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Vanquish Neo System Installation Qualification

Operating Instructions

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

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

The descriptions in this manual revision refer to sequence templates version: 1.0 or later. Changes on sequence templates with a version later than 1.0 are described in the annex attached to the Release Notes. The annex is a complement to this manual. The version number of the sequence template is indicated in the name of the report template.

Contents

1 Using this Manual

1.1 About these instructions

1.1.1 Scope

These Vanquish Neo System Installation Qualification operating instructions describe the procedures executed during the installation to confirm:

- that the principle communication between SII for Xcalibur / Chromeleon™ and the connected modules is successfully established. It is not intended to completely validate all module's communication and functions.
- The Vanquish Neo System (or with column compartment) basic chromatographic performance meets installation qualification (IQ) specifications.

The document **does not** describe a procedure for Operational Qualification of the connected modules.

1.1.2 Overview

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

Section	Content
2 Introduction	Describes the intention of the Installation Qualification, summarizes the supported devices and how to perform the qualification dependent on the installed system.
3 Requirements and Preparations	Describes the part and sample requirements for the 4 different Vanquish Neo System IQ procedures. The method parameters are provided as well.
4 Data Evaluation	Describes how to evaluate the installation qualification results and determine if the Vanquish Neo System performance meets the specifications. Both the SII for Xxalibur and Chromeleon data evaluation procedures are described.
5 Appendix	Provides an example of the IQ report that is available as separate excel file.

1.2 Conventions

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

1.2.1 Terminology

The descriptions in all sections use terminology used in SII for Xcalibur or Chromeleon 7.

1.2.2 Special Notices and Informational Notes

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

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

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

1.2.3 Typographical Conventions

These typographical conventions apply to the descriptions in this manual:

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

1.2.4 Other Conventions

1.2.4.1 Viewpoint

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

1.2.4.2 Electronic Manual Version (PDF)

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

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

1.2.4.3 Part Numbers

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

2 Introduction

2.1 Installation Qualification

The Vanquish Neo System Installation Qualification is used to confirm that the principle communication between SII for Xcalibur and Chromeleon and the chromatography system corresponds to the expected chromatographic behavior.

The IQ covers all procedures relating to the installation of instruments in a specific environment. Filling in the Installation and Familiarization check lists **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 delivered with the instrument; for example, operating instructions, manufacturer's specification, shipping details, and all materials required for operating the instrument. This can be either in paper or electronic form.
- The required computer hardware and the instrument firmware and software were correctly supplied.
- 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 chromatographic performance of the instrument is according to the specifications.
- The recommended service, maintenance, calibration, and qualification intervals were specified. A contact was named for performing service work and supplying spare parts.
- Information was provided about the consumable goods required for regular operation.

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

2.1.1 Vanquish Neo System IQ: Supported Workflows

The Vanquish Neo System Installation Qualification can be run in 4 different workflows:

- Direct Injection Nano
- Trap-and-Elute Nano
- Direct Injection Micro
- Trap-and-Elute Micro

NOTICE Only **ONE** workflow needs to be done to complete the IQ. More are not required for successful system installation. Additionally, heated Trap-and-Elute is not part of the supported IQ workflows.

The Vanquish Neo System is delivered as a stacked system, preconfigured and connected for *Direct Injection* workflows using Nano flow ranges. The ship kit contains the required materials to change the workflow chosen by the customer. An analytical column must be ordered separately. Details for system preparations are listed in Chapter 3.2.4

2.2 Supported Devices

The Vanquish Neo System Installation Qualification procedures are optimized for the following devices:

- Vanquish Neo System (Pump, Autosampler, Vanquish User Interface)
- Vanquish Neo System with Column Compartment
- Detectors (Vanquish VWD or Thermo Fisher Scientific Mass Spectrometers)

For these devices, the instrument qualification is performed according to the descriptions in 3.2.4 System preparation and IQ sequence acquisition.

2.3 Known Restrictions

Chromeleon AutoQ availability

Currently, no Vanquish Neo System AutoQ is available in the latest Chromeleon 7 release. The instrument qualification method and sequence setup and data evaluation are done manually in the used software.

When controlling the instrument under SII for Xcalibur, the AutoQ functions is per default not available and manual method and sequence setup and data evaluation need to be done as usual.

