmol Cyt_ C04 D:\RD\ D:\ND\ D:\ND\	4 Unknown Cytochrome C diget 500 fmol Cyt, C04 D:\RD\ D:\D_10 RA4 5 Unknown Cytochrome C diget 500 fmol Cyt, C05 D:\ND\ D:\D_10 RA5 6 Unknown Cytochrome C diget 500 fmol Cyt, C05 D:\ND\ D:\D_10 RA6 7 Unknown Cytochrome C diget 500 fmol Cyt, C06 D:\ND\ D:\D_10 RA6 7 Unknown Cytochrome C diget 500 fmol Cyt, C07 D:\ND\ D:\D_10 RA8 9 Unknown Cytochrome C diget 500 fmol Cyt, C08 D:\ND\ D:\D_10 RA8 9 Unknown Cytochrome C diget 500 fmol Cyt, C03 D:\ND\ D:\D_10 RA8 10 Unknown Cytochrome C diget 500 fmol Cyt, C13 D:\ND\ D:\D_10 RB2 13 Unknown Cytochrome C diget 500 fmol Cyt, C13 D:\ND\ D:\D_10 RB5 14 Unknown Cytochrome C diget 500 fmol Cyt, C13 D:\ND\ D:\D_10 RB5

FIGURE 6-9 Xcalibur RSLCnano IQ sequence example

6.4 Data Evaluation

- Data evaluation is done based on peak with *m/z* 728.84 (peptide sequence TGQAPGFSYTDANK, *z*=2; equivalent to peak #5 on the UV IQ test). Retention time reproducibility is evaluated for 6 consecutive runs and PWHH is evaluated for one run.
- Enter the MS result into the IQ report worksheet which will indicate if the IQ has passed or failed.
- Orange fields indicate information which needs to be provided.
- Once all information has been inserted the form can be printed using the standard Excel printing functions.
- The form can be reset with the button **Clear form** at the top.

1	- Arial B / U	- 9 · A A	· = = = *·		Format as Table *	3ª Delete -		Save	Print	
	h.	Font	Alignment	.40 00.	Styles	Cells	· 2* Filter * Select *	Save as Adobe PDF	copies x y	
		(- A 6	- Postantin	1	10-11		Contrag (contrag)	-	Print	
	758 *	14 0						😂 Open		
	8	c	0	2 1	G H	1 2	x 0 *	Close	Printer 0	
			oFisher		Clearfo	m		Info	R8:0 printer	
		SCIER	VIIFIC							
		Installation	Qualification		Use the clear	form button to reset the f	form.	Recent	Printer Properties	Thermo Fisher
					-			New	Settings	SCIENTIFIC
		RSL	Cnano		ind	lcates missing informatio	n	New	Dist Asher Dest.	Installation Qualification
								Print	Print Active Sheets Only print the active sheets	RSLC nano
	Reter	ntion Time Reprod	ucibility and PWH	H in MS						
	figuration				READY for p	int		Save & Send	Pages: \$ to \$	Relation Time Reproducibility and PMMH in MS Configuration
	unerel name	Model	Supplier's name	Sectal Number				Help	Print on Both Sides Flip pages on long edge	Induned name Biodal Busiler's name Bantal Rumber Anderne Difference Theme Section Autority
	sancier	WP5-3000	Theme Scientific	8031245					Inhits Collated	Renal Inco 200350 Themo Sciencific 0021240
	p Module	NCS-3500RS	Theme Scientife	8001246				Dptions	Collated 1.2.3 1.2.3 1.2.3	Courte Gran ACE-SECRED Theme Ecentric BETTLAT V/ Densor VII/D Sell Theme Ecentric BETTLAT
104	ma Oven	NCS-3500RSC	Themo Scientilio	8031247				🔀 Exit		DOVELAN WINEM & 10 Thems Scientific DEVID
	letector	VWD-3400	Thermo Scientito	8031248					Portrait Orientation •	till actual tallar theme acanthe Other
	SLink version		3 Thermo Scientific	0EMO					_	Paramban base reproduction to an advantage of the second s
6	ofware	Xealibur	Themo Scientillo	Other					A4	
									21 cm x 29.7 cm	Rus so Name Politime (non) mit 708 8 (h)
									Normal Margins	1 Groevere Clopest 21.57
ie:	ention time repro	ducibility calculation					-		Left 1.78 cm Right 1.78	1 Crotrome Cogen 21.01 3 Crotrome Cogent 21.03
	12	1	Bet.T	ine (min)					No Scaling	4 Grothome Diget 214 8 Grothome Diget 215
	Run no	Name	mie 7	28.3(2+)					100 Print sheets at their actual s	6 Gromone Clipter 2145
	1	Cytochrome C Digest		1157					Page Setup	860 8.01%
	2	Cyrocheome C Digest		21.61						
	3	Cytochrome C Digest		159						Intmob
	4	Cytochrome C Digest		215						Test Lant Dase-ved velues fesual
	5	Cytochrome C Digest Cytochrome C Digest		1158						Receive rime reps. 403019 RSD 0.12 Person Rimer (g) 412 and 6 Person
	6 age	Cyrooniome C Digest		R min						
				0 mn						
-3			0.0							
										Culture Is spreture Collection agriculter Olice
le:	results									
			10 10 IS							
les		Linit		Result						L
1	rition time repro. Hi (s)	<0.300 % RSD		Passed						
M	H LSI	< 12 sec	L	Passed						

