Vanquish

Neo System

VN-S10 and respective modules, (VN-A10, VN-C10, VN-P10)

User Guide

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Dornierstrasse 4, D-82110 Germering, Germany

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1 Using this Manual

This chapter provides information about this manual, the conventions used throughout the manual, and the reference documentation that is available in addition to this manual.

1.1 About this Manual

This document describes the setups, recommended experimental conditions and testing procedures required to run standard applications on the Thermo Scientific Vanquish Neo UHPLC system.

NOTICE This document is intended for Thermo Fisher Scientific (or authorized) service personnel as well as customers to assist in the installation and application testing of Vanquish Neo UHPLC systems. It does **not** replace the IQ or OQ procedures. It is assumed that the individual using this manual has had sufficient training in the installation and usage of analytical instrumentation and is aware of the potential hazards including (but not limited to) electrical hazards, chemical hazards, exposure to UV radiation and exposure to pressurized solvents.

This manual contains important information about the correct care and use of the Vanquish Neo UHPLC system. Please read this manual carefully before installing or running any of the applications described. Keep this manual close to the Vanquish Neo UHPLC system for future reference and pass it on to any subsequent user.

1.2 Conventions

This section describes the conventions used throughout this manual.

1.2.1.1 Special Notices and Informational Notes

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

NOTICE Highlights information necessary to prevent damage to the instrument or invalid test results.

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

1.2.2 Typographical Conventions

These typographical conventions apply to the descriptions in this manual:

References and Messages

References to figures and tables appear *italicized*.

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 instrument from the front.

Particularly Important Words

Particularly important words in the main flow of text appear ***in bold***.

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, figures or online reference materials

1.3 Reference Documentation

Further information relating to the Vanquish Neo system and associated applications and accessories available as follows:

- HPLC and UHPLC Resources
- Low-flow HPLC columns for Proteomics Applications
- Low-flow HPLC and UHPLC columns connection guide
- Low-flow HPLC and UHPLC columns web portal
- Vanquish Neo System Brochure
- Vanquish Neo System Customer Familiarization
- The Vanquish Neo System Operating Manual
- Vanquish Neo System Specification sheets
- Vanquish System Online configurator
- Vanquish User Interface (System Controller and Display) Installation Guide
- Viper™ and nanoViper™ EASY-Spray™ Column tips and tricks document
- Viper and nanoViper capillaries

  Thermo Scientific Viper and nanoViper Fingertight Fitting System - brochure

  Viper and nanoViper Fingertight Fitting Systems - specifications
- µPAC Neo HPLC columns: deeper proteomic coverage shorter runtime
- µPAC Neo Product Specification Sheet
- µPAC Neo HPLC Column – Use and Care Instructions

2 System Overview

This chapter provides details on the Vanquish Neo System hardware configurations

2.1 Hardware Components

The Vanquish Neo is a low-flow (nano-, capillary- and microflow-) UHPLC system designed for seamless integration for all high sensitivity LC-MS workflows.

2.1.1 System Components

The Vanquish Neo System (Figure 1) comprises the following hardware components.

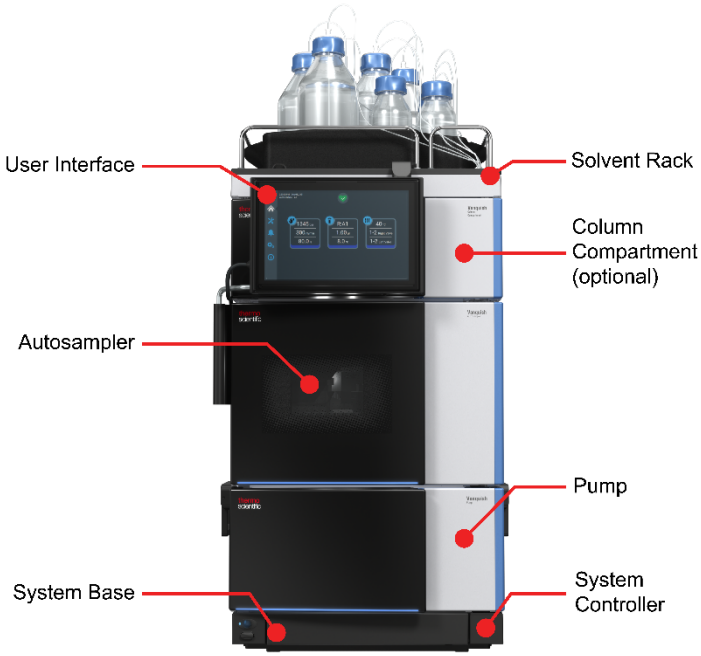


Figure 1: Vanquish Neo System Hardware Components

2.1.2 System Base

The system base carries the pump, autosampler, system controller and optionally the column compartment.

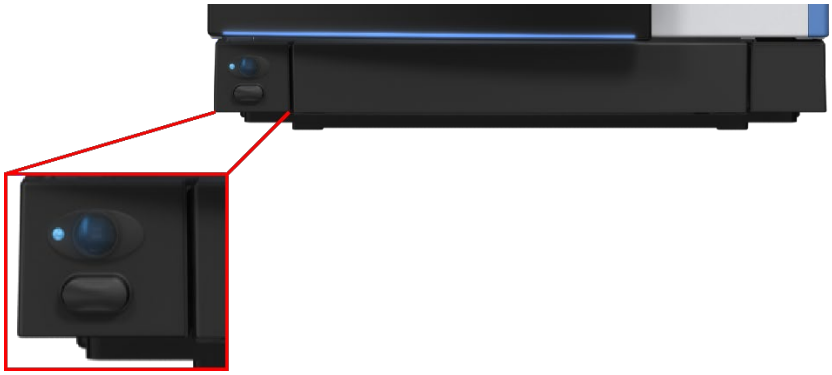


Figure 2: The Vanquish Neo System Base

The system base Figure 2 comprises:

- Power buttons for power on/off control of all modules and the system controller
- A drawer to store tools and small system parts
- Drain port for connecting a system waste line
- System base locking tool to toggle between moveable and stationary mode

2.1.3 System Controller and User Interface

The Vanquish System Controller is connected to the Vanquish User Interface:

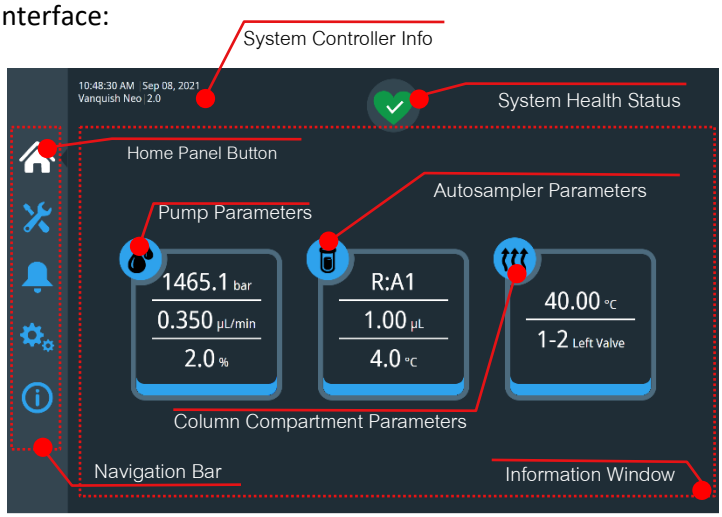


Figure 3: The Home Panel of the Vanquish User Interface

The System controller and Vanquish User Interface provide:

- Information on the
  - system health status
  - system module notifications
  - system settings including workflow and IP address
- Intelligent user guidance (scripts)
- Direct control of module main functions and parameters

2.1.4 Pump

The principle components of the continuous flow binary high pressure gradient pump equipped with integrated flowmeter are shown below (see Figure 4):

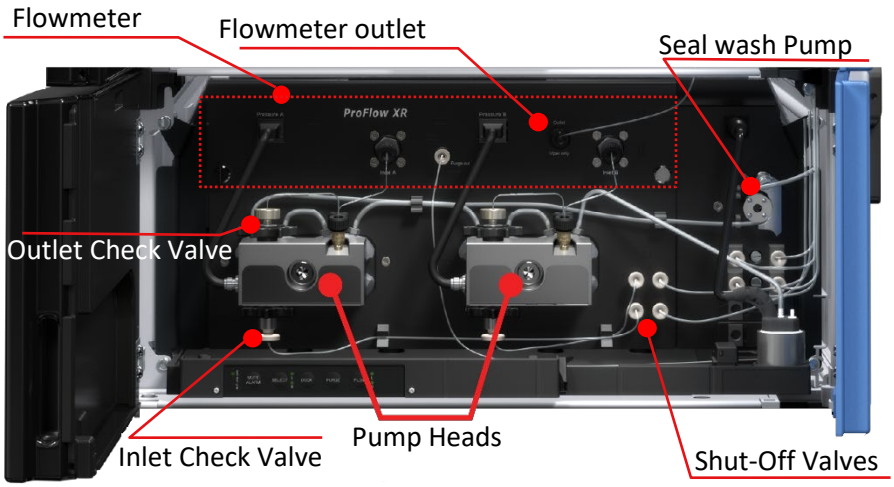


Figure 4: The Vanquish Neo Binary Pump

Each channel generates a partial flow controlled by a separate flow sensor. The partial flows are mixed and combined in a fluidic Tee piece located at the flow meter outlet. The combined flow leaves the flow meter with the selected target flow rate and solvent composition.

The pump has the following attributes:

- Up to 1500 bar operation from 100 nL/min to 100 µL/min
- Active flow control across the entire flow rate range
- Automatic purging
- Active solvent shut-off valves resulting in increased tolerance to solvent outgassing and more robust operation
- Multi-point flow-calibration algorithm for precise and reproducible flow delivery and high system-to-system reproducibility
- Gradient delay of < 25 nL

NOTICE Running the pump without solvents, particularly at microflow flow rates, can cause excessive wear to the piston seals and could result in flow sensor contamination.

2.1.5 Autosampler

The autosampler is based on the split-loop injection principle.

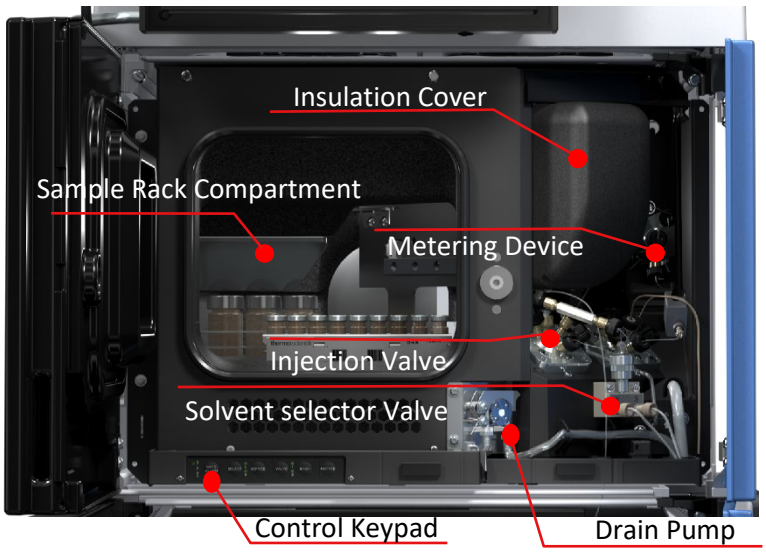


Figure 5: The Vanquish Neo Autosampler

Two 1500 bar capable proprietary 7 port 6 position valves enable offline switching of the injection fluidics during gradient separation. This ensures an extremely low GDV despite the split-loop sample architecture. The autosampler also has the following attributes:

- Air-to-air cooling – no condensation in the sampler compartment
- Multiple wash options for sampler fluidics using both weak and strong wash liquids to ensure extremely low carry-over
- Vial bottom detection technology for maximum sample extraction
- Metering Device with next generation SmartInject™ technology which also acts as a loading pump in trap-end-elute configuration
- High injection volume precision and accuracy over a wide injection volume range (10nL – 25µL with a standard loop, up to 500 µL with a 100 µL loop using Multidraw)
- Supports forward- and back-flush trap-and-elute workflows - no fluidic re-plumbing required

NOTICE The autosampler insulation cover (P/N 6252.1647) is essential for sample thermostatting. Failure to install it during instrument operation will cause the thermostatting to switch off after 20 min!

2.1.6 Column compartment

The horizontal Vanquish Neo Column Compartment is an optional module which supports optimized system stacking and capillary guiding for low-flow LC-MS connections. The main components are shown in Figure 6.