IQ with thermostatted columns

The Vanquish Neo System Installation Qualification is optimized for operation with a thermostatted column (i.e. Vanquish Neo System with Column Compartment). Thermal influences of the laboratory environment are known to influence the chromatographic behavior if no thermostatted column is used. Therefore, two IQ retention time precision (%RSD) specifications are set for both a thermostatted and non-thermostatted column

3 Requirements and Preparations

3.1 Software Requirement

Data acquisition must be performed using either one of below software packages:

- Chromeleon 7.2.10 MUd (or higher)
- SII for Xcalibur 1.5.1 (in combination with Xcalibur itself).

Ensure the latest compatible version of software is available and installed. For details, read the latest release notes of each software package.

Additionally, the Vanquish Neo system driver needs to be downloaded and installed separately. Ensure the latest version is used.

3.2 Materials Requirements

The Vanquish Neo System Installation Qualification primarily considers a mass spectrometer as detector. The Vanquish Neo System is also compatible with an Ultraviolet detector, which is described later in this chapter.

This section lists preparations for specific device components in the LC-MS setup. If details are not listed, they are considered as default setting or standard knowledge.

Please ensure all materials are available onsite before starting the installation qualification procedure. This avoids unnecessary complications and time loss.

3.2.1 Solvents

Regardless of detector used (UV or MS), always use Optima LC-MS grade solvents for operating the Vanquish Neo System. Use the Optima LC-MS grade premixed solvents where applicable for increased reliability and ease of use.

The following solvents are required:

Solvent	P/N
Water, Optima™ LC–MS grade	W6-212
Acetonitrile, Optima™ LC–MS grade	A955-212
Isopropanol, Optima™ LC–MS grade	A461–212
Water with 0.1% Formic Acid, Optima™ LC–MS grade	LS118–500
80% Acetonitrile with 0.1% Formic Acid, Optima™ LC–MS grade	LS122-500
Formic Acid (FA), Optima™ LC–MS grade	A117-10X1AMP

Prepare solvents

When handling the solvents, glassware and fluidic components, use gloves at all times.

1) Rinse all solvent bottles thoroughly with high organic, followed by aqueous solvent, before filling with the final solvent.

2) Additionally, ultrasonicate all solvent filter parts in a clean beaker glass as followed:

   - 15 minutes in 80% acetonitrile with 0.1% formic acid
   - 15 minutes in 100% water + 0.1 % formic acid

3) Continue to prepare the following mobile phase and wash solvents:

Solvent type	P/N
Mobile phase A	100% water + 0.1 % formic acid
Mobile phase B	80% acetonitrile with 0.1% formic acid
Rear seal wash	75% isopropanol in water + 0.1% formic acid
Weak wash solvent	100% water + 0.1 % formic acid
Strong wash solvent	80% acetonitrile with 0.1% formic acid

4) Install the prepared solvents and solvent filters on their appropriate location on top of the solvent rack.

3.2.2 Cytochrome C Digest and Blank

Cytochrome C Digest (P/N 161089) is used as IQ Standard and is delivered in the Vanquish Neo system Ship Kit. The procedure for sample preparation depends on the system flow range configuration used for the IQ (NAN vs MIC).

Note that the stock solution can be stored at 7°C for a few days or at -20°C for longer period. The dry standard can be stored at -20°C for longer period.

Prepare an 8 pmol/µl stock solution

1) Add 200 µl 95% H_2O / 5% ACN + 0.1% FA to the Cytochrome C digest vial.

2) Sonicate for 20 minutes and vortex (mix) briefly.

For MIC flow range

3) Transfer 100 µl stock solution into an autosampler vial.

For NAN flow range, dilute the stock solution to 1 pmol/µl:

4) Add 100 µL stock solution to 700 µL H_2O + 0.1% FA into a separate vial and mix.

5) Transfer 100 µl 1 pmol/µl solution into an autosampler vial.

6) Place the prepared Cytochrome C digest sample in the autosampler.

7) Fill a second autosampler vial with mobile phase A and place in the autosampler.

3.2.3 Analytical Columns and Trap Column Cartridge

An analytical column is not included with the Vanquish Neo System delivery. An analytical column needs to be ordered separately prior to the installation. The required IQ column is dependent on the flow range and ion source used.

A trap column cartridge and a holder are delivered in the ship kit with the system. This Trap column is compatible with all settable flow ranges of the Vanquish Neo System.