FIGURE 6-10 Example printing dialogs for the IQ report

6.5 Manual Selection of the IQ Method

- When local policies prevent the use of macros select the worksheet that provides the method for the configuration.
- Manually select the method, copy, and paste it in the Xcalibur method editor.
- The rest of the process is identical to the one outlined above.

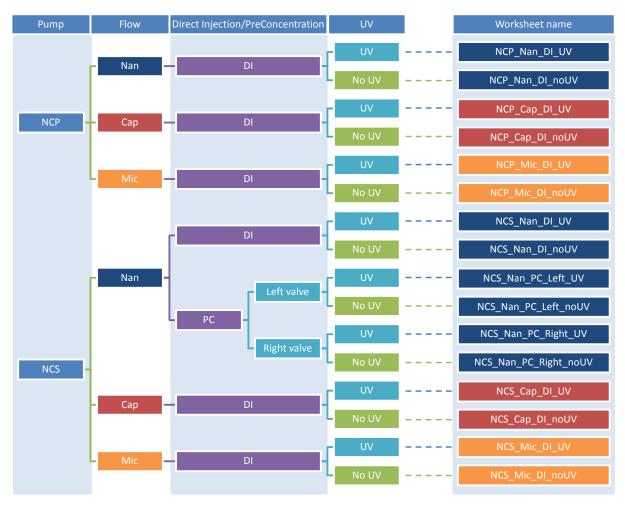


FIGURE 6-11 Supported set-ups and selection table for manually determining required program

7 SII in combination with Xcalibur (for ProFlow or Classic Flow Meter)

For the ProFlow flow meter, SII replaces DCMSLink as the interface for running LCMS analyses from Xcalibur. RSLCnano systems containing the nano Classic, Cap or Mic flow selectors can be run using either DCMSLink or SII. The level of automation available under Chromeleon is not available with SII control.

Important The macro enabled Excel file used to generate LC methods for DCMSLink is not compatible with SII (different file format). Therefore, the LC parameters have to be entered manually into the Xcalibur method file. The macro enabled Excel file should only be used for data evaluation and printing the report.

7.1 LC Method Parameter

Refer to the system conditions listed in 3.2.2 for details of the LC method parameters.

і Тір

The instrument parameters that are listed in the Excel file can be used to cross-check the method parameters as well as the default autosampler settings.

7.2 Creating the Instrument Method



• Open Xcalibur and click on the "Instrument Setup" Icon

- Select the Thermo Scientific SII icon to begin the LC method setup wizard
- Follow the wizard and enter the LC method parameters listed in 3.2.2.
- Finish the wizard for the LC method
- Complete the MS part of the method (make sure the acquisition times are adjusted accordingly). It is recommended to acquire data with Full MS1 scan.
- Save the method

I Tip: Ensure that the "Relay4Enabled" property is set to 5sec_InjectOut or higher (requires Expert mode).