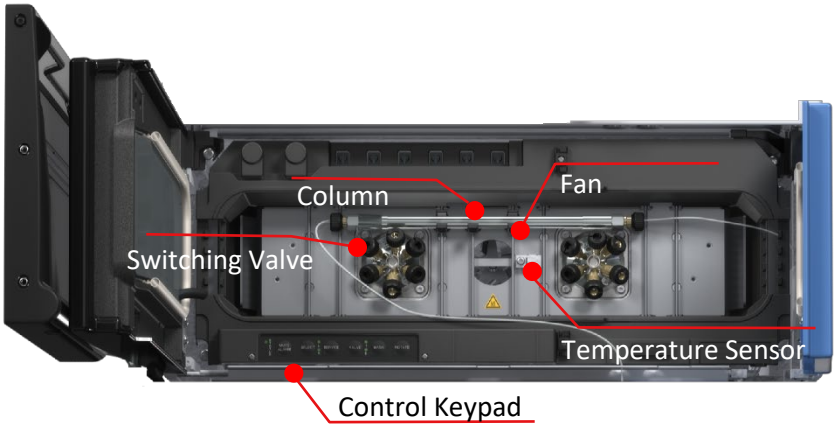


Figure 6: The Vanquish Neo Column Compartment

The Vanquish Neo Column Compartment works using advanced design forced air thermostatting to support column heating with an even airflow to ensure the temperature of the stationary phase remains constant over the entire column length and that the column and eluent have the same temperature during the analysis. Two optional low-dispersion 1500 bar switching valves for advanced workflow applications are also supported. Further attributes include:

- Fast heat-up from 35 °C to 60 °C ± 1 °C in < 12 minutes
- Temperature range:
  - +5 °C above ambient to +60 °C with Vanquish valves
  - +5 °C above ambient to +80 °C without valves
- Temperature precision of ± 0.1 °C
- Temperature stability of ± 0.05 °C
- VICI™ valve (50 °C maximum temperature - requires service for installation) and passive pre-heater compatible

2.1.7 System Rack and Bottle insert

The solvent rack (Figure 7) comes pre-attached to the top of the Vanquish Neo system, complete with a chemically resistant bottle insert which has the capacity to safely store:

- 5 positions for 1 L solvent reservoirs
- 2 positions for 2 L solvent reservoirs
- 4 positions for 0.25 L solvent reservoirs

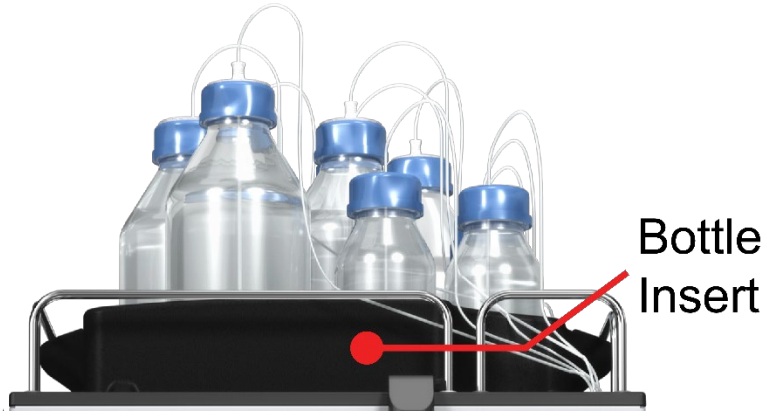


Figure 7: Solvent Rack Including Bottle Insert and Solvent Reservoirs

2.2 Control Elements

The Vanquish Neo system is designed for primary operation either from an instrument control PC for sample analysis or via the Vanquish User Interface when executing system set up, start-up or diagnostic tests.

In addition, keypads at the bottom of each module allow the user to execute specific functions directly from the module devices.

2.2.1 Status LEDs

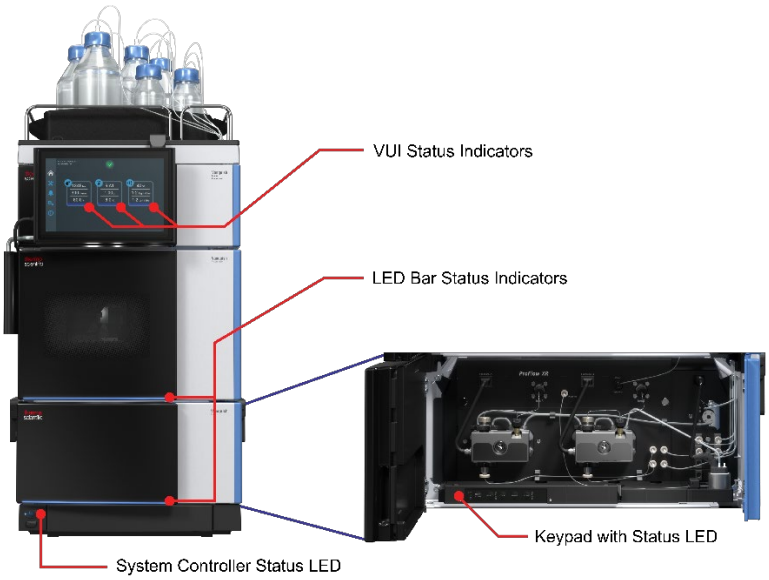


Figure 8: Vanquish Neo module status LEDs and key pads

The LED bars displayed on the front side of the modules and on the VSC screen (Figure 8) provide visual information including connectivity, health status as well as information about whether the system is powered up and acquiring data etc.

For details on what the colours and patterns (e.g. running vs flashing) mean for each module please refer to section 6.5.2. of the Vanquish Neo System Operating Manual.

2.2.2 Keypad Buttons

The Keypad buttons allow the user to perform specific functions directly from the modules. When a button is pressed, a short beep confirms that the function has been executed.

A list of the individual functions is given in section 6.5.1 of the Vanquish Neo Operating Manual.

TIP Not all keypad functionality is active for each module. Some functionality has been transferred to the Vanquish User Interface in which case the specific keypad is redundant. Some key pad functionality e.g manual valve switching on the autosampler has been intentionally deactivated as manual valve switching may cause damage to the system and / or its components.

3 The Vanquish User Interface

The Vanquish User Interface (VUI) provides the user with specific instrument control options without the system being connected to a control PC. The Navigation bar on the left side of the screen is home to five panel buttons, the details of which are described in the following sections.

TIP The Vanquish User Interface can also be accessed via internet browser if the Vanquish System Controller (VSC) is connected to a local area network or control PC.
Access is gained by inputting the IP address of the VSC which can be read out from the Settings Panel of the Vanquish User Interface (VUI).

3.1 Home Panel

The home panel (Figure 9) provides an overview of the functional parameters of the system displaying for example pressure and flow readouts along with the system health and operational status. It also provides access to direct control options for each of the modules.

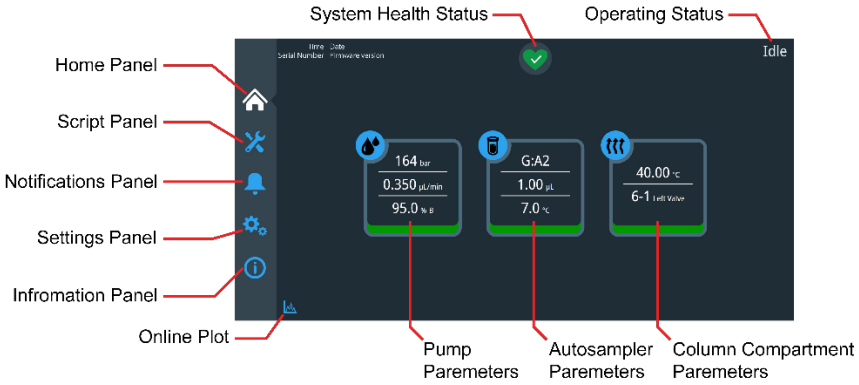


Figure 9: Vanquish User Interface (Home panel)

On the home panel, each module is represented by a box with a specific icon containing:

- Functional parameters – e.g., pump pressure, column compartment temperature, etc.
- A colored bar at the bottom of each box representing the LED bar status of the module.

The Direct control (DC) panel can be accessed by clicking on the respective module box. Whereas the box shows the current value, the DC window typically shows the nominal window. The direct control options available for the Vanquish Neo Binary Pump are shown in Figure 10 below.

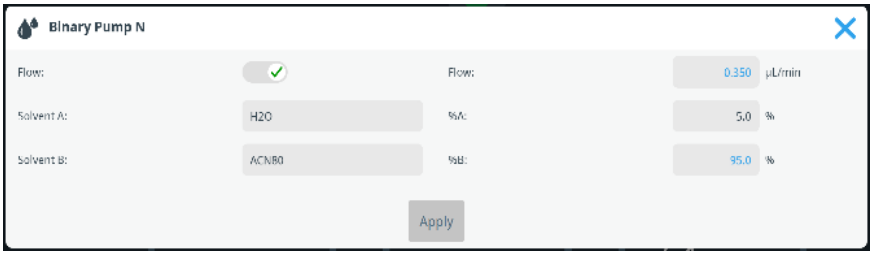


Figure 10: Vanquish Neo Binary Pump direct control panel

3.1.1 Online Plots

These are real time displays of selected system parameters and are accessed by clicking on the chromatography icon present in the bottom left corner of the home screen (see Figure 9).

A dialogue box opens which displays the connected modules on the top half of the screen and the available online plot associated with the specific module in the bottom half of the screen (see Figure 11).

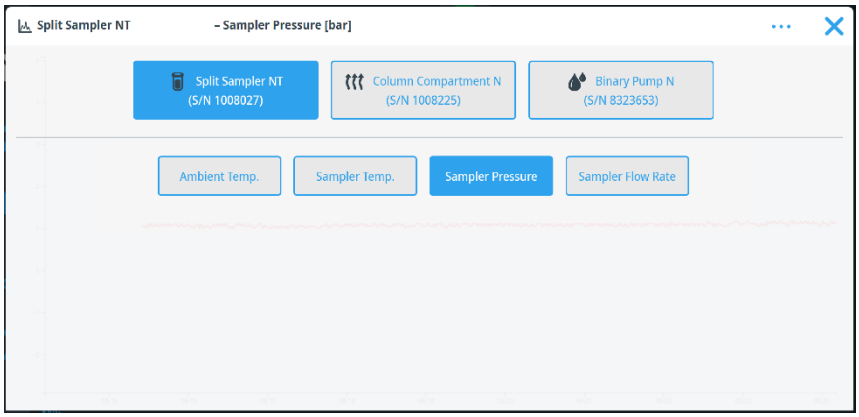


Figure 11: Online plot menu dialogue box

TIP Online plots are updated continuously and only show the last 10 minutes of available data from the specific channel. These plots are intended for quick reference only and their data rate is limited. Complete data sets with full signal information can be obtained via Chromeleon based .cmbx data files which are generated by executing the **E03 – Download Service Data** script. A full Chromeleon license is required to open and view the data file.

3.2 Script Panel

3.2.1 Overview

The script panel (Figure 12) provides automated scripts which cover multiple aspects of system care and use to ensure reliable, robust, and consistent operation.

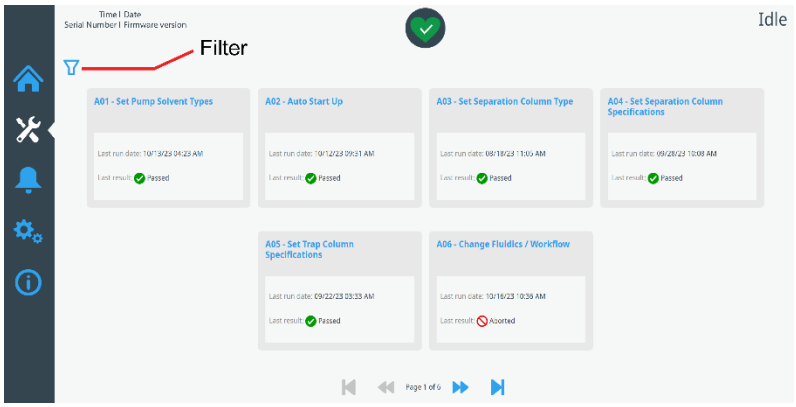


Figure 12: Page 1 of the VUI scripts

Each script dialogue box details the date and time when the script was last run and whether the script passed, failed, or whether it was cancelled prior to completion. Scripts can be filtered according to device and / or function (Figure 13). The filter function is accessed by clicking on the filter icon (shown in Figure 12)

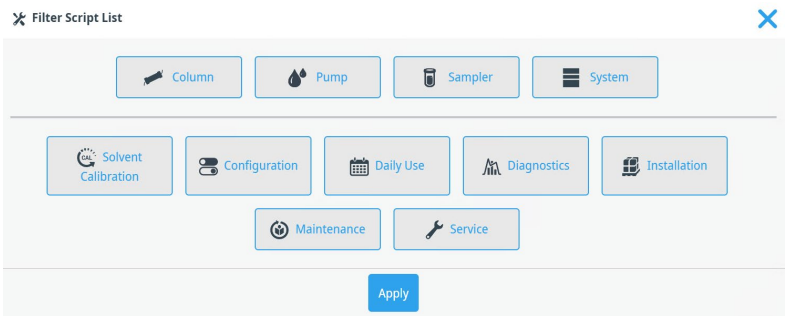


Figure 13: Script Filter List of Options

A list of the scripts available at time of publication (Vanquish Neo Bundle 1.4) , complete with description, are given in Table 1 below.