IQ using LC-MS setup

The following options for an **LC-MS setup** are available:

Flow range	Ion Source	Column	Part Number
Nano	EASY-Spray Source	EASY-SPRAY PEPMAP NEO C18 2UM 75UMX150MM 1500 BAR	ES75150PN
Nano	Nanospray Flex Ion Source	Double Nano Viper PEPMAP NEO C18 2UM 75UMX150MM 1500 BAR 1/16” to 1/32” microtight adapter Stainless steel emitters	DNV75150PN 00109-02-00055 ES542
Micro	HESI / Optamax Ion Source	Pepmap RSLC C18 2UM 1 MM x 150 MM	164711
All	n.a.	PepMap 100 C18 Column, 5µm, 0.3x5mm, 1500 bar Trap Column Cartridge Holders with nanoViper Fittings	174500 174502

Notice For using the HESI / Optamax source for MIC flow range, ensure that the **OPTON-30697** kit is installed.
This includes the 50 µm ID needle insert, compatible up to 100 µl/min.

IQ using LC-UV setup

If an IQ is chosen with LC-UV, only linear Pepmap Neo column formats are compatible. No EASY-Spray columns can be integrated with the specific UV flow cell. However, for the connections, additional parts are required. The following columns and connectors need to be ordered prior to the IQ.

Flow range	UV Cell	Column/ Accessory	Part Number
Nano	3 nL, 20 MPa, fused silica, PTFE tubing connection 6074.0270	Double Nano Viper PEPMAP NEO C18 2UM 75UMX150MM 1500 BAR 1/16" to 1/32" microtight adapter Black sleeves 280µm ID 1/32" OD	DNV75150PN 00109-02-00055 SC903
Micro	45 nL, 30 MPa, fused silica, nanoViper connection 6074.0285	Pepmap RSLC C18 2UM 1 MM x 150 MM	164711

3.2.4 System Preparation and IQ Sequence Acquisition

The Vanquish Neo System is delivered with all required tubing and capillaries for "Direct Injection" in Nano flow range installed. Follow the *Vanquish Neo System Operating Manual (P/N 4822.5001-EN-1.0)* to complete the hardware installation.

3.2.4.1 Vanquish Neo System Preparation Direct Injection Nano

1) Ensure the latest version of driver and software is installed on the acquisition PC.
2) Ensure the latest version of firmware is installed on the instrument.
3) Via the Vanquish User Interface, execute the following scripts in this order:
    a) A01: Set Solvent Pump Solvent Types (**A: H2O & B: ACN80**)
    b) A02: Auto Start Up - with "Diagnostics: **activated"**
    c) A03: Set Separation Column Type
    d) A04: Set Separation Column Specifications

    Once completed, the Vanquish Neo System is ready for running the IQ samples.

4) Program the acquisition method via the CM7 via the Instrument Method Wizard or the Xcalibur Method Editor. Use the following parameters:

LC Parameter	Value / Action		
Runtime	20.00 min		
Diagnostics Channels	Default		
Fluidic setup	Verify all information is correct		
Solvents	Verify the solvents are correctly set		
Flow Gradient	Note: only the following parameters need to be entered. The remainder will be filled automatically.		
	Run		
	Duration (min)	**Flow (µl/min)**	**%B**
	0.000	0.350	1.0
	0.100	0.350	4.0
	13.900	0.350	35.0
	Column Wash		
	0.100	0.350	99.0
	5.900	0.350	99.0
Wash Solvent Names	Default		
Temperature Control	Activate and set to “7.0 °C”		
Fast loading	Activate the checkbox		
Mode	PressureControl		
Pressure	1500 bar		
Loading Volume	Automatic		
Fast equilibration	Activate the checkbox		
Mode	PressureControl		
Pressure	1500 bar		
Equilibration Factor	2		

5) Once completed, finish the wizard, navigate in the method overview to the “Sampler” node, and verify the “*Vial Bottom Detection*” function is activated.

6) Setup the MS detector parameters in the method. Use the parameters listed below, leave all other parameters as default for the specific MS detector used.

MS Source and Scan Parameter	Value / Action
Method duration	20.00 min
Ionization mode	Positive
Voltage	1900 V
Ion transfer Tube Temp	275 °C
Start and End time	0 - 20 min
Scan Range	375 – 1200

7) Save the method and proceed to setup the sequence table.