7.3 Creating the Sequence

- Once the method has been created, make a sequence in the standard way in Xcalibur
- The sequence consists of 2 sets of 8 Cytochrome C digest runs and 1 mobile phase A injection after each set.

Sample Ty 1 Unknown 2 Unknown	pe SampleName Cytochrome C digest 500 fmol Cytochrome C digest 500 fmol	File Name Cyt_C01	Path D:\RD\	Inst Meth	Position BA1	Inj Vol Commo
2 Unknown			D:\RD\	D:\DI IQ	Bo1	1.000
	Cute also and Cuties at 500 ferral					
		Cyt_C02	D:\RD\	D:\DI_IQ	RA2	1.000
3 Unknown	Cytochrome C digest 500 fmol	Cyt_C03	D:\RD\	D:\DI_IQ	RA3	1.000
· oneoint				D:\DI_IQ		1.000
						1.000
- oneorin				D:\DI_IQ		1.000
• 0110109911	Cytochrome C digest 500 fmol	Cyt_C07	D:\RD\	D:\DI_IQ	RA7	1.000
	Cytochrome C digest 500 fmol	Cyt_C08	D:\RD\	D:\DI_IQ	RA8	1.000
	Mobile Phase A	Mob_A_1	D:\RD\	D:\DI_IQ	RA8	1.000
The ornerering	Cytochrome C digest 500 fmol	Cyt_C09	D:\RD\	D:\DI_IQ	RB1	1.000
	Cytochrome C digest 500 fmol	Cyt_C10		D:\DI_IQ		1.000
- onesen	Cytochrome C digest 500 fmol	Cyt_C11		D:\DI_IQ		1.000
on on on	Cytochrome C digest 500 fmol	Cyt_C12	D:\RD\	D:\DI_IQ		1.000
The windowini	Cytochrome C digest 500 fmol	Cyt_C13	D:\RD\	D:\DI_IQ		1.000
	Cytochrome C digest 500 fmol	Cyt_C14	D:\RD\	D:\DI_IQ	RB6	1.000
16 Unknown	Cytochrome C digest 500 fmol	Cyt_C15	D:\RD\	D:\DI_IQ	RB7	1.000
17 Unknown	Cytochrome C digest 500 fmol	Cyt_C16	D:\RD\	D:\DI_IQ	RB8	1.000
	Mobile Phase A	Mob_A_2	D:\RD\	D:\DI_IQ	RB8	1.000
*						0.001
	5 Unknown 6 Unknown 7 Unknown 8 Unknown 9 Unknown 10 Unknown 11 Unknown 12 Unknown 13 Unknown 14 Unknown 15 Unknown 16 Unknown 17 Unknown 17 Unknown	5 Unknown Cytochrome C digest 500 fmol 6 Unknown Cytochrome C digest 500 fmol 7 Unknown Cytochrome C digest 500 fmol 8 Unknown Cytochrome C digest 500 fmol 9 Unknown Cytochrome C digest 500 fmol 9 Unknown Mobile Phase A 10 Unknown Cytochrome C digest 500 fmol 12 Unknown Cytochrome C digest 500 fmol 13 Unknown Cytochrome C digest 500 fmol 14 Unknown Cytochrome C digest 500 fmol 15 Unknown Cytochrome C digest 500 fmol 16 Unknown Cytochrome C digest 500 fmol 17 Unknown Cytochrome C digest 500 fmol 16 Unknown Cytochrome C digest 500 fmol 17 Unknown Cytochrome C digest 500 fmol 18 Unknown Cytochrome C digest 500 fmol	5 Unknown Cytochrome C digest 500 fmol Cyt.C05 6 Unknown Cytochrome C digest 500 fmol Cyt.C06 7 Unknown Cytochrome C digest 500 fmol Cyt.C07 8 Unknown Cytochrome C digest 500 fmol Cyt.C08 9 Unknown Cytochrome C digest 500 fmol Cyt.C09 10 Unknown Mobie Phase A Mob A, 1 11 Unknown Cytochrome