3.2.2 Scripts available on the Vanquish User Interface

ID	Script	Description
A01	Set Pump Solvent Types	This script allows the operator to set the solvent types for the pump. This should be carried out when a solvent type is changed. After carrying out this script the correct factory pump calibration values are applied for the solvent type in use.
A02	Auto Start Up	This script purges the system and adjusts the offset of the sampler pressure sensor. This should be carried out after installation or long term shut down. After performing this script, the system is ready for operation. The system tightness can also be checked by running the D02 script after the auto start up.
A03	Set Separation Column Type	This script allows the operator to set the type of the separation column. This must be carried out whenever the column type is changed. The Vanquish Neo system and Chromatography Data System use this information for further operation. Information entered here affects the operating conditions available.
A04	Set Separation Column Specifications	Use this to set separation column specifications. The Vanquish Neo system and Chromatography Data System use this information to set instrument method limits and calculate gradient delay volumes. Implementing the correct parameters is essential for correct operation and to prevent column damage.
A05	Set Trap Column Specifications	Use this to set trap column specifications if one is in use i.e. for trap-and-elute or heated trap-and-elute workflows. The Vanquish Neo system and Chromatography Data System use the information to set instrument method limits and calculate gradient delay volumes. Implementing the correct parameters is essential for correct operation and to prevent column damage.
A06	Change Fluidics/Workflow	This script guides the user through a workflow and/or fluidic configuration change. After the change is complete – the system checks to ensure the fluidic configuration installed matches the one selected, then purges the system and tested for tightness.
A07	Change Sample Loop	This script guides the operator in how to change the sample loop. This should be carried out whenever replacing the loop or when using a different sample loop (e.g. 10µL or 100µL loop). Upon completion, the new sample loop is installed, purged, tested for tightness and the loop volume set for the instrument.
A08	Catch Valve	This script allows the operator to configure the Thermostatted Column Compartment to mount a compatible VICI valve.

ID	Script	Description
		This should be carried out whenever the operator configures the system to use a heated trap-and-elute workflow including a compatible VICI valve. After performing the script, the instrument is ready to use with compatible VICI valves for the heated trap-and-elute workflow. **Note: This script does not appear in the menu if the column compartment does not possess a VICI valve drive.**
B01	Change Liquids/Solvents	This script purges the system fluidics for the respective flow path components affected by the solvent being exchanged. Activate the “refresh only” option to exchange a solvent bottle with the same solvent type.This should be carried out whenever a bottle is exchanged.After performing the script, the instrument is purged with the new solvents.
B02	Clean and Equilibrate Column(s)	This script cleans and equilibrates the connected separation column or separation and trap column. It is intended for use offline of the MS to prevent impurities from the column from being washed through to the MS during the cleaning/equilibration script. Please ensure that the column is dismounted from the ion source and that the High Voltage in the ion source is switched off prior to script execution. This script can be used to remove unwanted substances from the column and to prime the column. After performing the script, the column is fully purged, primed and can be used for analysis.
B03	Clean Up System	This script is automatically triggered after an inject abort. It can also be triggered manually to clean up the injection fluidics. After performing the script, the sampler including trap column (if installed) is fully cleaned and ready for the next injection. Please note that the analytical column must either be washed and equilibrated manually. Alternatively, execute the B02 script.
B04	System Self-Test	This script performs a self-test for the entire system and sets the system as “ready” for a new injection. This should be carried out whenever a module failure state is reached. This is indicated by a red LED bar on the module door and on the VUI home panel. If the failure persists further troubleshooting is required using the notification panel and Vanquish Neo operating manual.
B05	Shutdown	This script will initialize a system shut down and prepare the system for either short-term or long-term storage.

ID	Script	Description
		This should be carried out whenever the instrument is not going to be used for an extended period of time. Refer to the operating manual for guidelines.
B06	Condition Columns	This script is used to prepare new columns or used columns for analysis after long term storage (> 1 week). The columns are flushed with 99% B from 20% to 80% of the upper pressure limit (volume to ramp up pressure + 3 column volumes).
C01	Adjust Pump Flow Sensor Offsets	This script adjusts the offset of the pump flow sensors. This should be carried out whenever peak retention times run out of specification because of pump flow sensor drift. After performing the script, the flow sensors are calibrated to perform within expectations.
C02	Purge Pump	This script offers two options to purge the entire pump or the flow meter only, e.g., to remove air, flush pump heads or to fill exchanged spare parts with solvent. This should be carried out whenever the flow rate is unstable, or modifications have been made to the pump fluidics. The script offers two intensity levels, fast and standard: Use purge intensity 'Fast' if the same solvent type is used. When changing solvent types, use 'Standard'.
C03	Adjust Sampler Pressure Sensor Offset	This script adjusts the offset of the sampler pressure sensor. This should be carried out whenever the capillary leading to the sampler pressure sensor has been detached. After performing the script, the sampler pressure sensor is calibrated to perform within expectations.
C04	Purge Sampler	This script offers various options to flush individual parts of the sampler, e.g., to remove air or to fill exchanged spare/optional parts with solvent. This should be used to remove air from the sampler or whenever modifications are made to the sampler fluidics. Note that with purge intensity set to “intense”, the metering device is purged internally with both strong and weak wash. For this reason, the script duration is significantly increased.
C05	Adjust System Pressure Sensors	This script adjusts all pressure sensors of the system (Pump and Sampler) to match each other. This should be carried out whenever a pressure sensor or a module or spare part with a pressure sensor has been exchanged. Note this script is not required following a calibration using the script “C03 - Adjust Sampler Pressure Sensor Offset”

ID	Script	Description
		After performing the script all pressure sensors of the instrument are aligned.
C10	Clean or Replace Pump Head Check Valves	This script will guide the operator through the cleaning or replacement process for the pump check valves. This should be carried out whenever a check valve is exchanged. The check valves should be replaced whenever they are worn or damaged and whenever a leaking check valve is observed. After following through with the script, the new pump check valves are installed, the pump is purged and tested for tightness.
C21	Replace Needle Unit And Seat	This script will guide the operator through the procedure of replacing the needle unit and needle seat of the sampler, should their exchange be required. At the end of the script, the sampler is purged and tested for tightness.
C22	Replace Metering Head	This script will guide the operator through the procedure to replace the metering head of the auto sampler should it require exchange. At the end of the script, the sampler is automatically purged and tested for tightness.
D01	Test System Back Pressure	This script identifies system blockages and/or defective parts in the event an overpressure is observed. If it fails, the part that causes the overpressure is identified. After exchanging the faulty part, the test should be carried out again to verify that the instrument is fully operational. Note: In Trap- and-Elute configuration, the option “test only trap column back pressure” is also available. This can be used regular to monitor trap column performance on the fly - see section 6.1.7 for details.
D02	Test System Tightness	This script checks the system for leakages. Options are available to perform the test for individual modules as well as the entire system. 5 test pressure limits are available. For trap-and-elute workflows, ensure that the trap column specifications are set correctly, before running the script, otherwise the trap column could be damaged. Note: The test pressure will never exceed the column pressure limits set with scripts A04 and A05 irrespective of what test pressure is set in the script. If the script fails, information is provided to direct the operator to the fluidic component causing the leak.
E01	Initialize System Setup	This script resets the currently installed system settings to reinitialize the system configuration. This should be carried out when scripts are not available on the script panel or fluidics/workflows have been configured incorrectly. See section 6.2.1 for details.

ID	Script	Description
E02	Reset Factory Defaults	This script sets the factory default values for the selected devices. This script should be carried out on demand as required by the operator or when explicitly requested by Thermo Fisher Scientific personnel. After performing the script, the default values of the instrument are restored.
E03	Download Service Data	This script allows the operator to download a CMBX Service Data Package. This should be carried out when requested by the Thermo Fisher Scientific service organization and as the last step in performing the System Check procedure, (see section 6.1.3.) After performing the script, a download link is provided from the web interface of the Vanquish User Interface which can be shared with Thermo Fisher Scientific service personnel.
E04	Detailed Leak Test	This script carries out a detailed diagnostic to determine the cause of leaks in the pump as identified in the D02 script. This script should only be carried out by or on the request of Thermo Fisher service personnel.
M31	Solvent Calibration Step 1: Purge Pump with New Solvent	Solvent calibration (SC) is required when a solvent type differs by ≥ 5% from a pre-calibrated factory solvent types (script A01). Script M31 is the first step in a 3 step procedure. It flushes the system with the new (custom) solvent
M32	Solvent Calibration Step 2: Calibrate Pump Block A or B	This script (step 2) calibrates the selected pump block (A (left) or B (right)) for the new solvent type. The script should only be run after successful completion of script M31 (step 1). The calibration takes approximately 2 hours
M32a	Solvent Calibration Step 2a: Calibrate Block A High Viscosity	This script calibrates pump block A (left) for a high viscosity solvent. It should only be run when script M32 explicitly tells the user to do so.
M33	Solvent Calibration Step 3: Restore Operational Configuration	This script restores the pump to an “operational status” after script M32(a) is finished. Please note that in the event of failed custom solvent calibration (script M32(a) failed) – Script M33 must be executed to take the pump out of “calibration status” before re-attempting the calibration or starting trouble shooting.
M34	Delete Solvent Calibration Data	Run this script to delete calibration data from a previously performed calibration, e.g. to free up memory on the VUI

Table 1: List of Scripts with Description for the Vanquish User Interface

TIP After completing a diagnostic script, a summary report is displayed.

To see detailed test results, click on the following icon  **displayed in the bottom right-hand corner of the summary screen**.

3.3 Notification Panel

The notification panel logs information about all the warnings and errors of the Vanquish Neo system. These are displayed in chronological order in an event list (see Figure 14 below)

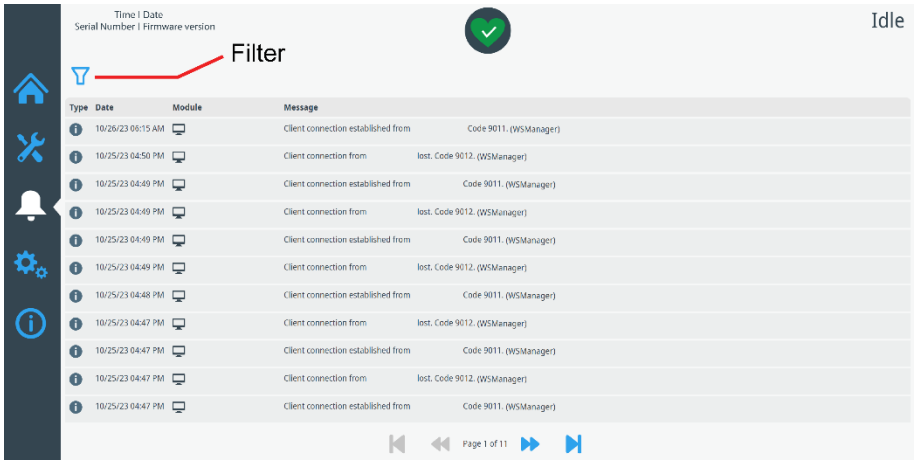


Figure 14: Notification Panel

The event list can be filtered according to module type and category (see Figure 15 below).