8) Setup and run an injection sequence of 1 µl per sample injection in this order:

   a) Blank

   b) 12 x Cytochrome C

   c) Blank

3.2.4.2 *Vanquish Neo System Preparation Trap-and-Elute Nano*

1) Follow steps 1, 2 and 3 of "*3.2.4.1 Vanquish Neo System Preparation Direct Injection Nano*".

2) Via the Vanquish User Interface, execute the following scripts in this order:

   a) A05: Set Trap Column Specifications → Set Support Backward Flush to "**Yes**"

   b) A06: Change Fluidics / Workflow

3) For the script A06: Change Fluidics / Workflow, select "***Fluidics: "Nano/Cap***" and "***Workflow: Trap-and-Elute***". Follow all instructions provided on the VUI to change the fluidics accordingly.

> **TIP** All required components to change the fluidic setup are provided in the Vanquish Neo System Ship Kit.

Once completed, the Vanquish Neo System is ready for running the IQ samples.

4) Program the acquisition method via the CM7 via the Instrument Method Wizard or the Xcalibur Method Editor. Use the following parameters:

Parameter	Value / action		
Runtime	20.00 min		
Diagnostics Channels	Default		
Fluidic setup	Verify all information is correct for both columns		
Solvents	Verify the solvents are correctly set		
Flow Gradient	Note: only the following parameters need to be entered. The remainder will be filled automatically.		
	Run		
	Duration (min)	**Flow (µl/min)**	**%B**
	0.000	0.350	1.0
	0.100	0.350	4.0
	13.900	0.350	35.0
	Column Wash		
	0.100	0.350	99.0
	5.900	0.350	99.0
Wash Solvent Names	Default		
Temperature Control	Activate and set to “7.0 °C”		
Loading Parameters → Mode	FlowControl		
Flow	60 µl/min		
Loading Volume	Automatic		
Fast equilibration	Activate the checkbox		
Mode	PressureControl		
Pressure	1500 bar		
Equilibration Factor	2		
Fast Wash and Equilibration	Activate the checkbox		
Wash Factor	100		
Equilibration Factor	Automatic		
Mode	Flow Control		
Flow	120 µl/min		

5) Once completed, finish the wizard, navigate in the method overview to the “Sampler” node, and activate “*Vial Bottom Detection*” function. Also ensure the Trap Column is set to “*Backward*” Trap Flush Direction.

6) Setup the MS detector parameters in the method. Use the parameters listed for "*3.2.4.1 Vanquish Neo System Preparation Direct Injection Nano*", leave all other parameters as default for the specific MS detector used.

7) Save the method and proceed to setup the sequence table.

8) Setup and run an injection sequence of 1 µl per sample injection in this order:

   a) Blank

   b) 12 x Cytochrome C

   c) Blank

3.2.4.3 Vanquish Neo System Preparation Direct Injection Micro

1) Follow steps 1, 2 and 3 of "3.2.4.1 Vanquish Neo System Preparation Direct Injection *NAN*".

2) Via the Vanquish User Interface, execute script A06: Change Fluidics / Workflow.

3) Select "***Fluidics: Micro***" and "***Workflow: Direct Injection***". Follow then all instructions provided to change the fluidics.

> **TIP** All required components to change the fluidic setup are provided in the Vanquish Neo System Ship Kit. From Nano/Cap to Micro, change the 20 µm capillaries to 50 µm capillaries as detailed by the script.

Once completed, the Vanquish Neo System is ready for running the IQ samples.

4) Program the acquisition method via the CM7 via the Instrument Method Wizard or the Xcalibur Method Editor. Use the following parameters:

Parameter	Value / action		
Runtime	20.00 min		
Diagnostics Channels	Default		
Fluidic setup	Verify all information is correct for both columns		
Solvents	Verify the solvents are correctly set		
Flow Gradient	Note: only the following parameters need to be entered. The remainder will be filled automatically.		
	Run		
	Duration (min)	**Flow (µl/min)**	**%B**
	0.000	50	1.0
	0.100	50	4.0
	13.900	50	35.0

Parameter	Value / action		
	Column Wash		
	0.100	50	99.0
	5.900	50	99.0
Wash Solvent Names	Default		
Temperature Control	Activate and set to “7.0 °C”		
Loading Parameters → Mode	PressureControl		
Pressure	800 bar		
Loading Volume	Automatic		
Fast equilibration	Activate the checkbox		
Mode	PressureControl		
Pressure	1200 bar		
Equilibration Factor	2		

5) Once completed, finish the wizard, navigate in the method overview to the “Sampler” node, and activate “*Vial Bottom Detection*” function.