C digest 500 fmol Cyt.C109 12 Unknown Cytochrome C digest 500 fmol Cyt.C10 13 Unknown Cytochrome C digest 500 fmol Cyt.C11 14 Unknown Cytochrome C digest 500 fmol Cyt.C12 14 Unknown Cytochrome C digest 500 fmol Cyt.C13 15 Unknown Cytochrome C digest 500 fmol Cyt.C14 16 Unknown Cytochrome C digest 500 fmol Cyt.C15 17 Unknown Cytochrome C digest 500 fmol Cyt.C15 17 Unknown Cytochrome C digest 500 fmol Cyt.C16 18	5 Unknown Cytochrome C digest 500 fmol Cyt_C05 D:RD\ 6 Unknown Cytochrome C digest 500 fmol Cyt_C06 D:RD\ 7 Unknown Cytochrome C digest 500 fmol Cyt_C07 D:RPD\ 8 Unknown Cytochrome C digest 500 fmol Cyt_C08 D:RPD\ 9 Unknown Cytochrome C digest 500 fmol Cyt_C08 D:RPD\ 10 Unknown Cytochrome C digest 500 fmol Cyt_C08 D:RPD\ 11 Unknown Cytochrome C digest 500 fmol Cyt_C10 D:RPD\ 12 Unknown Cytochrome C digest 500 fmol Cyt_C11 D:RD\ 13 Unknown Cytochrome C digest 500 fmol Cyt_C12 D:RPD\ 14 Unknown Cytochrome C digest 500 fmol Cyt_C13 D:RPD\ 14 Unknown Cytochrome C digest 500 fmol Cyt_C13 D:RPD\ 15 Unknown Cytochrome C digest 500 fmol Cyt_C15 D:RPD\ 16 Unknown Cytochrome C digest 500 fmol Cyt_C15 D:RPD\<	5 Unknown Cytochrome C diget 500 fmol Cyt. C05 D. YRD\ D. YDL (q) 6 Unknown Cytochrome C diget 500 fmol Cyt. C06 D. YRD\ D. YDL (q) 7 Unknown Cytochrome C diget 500 fmol Cyt. C07 D. YRD\ D. YDL (q) 8 Unknown Cytochrome C diget 500 fmol Cyt. C08 D. YRD\ D. YDL (q) 9 Unknown Cytochrome C diget 500 fmol Cyt. C08 D. YRD\ D. YDL (q) 10 Unknown Cytochrome C diget 500 fmol Cyt. C09 D. YRD\ D. YDL (q) 11 Unknown Cytochrome C diget 500 fmol Cyt. C10 D. YRD\ D. YDL (q) 12 Unknown Cytochrome C diget 500 fmol Cyt. C11 D. YRD\ D. YDL (q) 13 Unknown Cytochrome C diget 500 fmol Cyt. C13 D. YRD\ D. YDL (q) 14 Unknown Cytochrome C diget 500 fmol Cyt. C14 D. YRD\ D. YDL (q) 15 Unknown Cytochrome C diget 500 fmol Cyt. C15 D. YRD\ D. YDL	5 Unknown Cytochrome C diget 500 lmol Cyt. C05 D.NB/\ D.ND/LQ PA5 6 Unknown Cytochrome C diget 500 lmol Cyt. C06 D.NB/\ D.ND/LQ RA5 7 Unknown Cytochrome C diget 500 lmol Cyt. C07 D.NB/N D.VD/LQ RA6 8 Unknown Cytochrome C diget 500 lmol Cyt. C08 D.NB/N D.VD/LQ RA8 9 Unknown Cytochrome C diget 500 lmol Cyt. C08 D.NB/N D.VD/LQ RA8 10 Unknown Cytochrome C diget 500 lmol Cyt. C08 D.NB/N D.VD/LQ RA8 11 Unknown Cytochrome C diget 500 lmol Cyt. C10 D.NB/N D.VD/LQ RB8 12 Unknown Cytochrome C diget 500 lmol Cyt. C11 D.NB/N D.VD/LQ RB2 13 Unknown Cytochrome C diget 500 lmol Cyt. C12 D.NB/N D.VD/LQ RB5 15 Unknown Cytochrome C diget 500 lmol Cyt. C14 D.NB/N D.VD/LQ RB7