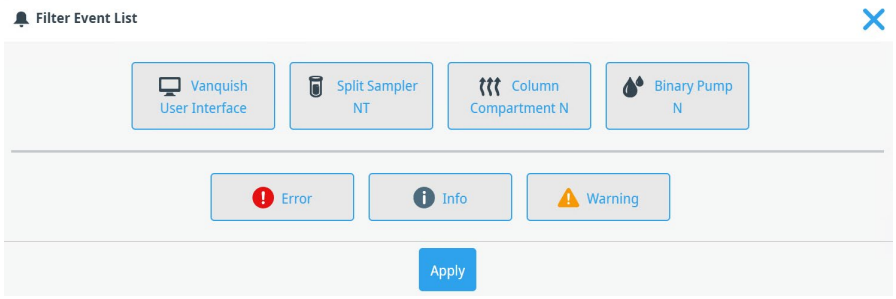


Figure 15: The filter event list dialogue box for the notification panel

TIP The filter event categories (e.g., Error, Info, Warning) only appear if the respective category contains an entry in the notification log. If the category is unavailable, then no event corresponding to that category has been logged.

3.4 Settings Panel

The main menu screen of the settings panel is shown in Figure 16 below.

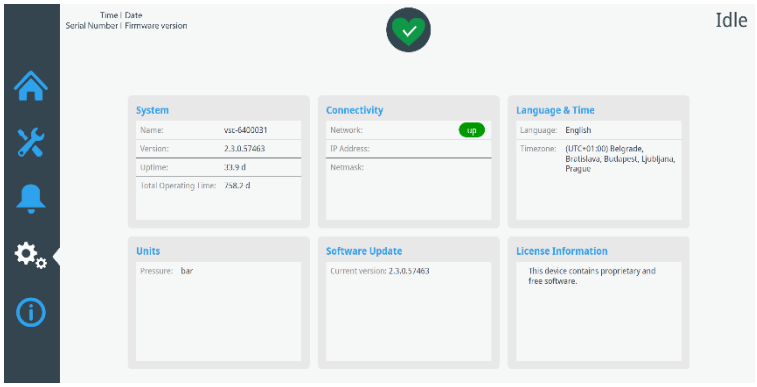


Figure 16: Settings Panel

The settings panel comprises six boxes, some of which are display only e.g., the system settings and license information, whereas connectivity and units can be adjusted.

TIP i) The Language settings can be set independently on the web browser and the VUI. ii) The time zone settings can only be set on the VUI when the VSC is not connected in the instrument configuration manager.

TIP VSC software updates are not available via the software update tab. This is intended as a read only field.

3.5 Information Panel

The Information panel comprises 3 boxes and is shown in Figure 17 below:

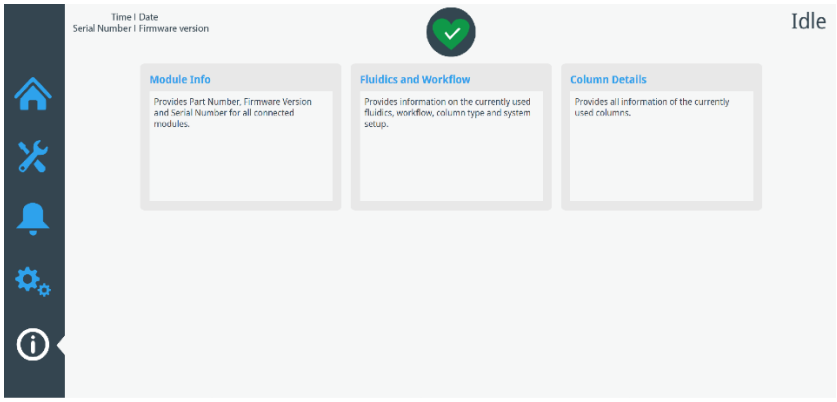


Figure 17 The VUI Information Panel

The information panel provides the user with quick and direct access to useful information relating to the Vanquish Neo system:

- Module Info
  - Firmware Version and Serial Numbers
- Fluidics and Workflow
  - System setup, fluidics and column type and workflow
- Column Details
  - Dimensions, flow, pressure, temperature and pressure change specifications
  - Whether backward flush is supported or not (for trap-and-elute workflows only)

4 Operating the System

4.1 Preparing for Operation

4.1.1 Preparing Solvents

For best results, when running conventional reversed phase LC-MS based analytics, use premixed Optima™ LC-MS solvents (see Table 2 for a complete list of available solvents).

TIP To ensure system cleanliness, it is imperative that solvent bottles are thoroughly rinsed and filter frits, filter holders and solvent line adapters are rinsed with ultrasonication prior to first use.

- Only use fresh LC-MS grade solvents
- Degas (sonicate) solvents for 15 minutes prior to installation
- Avoid the use of detergents when cleaning glassware. All glassware used for LC-MS applications (including graduated cylinders) should be rinsed with LC-MS grade solvents prior to use and should be labelled and stored separately.

NOTICE To avoid bacterial growth and / or changes to the solvent composition, solvents must be refreshed every two weeks. Do not top up solvent bottles. Replace with freshly prepared solvents.

4.1.2 Mobile Phases

For trouble-free operation we recommend Optima™ LC-MS solvents.

Part No.	Description	Fisher Scientific Web Link
LS118-500	Water with 0.1% Formic Acid (v/v)	https://www.fishersci.com/shop/products/0-1-formic-acid-water-optima-lc-ms-solvent-blends-fisher-chemical-4/LS118500
LS122-500	80% Acetonitrile, 20% Water with 0.1% Formic Acid	https://www.fishersci.com/shop/products/0-1-formic-acid-water-optima-lc-ms-solvent-blends-fisher-chemical-4/LS122500
A117-50	Formic Acid, 99.0+%	https://www.fishersci.com/shop/products/formic-acid-optima-lc-ms-grade-fisher-chemical-5/A11750
A461-212	Isopropanol	https://www.fishersci.com/shop/products/2-propanol-optima-lc-ms-fisher-chemical-4/A461212#?keyword=A461-212
LS120-1	Acetonitrile with 0.1% Formic Acid (v/v)	https://www.fishersci.com/shop/products/0-1-formic-acid-acetonitrile-optima-lc-ms-solvent-blends-fisher-chemical-4/LS1201
LS118-212	Water with 0.1% Formic Acid (v/v)	https://www.fishersci.com/shop/products/0-1-formic-acid-water-optima-lc-ms-solvent-blends-fisher-chemical-4/LS118212
LS120-212	Acetonitrile with 0.1% Formic Acid (v/v)	https://www.fishersci.com/shop/products/0-1-formic-acid-acetonitrile-optima-lc-ms-solvent-blends-fisher-chemical-4/LS120212
A117-1AMP	Formic Acid, 99.0+%	https://www.fishersci.com/shop/products/formic-acid-optima-lc-ms-grade-fisher-chemical-5/A1171AMP

Table 2: Recommended Solvents

4.1.3 Switching on the system

- Turn on all main power switches on the back of each of the instrument modules (including the system controller).
- Turn on the system controller by pressing the UPPER button on the system base – an illuminated blue LED on the front of the system confirms that the VSC is powered up (see Figure 2).

TIP It is recommended that the rear seal wash liquid (comprising 75% IPA / 25% H_2O, 0.1% FA) is already installed prior to powering up the system as turning on the power supply to the pump automatically primes the rear-seal wash pump.

4.1.4 Configuring the Local Area Network (LAN) connection on the VSC

The majority of Vanquish Neo systems operate as front ends to mass spectrometers which are supplied with their own control PC and instrument LAN. For security reasons, it is strongly recommended to connect the Vanquish Neo to the instrument LAN rather than to an office network.

The IP address and netmask settings must be custom configured. Recommend IP and netmask settings which are compatible with Thermo Scientific mass spectrometers are given in Figure 18 below:

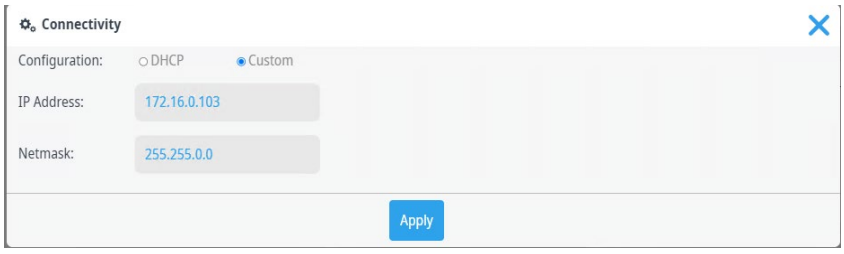


Figure 18: Manual configuration of the VSC IP address

4.1.5 Configuring the Vanquish Neo System in SII / Chromeleon

1. Open the Chromeleon Services Manager and start the Instrument Controller (if not started automatically on system start).

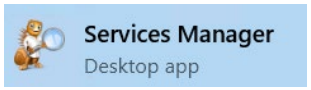


Figure 19: Service Manager application

2. Open the Chromeleon Instrument Configuration Manager and add a new Instrument. For Thermo Xcalibur/SII for Xcalibur installations, open Xcalibur Instrument Configuration, select SII for Xcalibur and configure device, press Configure / Configure Device and add a new Instrument (Figure 20).

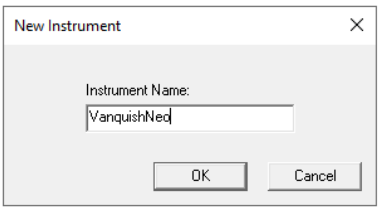


Figure 20: New instrument

3. Add a new module and select “HPLC: Vanquish” followed by “Vanquish Neo System”. Enter the Vanquish System Controller IP address (Figure 21). The IP address of the Vanquish Neo system can be displayed and modified on the Vanquish UI – Settings Panel – Connectivity. For details, please refer to section 4.1.4.

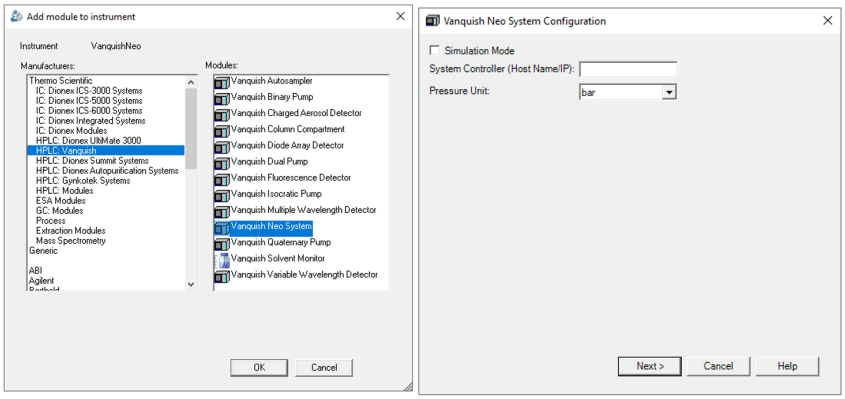


Figure 21: Add new module to the instrument and set the system controller IP address

4. After the IP address is entered and the system is successfully connected to the instrument control PC, the configured modules of the Vanquish Neo system are displayed (Figure 22).

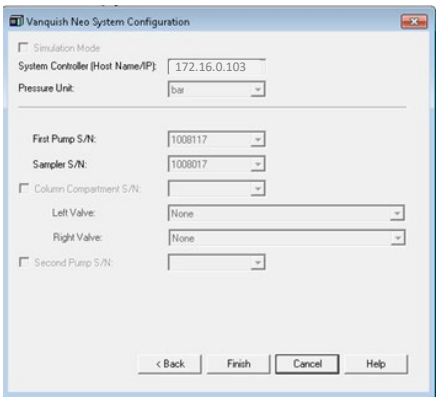


Figure 22: Vanquish Neo system and connected modules

5. Optional: Add a Vanquish Variable Wavelength Detector to the instrument. Choose HPLC: Vanquish followed by the respective detector type. For Chromeleon LC-MS installations also add the mass spectrometer to the instrument. For Thermo Xcalibur installations add the mass spectrometer following the instructions in Thermo Xcalibur Data Acquisition and Processing User Guide.

6. Save the configuration and launch Chromeleon. For Thermo Xcalibur/SII for Xcalibur installations, open Xcalibur.

NOTICE For all Thermo Xcalibur related topics please follow the respective instructions in the Thermo Xcalibur Data Acquisition and Processing User Guide and the Thermo Xcalibur Qual Browser User Guide.

4.2 Starting up the system

4.2.1 Setting Solvent Types (script A01)

- Choose the desired solvent type for the respective solvents from the drop-down menu
- Click on “Apply”

NOTICE For solvent types which differ by ≥ ±5% from one of the four pre-calibrated solvent types please run the custom solvent calibration scripts M31 to M33.