6) Setup the MS detector parameters in the method. Use the parameters listed for “*3.2.4.1 Vanquish Neo System Preparation* ***Direct Injection NAN***”, leave all other parameters as default for the specific MS detector used.

7) Save the method and proceed to setup the sequence table.

8) Setup and run an injection sequence of 1 µl per sample injection in this order:

   a) Blank

   b) 12 x Cytochrome C

   c) Blank

3.2.4.4 Vanquish Neo System Preparation Trap-and-Elute Micro

1) Follow steps 1, 2 and 3 of “3.2.4.1 Vanquish Neo System Preparation Direct Injection *NAN*”.

2) Via the Vanquish User Interface, execute the following scripts in this order:

   a) A5: Set Trap Column Specifications → Set Support Backward Flush to “Yes”

   b) A6: Change Fluidics / workflow

3) Select "*Fluidics: Micro*" and "*Workflow: Trap-and-Elute*". Follow all instructions provided to change the fluidics.

> **TIP** All required components to change the fluidic setup are provided in the Vanquish Neo System Ship Kit.

Once completed, the Vanquish Neo System is ready for running the IQ samples

4) Program the acquisition method via the CM7 via the Instrument Method Wizard or the Xcalibur Method Editor. Use the following parameters:

Parameter	Value / action		
Runtime	20.00 min		
Diagnostics Channels	Default		
Fluidic setup	Verify all information is correct for both columns		
Solvents	Verify the solvents are correctly set		
Flow Gradient	Note: only the following parameters need to be entered. The remainder will be filled automatically.		
	Run		
	Duration (min)	**Flow (µl/min)**	**%B**
	0.000	50	1.0
	0.100	50	4.0
	13.900	50	35.0
	Column Wash		
	0.100	50	99.0
	5.900	50	99.0
Wash Solvent Names	Default		
Temperature Control	Activate and set to "7.0 °C"		
Loading Parameters → Mode	FlowControl		
Flow	60 µl/min		
Loading Volume	Automatic		
Fast equilibration	Activate the checkbox		
Mode	PressureControl		
Pressure	800 bar		
Equilibration Factor	2		
Fast Wash and Equilibration	Activate the checkbox		
Wash Factor	100		

Parameter	Value / action
Equilibration Factor	Automatic
Mode	Flow Control
Flow	120 µl/min

5) Once completed, finish the wizard, navigate in the method overview to the "Sampler" node, and activate "*Vial Bottom Detection*" function. Also ensure the Trap Column is set to "*Backward*" Trap Flush Direction.

6) Setup the MS detector parameters in the method. Use the parameters listed for "*3.2.4.1 Vanquish Neo System Preparation* ***Direct Injection NAN***", leave all other parameters as default for the specific MS detector used.

7) Save the method and proceed to setup the sequence table.

8) Setup and run an injection sequence of 1 µl per sample injection in this order:

   a) Blank

   b) 12 x Cytochrome C

   c) Blank

3.2.5 Vanquish Neo System with Column Compartment

The Vanquish Neo System can be expanded with a Vanquish Neo Column Compartment (VN-C10-A). This column compartment allows the linear Pepmap Neo analytical columns to thermostatted for improved chromatography stability.

This Vanquish Neo System Installation Qualification has been setup for the thermostatted analytical columns. If no analytical column thermostatting option is available, the retention time stability IQ specifications for non-thermostatted columns need to be used.

The IQ procedures listed here are NOT compatible with **heated** Trap-and-Elute configurations.

3.2.5.1 Preparations

Follow the column compartment installation procedure described in the Vanquish Neo System Operating Manual. A switching valve can be installed (e.g. to enable Heated Trap-and-Elute workflows), however is not required for the IQ.

When bypassing the column compartment switching valve, the analytical column will be connected via a Viper union to the transfer line coming from the left valve of the autosampler.

However, if the column compartment switching valve is used, ensure the valve is installed in the **LEFT** position to be compatible with the system preparation scripts.

For all acquisition method described, set the temperature of the column compartment to 40°C in the "Temperature" field of the instrument method editor. All other parameters are left default or are automatically recognized.