FIGURE 7-1 Xcalibur RSLCnano IQ sequence example

7.4 Data Evaluation

- Data evaluation is done based on peak with *m/z* 728.84 (peptide sequence TGQAPGFSYTDANK, *z*=2; equivalent to peak #5 on the UV IQ test). Retention time reproducibility is evaluated for 6 consecutive runs and PWHH is evaluated for one run.
- Enter the MS result into the IQ report worksheet which will indicate if the IQ has passed or failed.
- Orange fields indicate information which needs to be provided.
- Once all information has been inserted the form can be printed using the standard Excel printing functions.
- The form can be reset with the button **Clear form** at the top.

8 Troubleshooting

8.1 General Notes

This section provides troubleshooting information in relation with the IQ procedure. More information about instrument-specific problems is provided in the documentation shipped with the system and in the service instructions available for the various modules.

The IQ procedures described in this manual have been optimized for the conditions shown. When troubleshooting the IQ results, please verify that the experimental setup is used as described in this manual.

8.2 Symptoms and Possible Causes

8.2.1 IQ Failed on Reproducibility

Here is a list of the most commonly encountered causes when an IQ does not meet the IQ reproducibility criteria.

- The system has not been sufficiently equilibrated. This particular pump may require more runs than the standard 16 injections in order to operate within the specified limits. Repeat the IQ procedure (partially).
- Dead volumes could be present in the system. Check that all connections (particularly those which are non nanoViper) are free of dead volumes.

8.2.2 IQ Failed on Resolution and/or Peak Width

Here is a list of the most commonly encountered causes when an IQ does not meet the IQ criteria for either resolution or peak width.

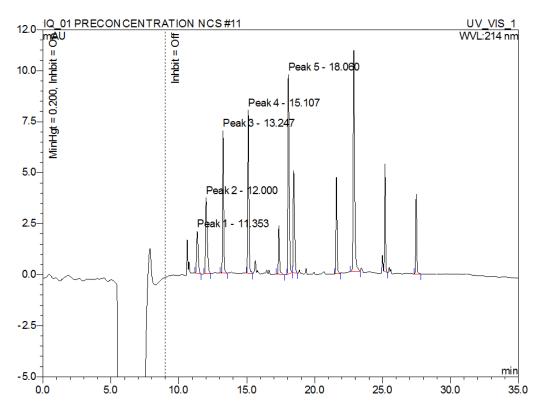
- The sample has been diluted in the wrong solvent resulting in poor trapping of the analytes at the top of the column. Check your solvents and prepare your sample with fresh solvent.
- The mobile phases were not prepared correctly resulting either in break-through or in poor elution.
- Dead volumes are present in the system resulting in peak broadening. Make sure that all connections are free of dead volumes, particularly those which are non nanoViper.
- The (trap) column is not performing correctly. Exchange the (trap) column.

8.2.3 IQ Failed on Peak Height

Here is a list of the most commonly encountered causes when an IQ does not meet the IQ criteria for peak height (sensitivity).

- The sample was prepared incorrectly.
- The sample has been diluted in the wrong solvent resulting in poor dissolution of the analytes. Check your solvents and prepare your sample with fresh solvent.
- The sample was prepared in a plastic autosampler vial. Only use glass insert vials.
- The detector lamp intensity is too low.
- The flow cell transparency is too low.
- The detection parameters are not correct, check your integration method.
- Check for air in the syringe. Check in the autosampler manual.

9 Appendices



9.1 Example Chromatogram Nano-LC Configuration

FIGURE 9-1 Example chromatogram in preconcentration configuration

9.2 Example of Report

Sequence: ChromeleonLocal\IQ_V04\NANO_CAP_MIC_LC_DEMO\IQ_01 DIRECT INJECTION NCx Page 1 of 4 Sample: CCD 13 Runtime: 13/Nov/2013 02:05



Installation Qualification Ultimate 3000 - Systemtest (NAN-HPLC)