4.2.2 Auto Start Up (script A02)

- Toggle the Diagnostics to “on” (see Figure 23) and click “Apply” to execute (duration 45 – 60 minutes)

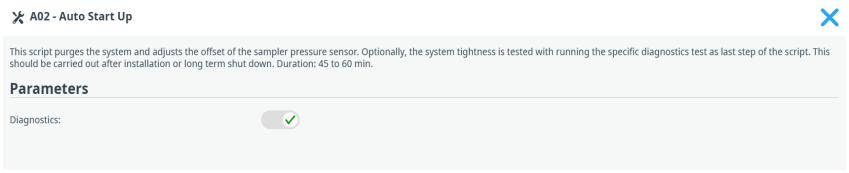


Figure 23: Dialogue box for script A02 showing the diagnostics set to “on”

The automated script:

- flushes the system modules
- flushes the complete flow path up to the inlet capillary
- Performs a leak tightness test

Run this script:

- Before operating the system for the first time
- After installing or replacing components in the system flow path
- After a long period of system shutdown

The results are displayed upon completion of the script.

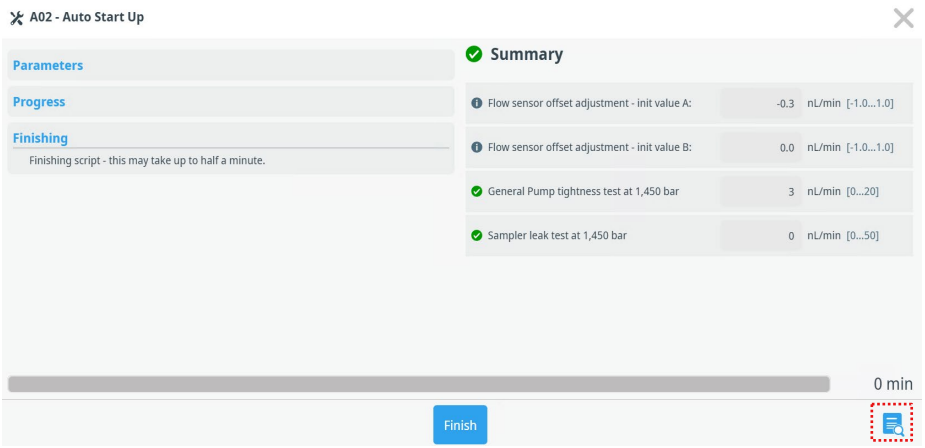


Figure 24: Example results summary table following the completion of the auto start up script

TIP Clicking on the icon in the bottom right-hand corner of the screen opens up a dialogue box containing detailed test results. This holds true for all diagnostic scripts.

TIP The **D01 – Test System Back Pressure** script can also be run after successful completion of the auto startup script. This will check whether or not the system has any blockages

4.3 Direct Control Options

The Vanquish Neo system can be controlled either via the system ePanel in the Chromeleon console or via the direct control option in Xcalibur.

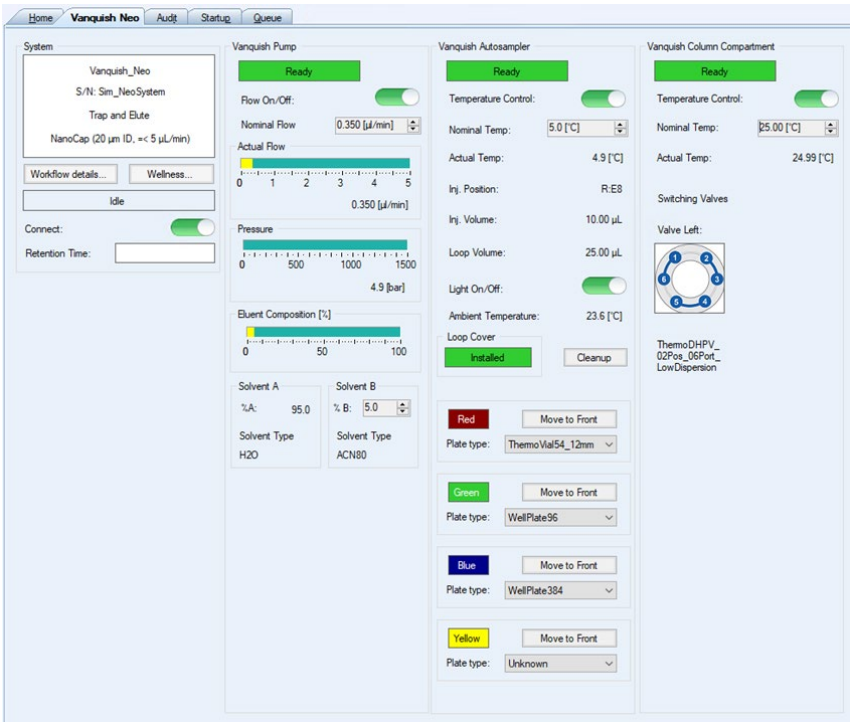


Figure 25: Vanquish Neo System ePanel

Alternatively, the system can be controlled using the VUI and the direct control option on the home panel.

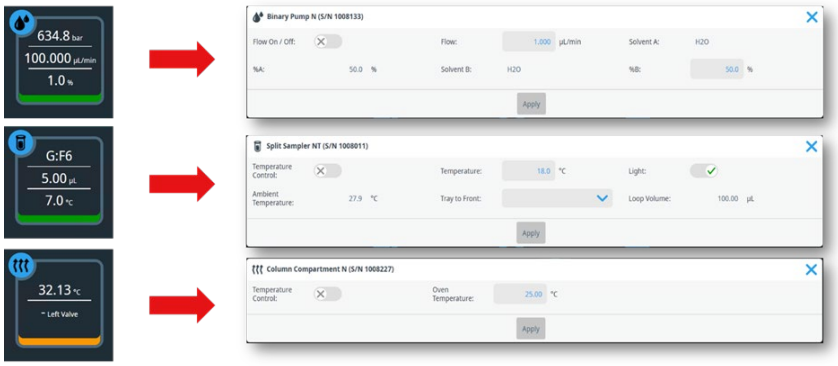


Figure 26: Direct Control Options on the VUI home screen

4.4 Setting up Method and Workflow parameters on the VUI

4.4.1 Setting analytical column type (script A03)

- Install the column
- Select the column type from the dropdown menu in script A03

- **TIP** The menu option to select whether the linear column is to be installed in the column compartment or not will only appear if a Vanquish column compartment N is installed.

4.4.2 Setting the analytical column specifications (A04)

A typical example of column specifications for a PepMap Neo column is shown in Figure 27 below.

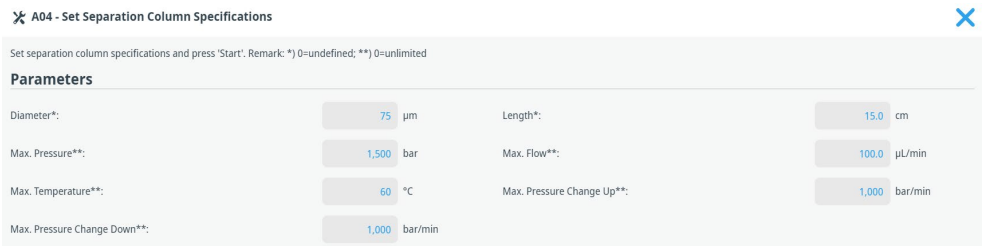


Figure 27: Example analytical column specifications for a 75 µm x 15 cm ID PepMap Neo column

4.4.2.1 *Standard Column settings for Thermo Scientific™ EASY-Spray™ and Linear Columns*

A number of the column settings required for script A04 are not specified in the accompanying column literature. For Thermo Scientific columns, unless specified otherwise, we suggest the following standard settings:

Parameter	Value
Maximum Flow	100 µL/min
Maximum Temperature	60 °C
Maximum Pressure Change Up	1000 bar/min
Maximum Pressure Change Down	1000 bar/min

Table 3: Standard Separation Column Specifications

The standard settings described above have also been adopted for the instrument methods detailed in section 5.2 and which are available for download. These method settings should be adopted to avoid ready check errors when using these methods.

TIP The maximum flow rate of 100 µL/min can be considered a universal parameter for nano and capillary as well as microflow columns. Whilst this flow rate will never be reached at nano and capillary flow rates, using this setting will ensure method execution is limited only by the pressure specifications of the columns and / or the Vanquish Neo System.

NOTICE These values **DO NOT apply** to the **Thermo Scientific™ µPac™ HPLC columns**. Please always adhere strictly to the accompanying column literature when using these columns. Failure to do so could result in irreversible damage to the column.

4.4.3 Setting trap column specifications (script A05)

4.4.3.1 PepMap Neo Trap Cartridges

The PepMap Neo Trap Cartridges are 1500 bar capable 300 µm . x 0.5 cm columns which can be operated in both forward and backflush mode.

The default settings for the 1500 bar PepMap Neo Trap Cartridges are shown given below:

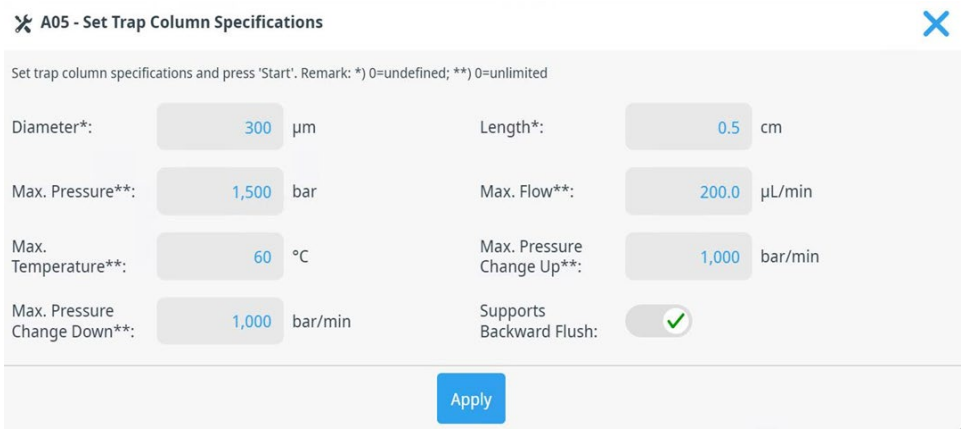


Figure 28: Default specification settings for PepMap Neo Trap Cartridges

4.4.3.2 PepMap NanoTrap Columns

The default settings for the 500 bar pressure rated fused silica based PepMap NanoTrap columns are shown (Figure 29) below:

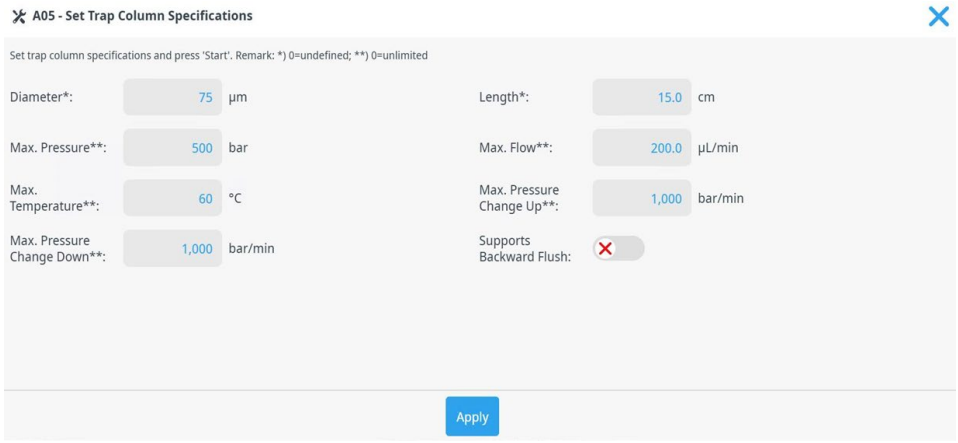


Figure 29: Default specification settings for the PepMap NanoTrap columns

NOTICE: Note that the column length is defined as 15 cm although the packed bed length (as defined on the column label) is only 2 cm. The column length must be set to 15 cm (the total length of the column capillary) to ensure correct system operation (GDV calculation by the Vanquish Neo System fluidic framework).

NOTICE: Ensure that the option “supports backflush” is set to “off” and pay careful attention to the flow direction when installing nano trap columns (direction should be port 4 (inlet) to port 6 (outlet). These typically do not have a frit installed on the front side of the column. Installing these columns in black flush mode may lead to the trap columns being emptied into the system and capillaries.