3.2.6 Ultraviolet Detector

Besides using a mass spectrometer as detector, the Vanquish Neo System Installation Qualification can also be performed with an ultraviolet detector (UV). The Vanquish Flex variable wavelength detector (VF-D40-A) with the appropriate flow cell can be integrated with the Vanquish Neo System.

Notice The Vanquish Neo System Installation Qualification using a VWD detector is **ONLY** compatible with the linear PepMap column types.
An EASY-Spray column cannot be connected to a UV detector.
Additionally, an LC-UV-MS hardware setup is not suitable for IQ acquisition.

3.2.6.1 Preparations

Lamp warm up

Turn the lamp on as soon as possible. Allow the lamps to warm up and reach operating temperature. The lamps should be running for at least 1 hour before you start data acquisition.

Flow cell installation and connection

Depending on the flow range chosen to run the IQ, ensure the correct flow cell is delivered and installed. Refer to the *VF-D40-A Operating Manual* for details on the flow cell installation procedure.

Flow range	Flow Cell	Part Number
Nano	3 nL, 20 MPa, fused silica, PTFE tubing connection	6074.0270
Micro	45 nL, 30 MPa, fused silica, nanoViper connection	6074.0285

For Nano flow range

Connect the Nano linear Pepmap Neo column outlet nanoViper to the 1/16” side of the microtight adapter. Then, slide a black sleeve (P/N SC903) over the fused silica inlet capillary of the 3 nL flow cell. Insert this combination into the 1/32” fitting of the microtight adapter side. While tightening, ensure that the sleeve and fused silica capillary are aligned completely against the installed nanoViper fitting installed. Tighten properly and test connection by gently pulling on the fused silica capillary.

Ensure a suitable waste tubing (e.g. PTFE) is installed to the flow cell outlet and guided to the draining system.

For Micro flow range

Connect the Micro column via a nanoViper capillary and Viper union to the nanoViper connection of the 45 nL flow cell. Ensure a suitable waste tubing (e.g. PTFE) is installed to the flow cell outlet and guided to the draining system.

Acquisition Method

For all acquisition methods described, match the run time for UV data acquisition to the method run time. Set the acquisition wavelength to 214 nm. All other parameters are left default or are automatically recognized.

4 Data Analysis

4.1 IQ specification

The Cytochrome C digest runs are evaluated to determine if the instrument performance is within the IQ specification limits.

Peak 5 (m/z 728.84) will be used to determine the retention time precision of the last 6 Cytochrome C runs and peak with at half maximum (PWHM) of the last 6 Cytochrome C runs

Note, compared to the IQ with an MS, for a Vanquish Neo System IQ with UV are two more specifications evaluated: the resolution of peak 1 to 4 and the UV absorbance (peak height) of peak 5.

Regardless of IQ workflow and flow range used, the same IQ criteria apply for all 4 IQ workflows. The following IQ criteria need to be met for a successful installation:

Specification MS	**Criteria**	
Retention time precision [% RSD] (Peak #5, m/z 728.84, 2+)	Thermostatted	Non-thermostatted
	≤ 0.30	≤ 0.40
PWHM [s] (Peak #5, m/z 728.84, 2+)	≤ 7	
Specification UV		
Retention time precision [% RSD] (Peak #5)	Thermostatted	Non-thermostatted
	≤ 0.30	≤ 0.40
PWHM [s] (Peak #5)	≤ 7	
Resolution of peaks (Peaks #1 to #4)	> 1.5	
UV absorbance [mAU] (Height Peak #5)	≥ 3	

The data processing steps differ based on which software is used. Below are the processes for SII for Xcalibur and Chromeleon described.