Instrument name	Model	Supplier's name	Serial Number
Autosampler	WPS-3000	Thermo Scientific	4170504
Pump Module	NCS-3500RS	Thermo Scientific	8055871
Column Oven	NCS-3500RSC	Thermo Scientific	8055871
UV Detector	VWD-3400	Thermo Scientific	8054244
Chromeleon Datasystem	7.2.0.4121	Thermo Scientific	100010
Accessories	Name	·	-
Accessories	Name NAN-15-02-C18PM, P/N 10	4534	-
Accessories Column Type			-
Accessories Column Type Sample	NAN-15-02-C18PM, P/N 1		-
• Accessories Accessories Column Type Sample Sample Solvent Solvent A for Gradient	NAN-15-02-C18PM, P/N 1 Cytochrome C digest, 0.5 p		-
Accessories Column Type Sample Sample Solvent Solvent A for Gradient	NAN-15-02-C18PM, P/N 1 Cytochrome C digest, 0.5 p Solvent A/Solvent B (98:2)	mol/µL	-
Accessories Column Type Sample Sample Solvent	NAN-15-02-C18PM, P/N 10 Cytochrome C digest, 0.5 p Solvent A/Solvent B (98:2) Water + 0.1% FA	mol/µL	-
Accessories Column Type Sample Sample Solvent Solvent A for Gradient	NAN-15-02-C18PM, P/N 10 Cytochrome C digest, 0.5 p Solvent A/Solvent B (98:2) Water + 0.1% FA	mol/µL	-

Chromeleon (c) 2013 Thermo Fisher Scientific Inc. Version 7.2 Build 3972 (223955) IQ_NANO_CAP_MIC_NCx_DirectInject_V04 / TitlePage Printed: 1/23/2014 1:40 PM

Sequence: ChromeleonLocal\IQ Sample: CCD 13	_V04\NANO_CAP_M	MIC_LC_DEMO\IQ_0	Page 2 of 4 time: 13/Nov/2013 02:05



	digest, concentration 0.5 pmol/µL for NAN, 8 pmol/µL for CAP and 16 pmol/µL for MIC) is injected
	The peptides are separated on a PepMap C18 column with an Acetonitrile gradient.
	(RSD) of the retention times of peak #5 is calculated. The resolution of peaks # 1-4,
ne neight and peak width	at half height of peak #5 are determined.
System conditions	
Eluent A:	Water + 0.1% FA
Eluent B:	Water/ACN 20/80 + 0.08% FA
Flow rate:	0.300 µL/min
Column:	NAN-15-02-C18PM, P/N 164534
Sample:	Cytochrome C digest, 0.5 pmol/µL
injection volume:	1.0 µL
Column temperature:	35°C
Sample temperature:	5*C (only WPS 3000 (T))
Gradient:	
Time (min)	%B
0	4
30	55
31	90
36	90
37	4
60	4

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Sequence: ChromeleonLocal\IQ_V04\NANO_CAP_MIC_LC_DEMO\IQ_01 DIRECT INJECTION NCx Page 3 of 4 Sample: CCD 13 Runtime: 13/Nov/2013 02:05

Thermo Fisher S C I E N T I F I C Installation Qualification Ultimate 3000 - Systemtest (NAN-HPLC)

Retention Time Reproducibility

Instruments and Fluidics

Instrument name	Model	Supplier's name	Serial Number
Autosampler	WPS-3000	Thermo Scientific	4170504
Pump Module	NCS-3500RS	Thermo Scientific	8055871
Column Oven	NCS-3500RSC	Thermo Scientific	8055871
UV Detector	VWD-3400	Thermo Scientific	8054244
Chromeleon Datasystem	7.2.0.4121	Thermo Scientific	100010

Accessories	Name
Column Type	NAN-15-02-C18PM, P/N 164534
Sample	Cytochrome C digest, 0.5 pmol/µL
Sample Solvent	Solvent A/Solvent B (98:2)
Solvent A for Gradient	Water + 0.1% FA
Solvent B for gradient	Water/ACN 20/80 + 0.08% FA