4.4.4 Setting up system fluidics and workflow (script A06)

The Vanquish Neo system has an inbuilt fluidic framework which contains information on the system capillary dimensions (and volumes) used for different workflows. The system is shipped preconfigured in the nano/cap fluidic configuration for direct injection workflows.If an alternative fluidic or workflow configuration is desired execute script A06 and select the desired configuration follow by “apply” (Figure 30)

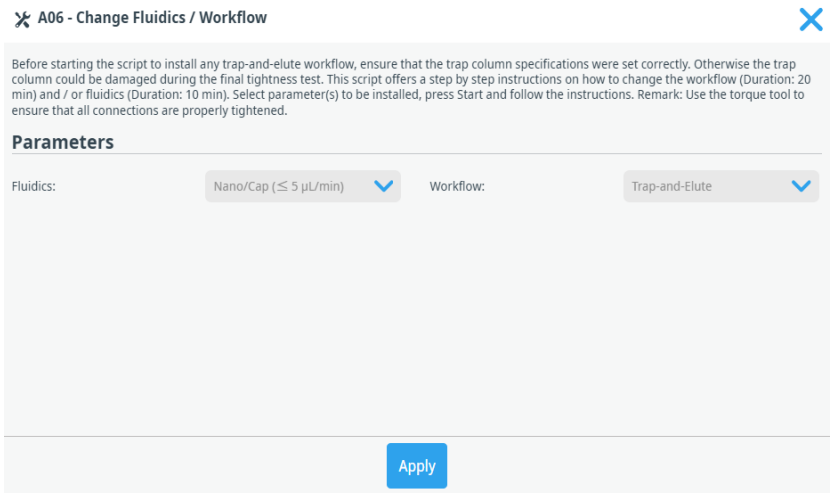


Figure 30: The change fluidics / workflow script

For flow rates ≤ 5 µL/min, the nano/cap fluidic capillaries should be used. For flow rates > 5 µL/min, the micro flow option should be selected.

The script guides the user through the necessary changes required via a series of step-by-step messages and animations.

4.4.4.1 *Configuring heated trap-and-elute workflows*

If the Vanquish Neo Column Compartment N is installed complete with left valve the VUI will offer the user, the option to configure the system in heated-trap mode in either forward (FWD) or backward (BWD) flush configuration Figure 31.

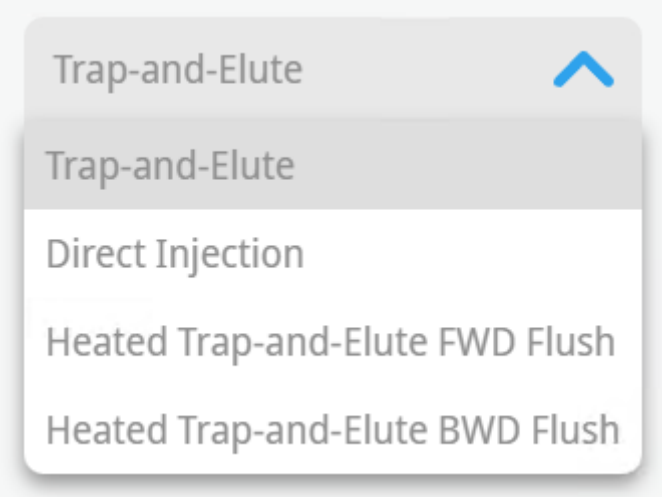


Figure 31: Workflow options in heated trap-and-elute mode

TIP: The heated trap-and-elute based workflow employs a 6-port-2-position valve. For this valve type the flush direction is dependent on the precise fluidic capillary configuration on the valve ports. As such the flush direction must be defined at when the workflow type is selected on the VUI. This is in contrast to the conventional trap-and-elute workflow which uses a 7-port-6-position valve. Here the choice between forward and backward flush is independent on the valve port plumbing and so can be decided / changed simply by adjusting the settings in the instrument method editor.

5 Methods and Applications

5.1 Programming Instrument Methods

The Vanquish Neo Instrument Method Wizard/Editor is the interface for the user to create new methods and change existing ones. An instrument method contains the control commands executed by the system when running an analysis. The tool used to create an instrument method is the Instrument Method Wizard (IMW); the tool used to view and modify methods is the Instrument Method Editor (IME).

NOTICE The pages shown below for the Instrument Method Wizard/Editor are including all possible options. Specific parameters are limited to specific workflows and configurations. The view you may get for your selected workflow may differ from the view shown in this document.

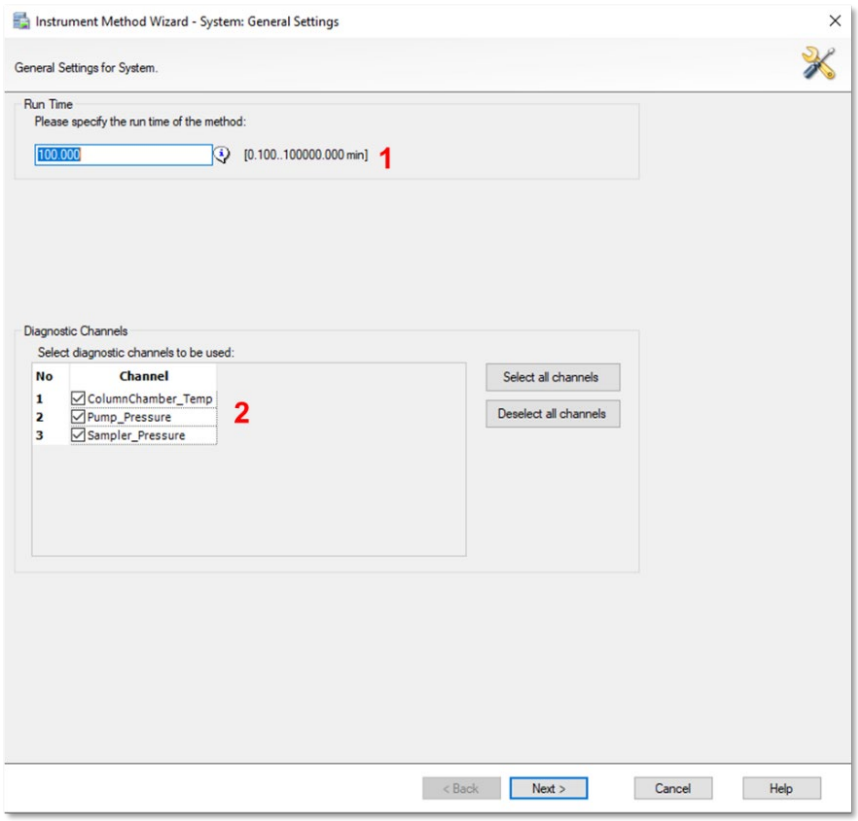


Figure 32: Page 1 - System: General Settings

#	Description
1	Specifies the duration of data acquisition for the LC method. The MS method runtime should ideally have the same duration.
2	Lists the available diagnostic channels. *Note: The list of channels depends on the configured modules.*

Table 4: Key to Figure 32 Page 1 system general settings

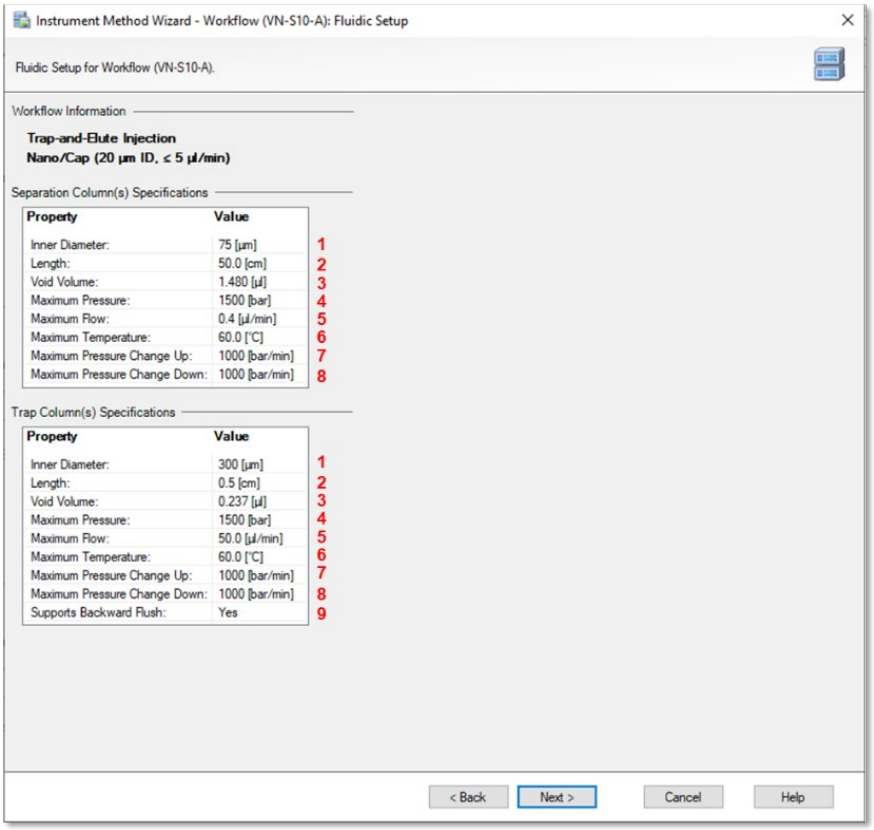


Figure 33: Page 2 – Workflow: Fluidic Setup

#	Description
1	Inner diameter of the separation/trap column*.
2	Length of separation/trap column*.
3	Calculated separation/trap column void volume based on the dimensions of the column and considering 67% of the total open tube volume.
4	Maximum pressure of the separation/trap column*.
5	Maximum recommended flow rate of the separation/trap column*.
6	Maximum recommended temperature of the separation/trap column*..
7	Maximum pressure change up for the separation/trap column. Note: Unless stated differently in the column specifications use 1000 bar/min.
8	Maximum pressure change down for the separation/trap column. Note: Unless stated differently in the column specifications use 1000 bar/min.
9	Specifies if the installed trap column supports backflush operation. This means that the mobile phase can flow through the trap column in both directions without disrupting the column packing. Note: See trap column/cartridge specification sheet for details.

*Table 5: Key to Figure 33 – Workflow fluidic setup *see column label or specification sheet for details.*

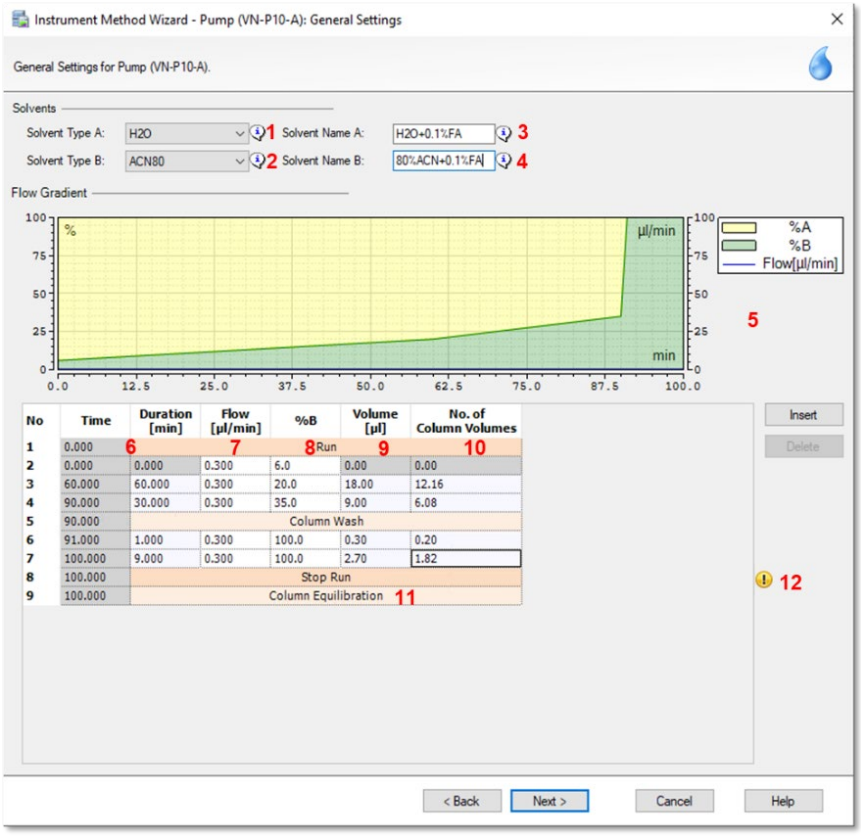


Figure 34: Page 3 – Pump: General Settings

#	Description
1	Specifies the calibrated solvent type for pump A. Note: Factory pre-calibrated solvents are 100% water, 100% acetonitrile, water/acetonitrile (20:80, v/v) and water/methanol (10:90, v/v). Additional user defined solvents can be calibrated and will be added to this list after successful flow meter calibration.
2	Specifies the calibrated solvent type for pump B. Note: Factory pre-calibrated solvents are 100% water, 100% acetonitrile, water/acetonitrile (20:80, v/v) and water/methanol (10:90, v/v). Additional user defined solvents can be calibrated and will be added to this list after the successful flow meter calibration.
3	Text field for entering individual description of solvent A
4	Text field for entering individual description of solvent B
5	Flow gradient plot
6	Start Time and Duration of the gradient step
7	Flow rate of the gradient step
8	Percentage of solvent B of the gradient step
9	Volume of the delivered eluent based on the flow rate and duration of the gradient step

10	Number of column volumes based on the delivered eluent volume and the calculated void volume of the separation column on the “Fluidic Setup” tab in the workflow section.
11	The equilibration of the column(s) takes place after “Stop Run” without data acquisition. The equilibration parameters can be set on the “Wash and Equilibration Settings” tab in the workflow section.
12	Warning icon to indicate if the column wash is less than the recommended wash volume based on number of column volumes. It should be at least one column volume equivalent. Note: With the Column Wash settings used in this example, the warning icon will not be visible.