4.1.1 Data Analysis with SII 1.5.1 for Xcalibur

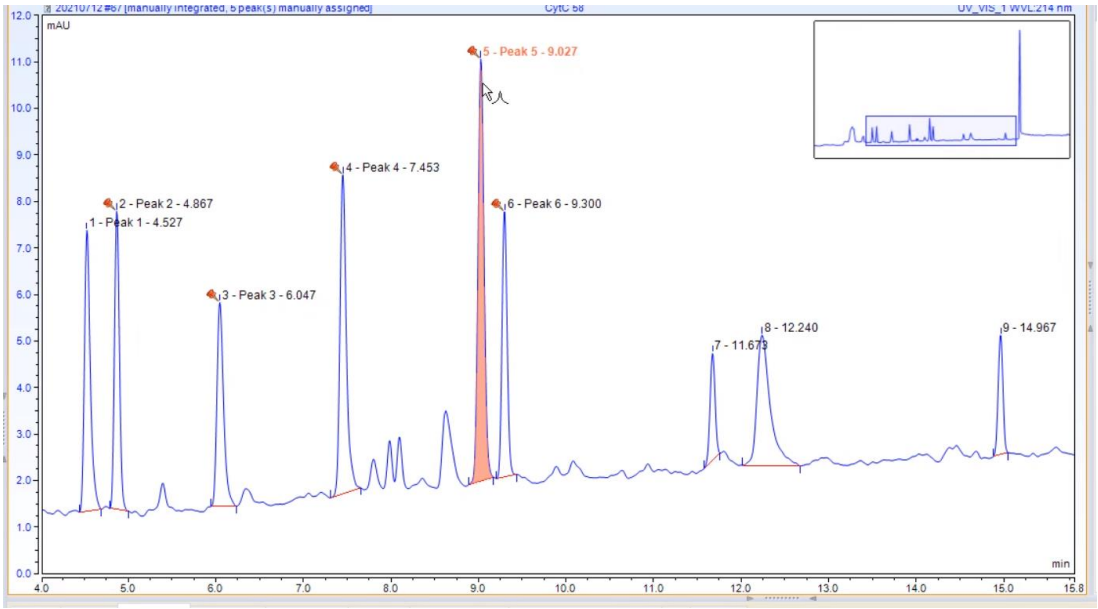
Within Xcalibur, the RAW data can be processed with either Qualbrowser or Freestyle.

1) Open the RAW file in the data processing tool.
2) Generate an Extracted Ion Chromatogram of mass m/z 728.84.
3) Apply a 7-point Gaussian smoothing.
4) In the chromatogram pane, determine the peak retention time at the peak apex. Write the RT of each of the last 6 runs in the IQ report.
5) Change the X-axis view settings to 50-100%. This sets the X-axis and peak intersection at the correct PWHM.
6) Determine the PWHM in seconds. Write the PWHM of each of the last 6 runs in the external IQ report.
7) Review the results and determine if IQ is completed.

4.1.2 Data Analysis Chromeleon 7

1) Open the Injection List in Chromeleon 7.
2) Locate the RSLCnano Report Template "*IQ_NANO_CAP_MIC_NCS_Preconcentration_V04_8*" and copy to the Injection List folder.
3) In the Injection List, set the "Processing Method" value for the last 6 Cytochrome C injections to "NAN" or "MIC", whichever workflow flow range was used.
4) In the Injection List, write in the "Comment" column for Cytochrome C 1-5 "Date evaluation" and for the final Cytochrome C "Print Data evaluation".

5) Navigate to the “Data Processing” workspace and evaluate the “Peak 5” area integration for the last 6 Cytochrome C runs. Adjust the peak assignments manually in case the peaks are not recognized.



6) Save Changes before moving to the “Report Designer” workspace.

7) Open the “Report Designer” workspace and navigate to the Retention time tab.

8) Click on “Update external references”.

9) Find the “Ret. Time” and “Peak Width” column.

No.	Name	Ret.Time min UV_VIS_1 Peak 5	Height mAU UV_VIS_1 Peak 5	Peak Width (50%) seconds UV_VIS_1 Peak 5	Resolution UV_VIS_1 Peak 1	Resolution UV_VIS_1 Peak 2	Resolution UV_VIS_1 Peak 3
64	CytC 55	9.03	9.259	4.46	2.95	9.99	10.69
65	CytC 56	9.03	9.243	4.37	3.02	9.98	10.36
66	CytC 57	9.03	9.163	4.43	2.97	9.22	9.95
67	CytC 58	9.03	9.072	4.43	3.06	9.91	10.83
68	CytC 59	9.03	9.327	4.36	3.05	10.00	10.25
69	CytC 60	9.03	9.467	4.25	3.03	10.28	10.76
Average:		**9.027**	**9.255**	**4.384**	**3.014**	**9.898**	**10.472**
RSD:		**0.001**					
Min.Resolution:					**2.95**	**9.22**	**9.95**

Retention time | RSD calculation | MS results | Results_and_headers | Audit trail

10) Transfer the values to the external IQ report.

11) Review the results and determine if IQ is completed.

5 Appendix

5.1 IQ_Report_Comm_Test_x_y.docx form

PLACEHOLDER FOR EXAMPLE REPORT