Limits, Values and Test Results

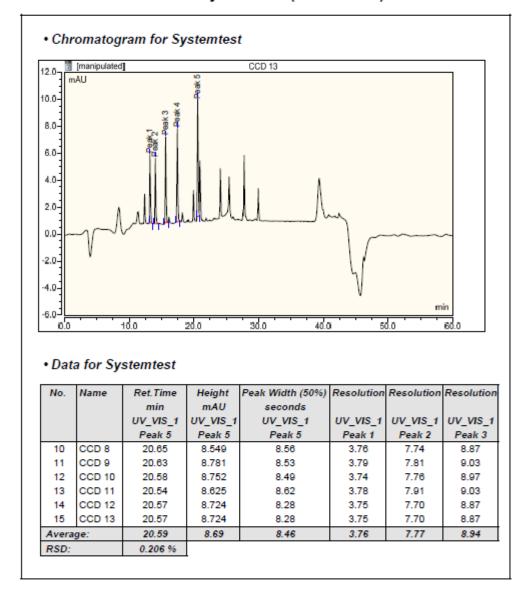
Resolution of peaks 1 to 4 >1.50 3.74 T Peakwidth at half height (peak 5) <12 s 8.5 s T		Limit	Observed values	Result
Peakwidth at half height (peak 5) <12 s 8.5 s T UV absorbance at 214 nm (peak 5) >3.00 mAU 8.69 mAU T	ention time repro. (peak 5)	<0.300 % RSD	0.206 % RSD	Test passed
UV absorbance at 214 nm (peak 5) >3.00 mAU 8.69 mAU T	olution of peaks 1 to 4	>1.50	3.74	Test passed
	kwidth at half height (peak 5)	<12 s	8.5 s	Test passed
Customer's signature Operator's signature	absorbance at 214 nm (peak 5)	>3.00 mAU	8.69 mAU	Test passed
Customer's signature Operator's signature				

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IQ_NANO_CAP_MIC_NCx_DirectInject_V04 / Retention time Printed: 1/23/2014 1:40 PM Sequence: ChromeleonLocal\Q_V04\NANO_CAP_MIC_LC_DEMO\\Q_01 DIRECT INJECTION NCx Page 4 of 4 Sample: CCD 13 Runtime: 13/Nov/2013 02:05



Installation Qualification Ultimate 3000 - Systemtest (NAN-HPLC)



Chromeleon (c) 2013 Thermo Fisher Scientific Inc. Version 7.2 Build 3972 (223955) IQ_NANO_CAP_MIC_NCx_DirectInject_V04 / RSD calculation Printed: 1/23/2014 1:40 PM Sequence: ChromeleonLocal\IQ_V04\NANO_CAP_MIC_LC_DEMO\IQ_01 DIRECT INJECTION NCx Page 3 of 4 Sample: CCD 13 Runtime: 13/Nov/2013 02:05



Installation Qualification Ultimate 3000 - Systemtest (NAN-HPLC)

Model	Supplier's name	Serial Number
WP \$-3000	Thermo Scientific	4170504
NC S-3500R S	Thermo Scientific	8055871
NCS-3500RSC	Thermo Scientific	8055871
VWD-3400	Thermo Scientific	8054244
7.2.0.4121	Thermo Scientific	100010
Name	Ret.Time (min)	l
	m/z 728.9 (2+)	
Cytochrome C Digest	20.65	
Cytochrome C Digest	20.63	
Cytochrome C Digest	20.58	
Cytochrome C Digest	20.54	
Cytochrome C Digest	20.57	
Cytochrome C Digest	20.57	
	0.206 %	
Limit	Observed values	Result
<0.300 % RSD	0.206 % RSD	Test passed
< 12 seconds	9 seconds	Test passed
	WP S-3000 NC S-3500R S NC S-3500R SC VWD-3400 7.2.0.4121 Name Cytochrome C Digest Cytochrome C Digest	WP S-3000 Thermo Scientific NC S-3500R S Thermo Scientific NC S-3500R SC Thermo Scientific VWD-3400 Thermo Scientific 7.2.0.4121 Thermo Scientific Name Ret.Time (min) m/z 728.9 (2+) Cytochrome C Digest 20.65 Cytochrome C Digest 20.63 Cytochrome C Digest 20.58 Cytochrome C Digest 20.57 Cytochrome C Digest 20.5

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