Table 6: Key to Figure 34- Page 3 Pump General Settings

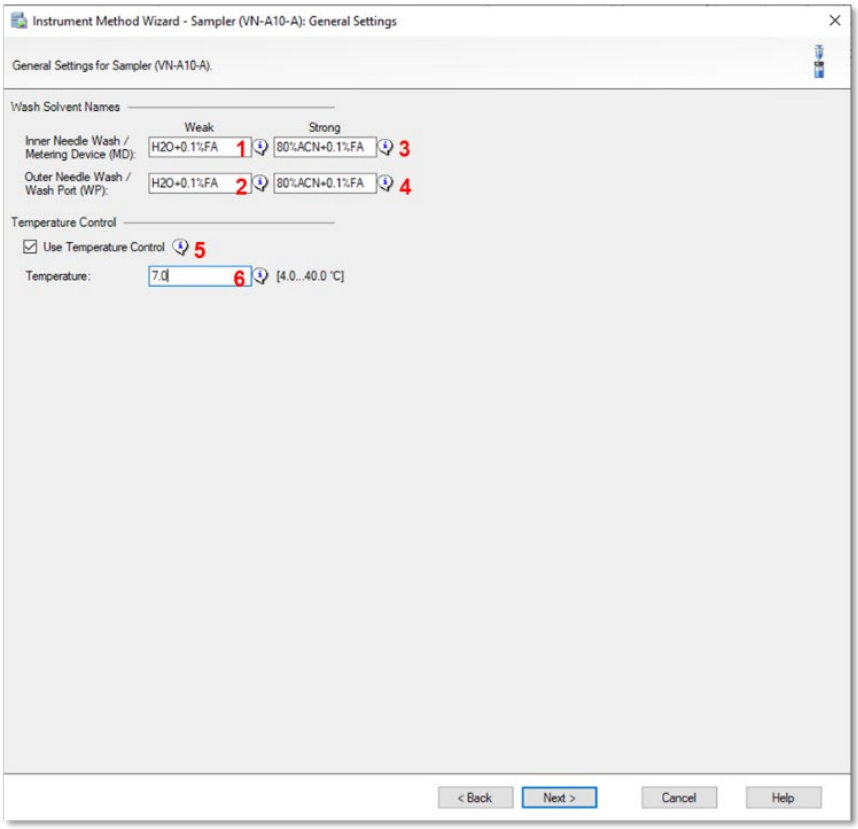


Figure 35: Page 4 – Sampler: General Settings

#	Description
1	Text field for entering individual description for the weak liquid for the inner needle wash used from the metering device during the injection procedure. Note: Wash parameters for the inner needle wash are pre-optimized and are not part of the instrument method editor. This liquid is also utilized for the sample loading onto the trap column and for Fast Equilibration with the trap-and-elute workflows. (Default: WeakSolvent)
2	Text field for entering individual description for the weak liquid for the outer needle wash performed in the wash port of the sampler during the injection procedure. Note: The Outer Needle Wash settings can be modified in the "Advanced Settings" tab in the sampler section. (Default: WeakSolventWp)
3	Text field for entering individual description for the strong liquid for the inner needle wash used from the metering device during the injection procedure. Note: Wash parameters for the inner needle wash are pre-optimized and are not part of the instrument method editor. (Default: StrongSolvent)
4	Text field for entering individual description for the strong liquid for the outer needle wash performed in the wash port of the sampler during the injection procedure. Note: The Outer Needle Wash settings can be modified in the "Advanced Settings" tab in the sampler section. (Default: StrongSolventWp)
5	Temperature control of the sample compartment. Note: If the autosampler insulation cover is not installed properly, thermostatting cannot be enabled or will be turned off automatically.
6	Temperature setpoint for the sample compartment

Table 7: Key to Figure 35- Sampler general settings

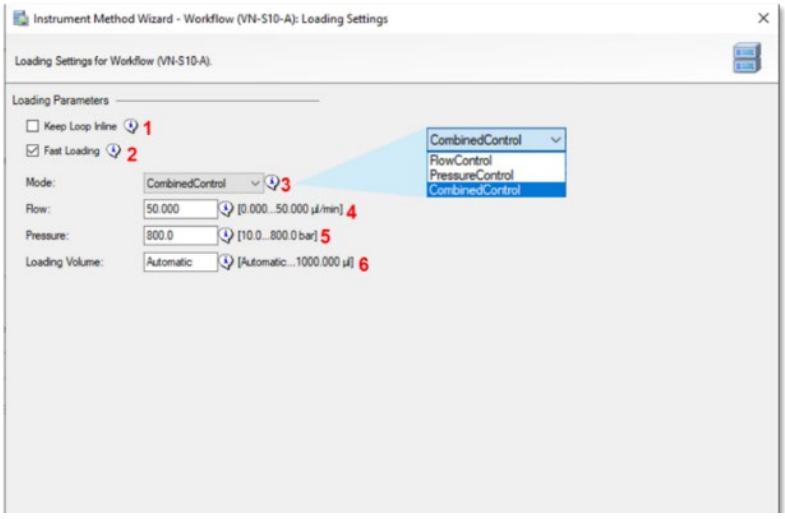


Figure 36: Page 5 – Workflow: Loading Settings

#	Description
1	Specifies whether the sample loop should remain in the flow path (inline) after the sample pick-up and loading procedure. *Note: Option only available for the Micro Flow Direct Injection workflow.It is only recommended for higher flow rates due to the additional gradient delay volume if the loop is kept inline.*
2	Specifies whether the column loading will be accomplished faster, and overall sampling time will be reduced. Selecting this option enables the parameters #3 - #6. *Note: Option only available for the Direct Injection workflows. For the Trap-and-Elute workflows this option is always enabled, because trapping is performed using the metering device.*
3	Specifies the Fast-Loading Mode. Following modes are available: Flow Control: The pump/metering device uses the defined flow rate (#4) for the sample loading. Pressure Control: The pump/metering device increases the flow for the sample loading until the specified pressure (#5) is reached. CombinedControl (default selection): The pump/metering device speed is limited by either the flow (#4) or the pressure (#5) depending on which limit is reached first.
4	Specifies the target flow during Fast Loading for loading modes FlowControl and CombinedControl.
5	Specifies the target pressure during Fast Loading for loading modes PressureControl and CombinedControl. *Note: For the trap-and-elute workflow this parameter is limited to 800 bar, because this is the maximum pressure for the metering device flow delivery.*
6	Specifies the eluent loading volume during column loading. When “automatic” loading volume is selected, it automatically calculates the volume of eluent required to transfer the complete sample plug to the separation column. Using a nominal loading volume, this particular volume of eluent will be used for loading.

Table 8: Key to figure 36 Workflow – Loading settings

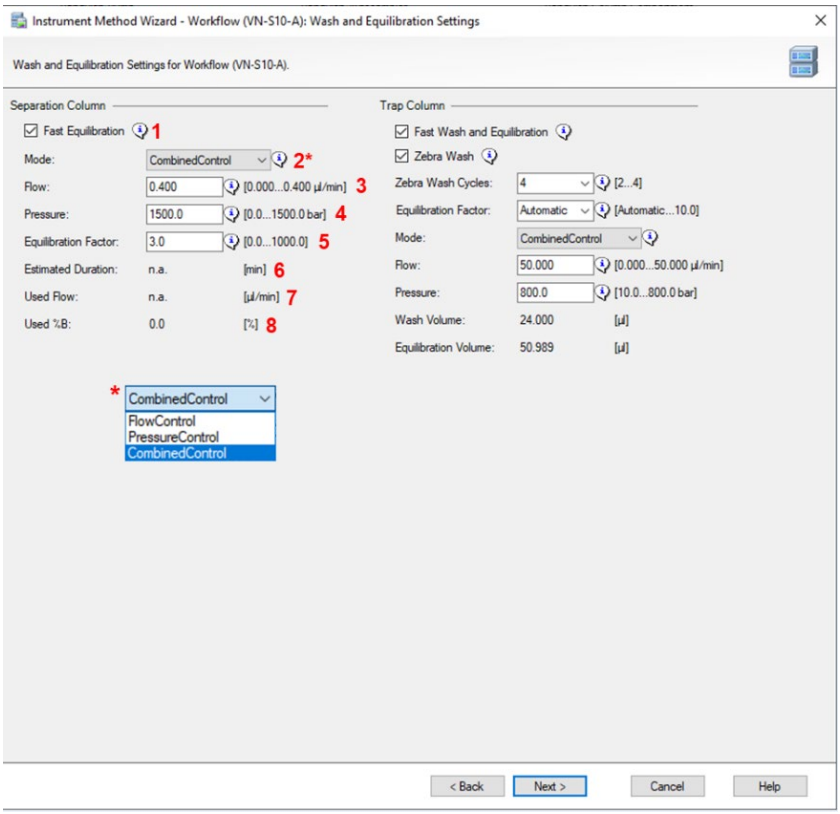


Figure 37: Page 6 - Workflow: Wash and Equilibration Settings (1)

Figure 37	Description
1	Specifies whether column equilibration is accomplished faster, and overall sampling time is reduced. Selecting this option enables the parameters #2 - #8).
2	Specifies the Fast Equilibration Mode. Following modes are available: FlowControl: The pump uses the defined flow (#3) for the column equilibration. PressureControl: The pump increases the flow for the column equilibration until the user specified pressure (#4) is reached. CombinedControl (default selection): The pump speed will be limited by either the flow (#3) or the pressure (#4) depending on which limit is reached first.
3	Specifies the target flow during Fast Equilibration for equilibration modes FlowControl and CombinedControl.
4	Specifies the target pressure during Fast Equilibration for equilibration modes PressureControl and CombinedControl.
5	Determines the eluent volume used during column equilibration. The used volume is the void volume of the column(s) to be equilibrated multiplied with this factor. The volume calculation depends on several other factors, such as selected workflow, instrument method parameters, and

	whether a trap column is installed or not. By default, the value is set to 3.0. A higher equilibration factor means a higher equilibration quality but results in a longer run time and higher solvent consumption. Determine a factor that best fits your method and column properties.
6	Estimated duration for the column equilibration. Note: Only available for FlowControl equilibration mode or if Fast Equilibration is disabled.
7	Flow used for the column equilibration. Note: Only available for FlowControl equilibration mode or if Fast Equilibration is disabled, in this case the value matches the value shown in the first row of the gradient table.
8	Indicates the percentage of solvent B used for equilibration. The value matches the value shown in the first row of the gradient table.

Table 9: Key to Figure 39: Page 7 - Column Compartment: General Settings Note: This page is only shown if a Vanquish Neo Column Compartment is part of the instrument configuration.Wash and Equilibration Settings (1)

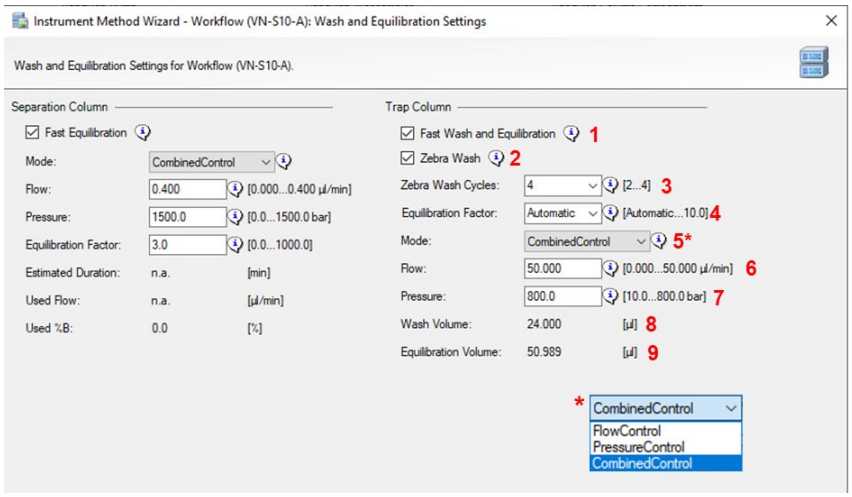


Figure 38: Page 6 - Workflow: Wash and Equilibration Settings (2)

#	Description
1	Specifies whether trap column wash and equilibration are accomplished faster, and overall sampling time is reduced. Enabling this option will switch the trap column offline and the metering device will wash and equilibrate the trap column. If this option is disabled, the trap column is kept inline and washed and equilibrated together with the separation column from the analytical pump. Selecting this option will enable the parameters #2 - #8.
2	Specifies whether the Zebra Wash option is activated. The Zebra wash option uses alternate solvent plugs of strong and weak wash liquid, drawn from the needle wash port, and pushed to the trap column during the trap column wash process to minimize trap column carry over.
3	Zebra Wash deactivated (see 2): Specifies the volume of the strong wash liquid from the wash port used for the trap column wash procedure. The used volume is the void volume of the trap column to be washed multiplied with this factor. Zebra Wash activated (see 2): Specifies the number of Zebra Wash cycles. One cycle refers to a combination of one strong and one weak solvent plug. The size of each plug and the number of available cycles depends on the installed loop.
4	Determines the volume of weak liquid from the metering device used during column equilibration. The volume is the void volume of the column(s) to be equilibrated multiplied with this factor and multiplied with the Wash Factor. By default, the value is set to 2.0. A higher equilibration factor means a higher equilibration quality but results in a longer run time and higher solvent consumption. Determine a factor that best fits your method and column properties.
5	Specifies the Fast Equilibration Mode. Following modes are available: FlowControl: The metering device uses the defined flow (#4) for the trap column equilibration. PressureControl: The metering device increases the flow for the trap column equilibration until the specified pressure (#5) is reached.

	CombinedControl (default selection): The metering device speed is limited by either the flow (#4) or the pressure (#5) depending on which limit is reached first.
6	Specifies the target flow during Fast Equilibration for equilibration modes FlowControl and CombinedControl.
7	Specifies the target pressure during Fast Equilibration for equilibration modes PressureControl and CombinedControl. Note: This parameter is limited to 800 bar, because this is the maximum pressure for the metering device flow delivery.
8	Zebra Wash deactivated (see 2): Estimated volume of strong wash liquid from the wash port used for the trap column wash. Zebra Wash activated (see 2): Estimated volume of strong and weak wash liquid used during trap column Zebra Wash.
9	Estimated volume of weak liquid from the metering device used for the trap column equilibration.

Table 10:Key to Figure 38 Wash and Equilibration Settings (2)

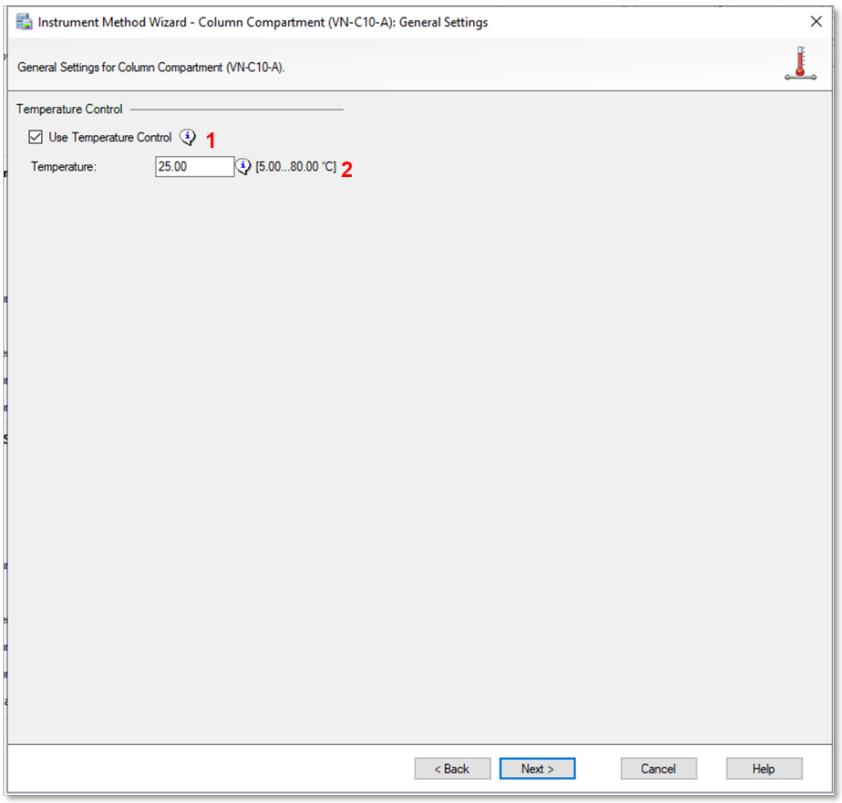


Figure 39: Page 7 - Column Compartment: General Settings
Note: This page is only shown if a Vanquish Neo Column Compartment is part of the instrument configuration.

#	Description
1	Enable temperature control of the column compartment.
2	Temperature setpoint of the column compartment. Note: Limits are automatically adjusted depending on the selected workflow and the individual temperature limits of installed valves and columns in the column chamber.

Table 11: Key to Figure 39: Page 7 - Column Compartment: General Settings
Note: This page is only shown if a Vanquish Neo Column Compartment is part of the instrument configuration.

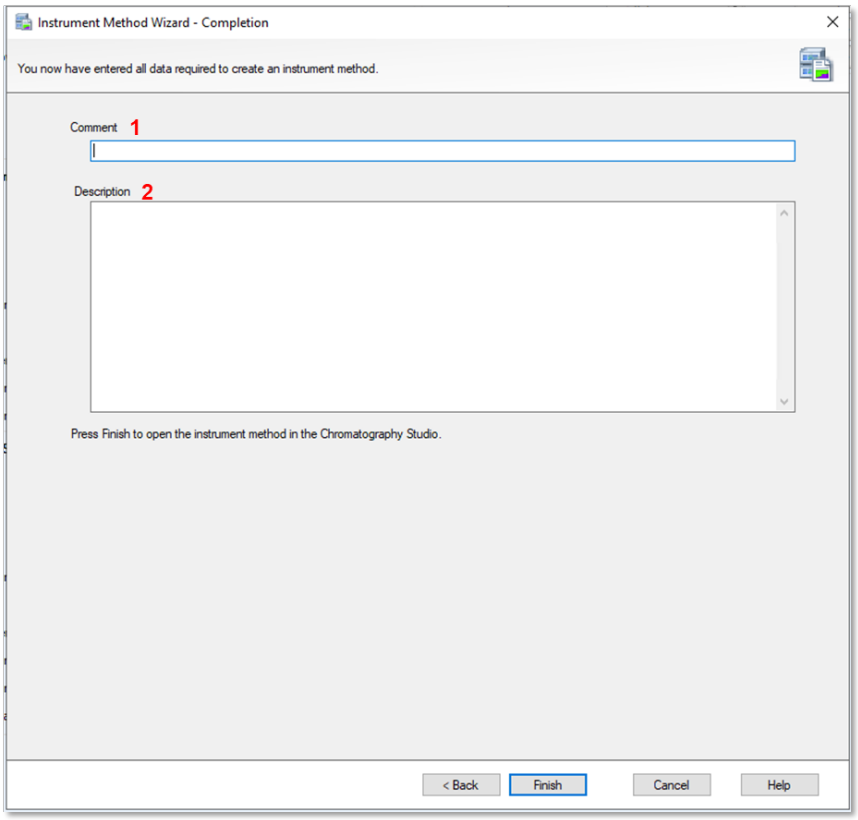


Figure 40: Page 8 – Completion

#	Description
1	Text field for comments
2	Text field for method descriptions or other details

Table 12: Key to Figure 40: Page 8 – Completion

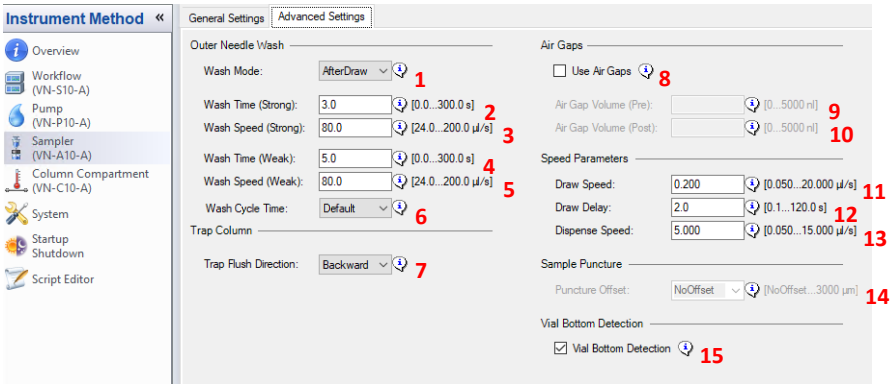


Figure 41: Instrument Method Editor – Sampler: Advanced Settings
Note: This page will be only present in the instrument method editor and not in the instrument method wizard.

#	Description
1	Outer Needle Wash mode performed in the autosampler wash port during the injection procedure.
2	Specifies the duration of the outer needle washing procedure in the autosampler wash port using the strong wash liquid (StrongSolventWp).
3	Specifies the flow speed of the strong wash liquid (StrongSolventWp) delivered by the wash pump to the wash port.
4	Specifies the duration of the outer needle washing procedure in the autosampler wash port using the weak wash liquid (WeakSolventWp).
5	Specifies the flow speed of the weak wash liquid (WeakSolventWp) delivered by the wash pump to the wash port.
6	Defines the speed of the needle leaving the wash port after the outer needle wash procedure. The setting “fast” can be used for high-throughput methods to further decrease the sampler cycle time.
7	Specifies the direction in which the trap column is flushed after loading. The available directions are Forward and Backward. Note: This option is not visible if the installed trap column does not support back-flush operation and if the heated trap-and-elute workflow is configured. Defined on the “Fluidic Setup” tab in the workflow section.
8	Select this option to activate or deactivate using air gaps for the sample pickup. Note: Using air gaps can have negative effects on autosampler precision.
9	Specifies the air gap before the sample plug introduced prior to sample pickup.
10	Specifies the air gap after the sample plug which is introduced after sample pickup.
11	Specifies the speed at which the sample is drawn into the needle.
12	Specifies the delay between end of sample draw and the beginning of needle movement to fill the loop.
13	Specifies the speed at which the loop is emptied into the waste. This step is part of the injection procedure prior to sample draw.
14	Specifies the distance the rack carousel moves horizontally after the needle has punctured the septum to open the septum. Note: Option not available in combination with Vial Bottom Detection.
15	Select this option to activate the vial bottom detection. Note: Enabling this option will disable the puncture offset property and set it to NoOffset (0 µm).

Table 13Key to Figure 41: Instrument Method Editor – Sampler: Advanced Settings

Note: This page will be only present in the instrument method editor and not in the instrument method wizard.

5.2 Optimized Default Methods

This section details a series of “proof-of-principle” methods optimized for the Vanquish Neo system. Each method has been tailored to a specific application and published as a technical note (TN).

Content	Flow Rate Range	Fluidic Config.	Workflow	TN reference
Deep Dive Proteomics	200 to 500 nL/min	Nano/Cap	Direct Injection	5.2.1
Targeted peptide Quantification	0.3 and 3 µL/min	Nano/Cap	Direct Injection	5.2.2
High throughput proteome profiling	0.3 – 1.3µL/min	Nano/Cap	Trap-and-Elute	5.2.3
Large cohort, ultra-robust proteomics	50 – 100 µL/min	Micro	Direct Injection	5.2.4
VQ Neo inter-system reproducibility	300 and 350 nL/min	Nano/Cap	Direct Injection	5.2.5
VQ Neo System Robustness	300 nL/min	Nano/Cap	Direct Injection	5.2.6
ZebraWash: Novel Trap Column Wash Protocols for ultra-low carryover	300 nL/min	Nano/Cap	Trap-and-Elute	5.2.7
Injection volumes up to 500µL	1.3µL/min	Nano/Cap	Trap-and-Elute	5.2.8
Ultra-High sensitivity Proteomics	100 nL/min	Nano/Cap	Direct Injection	5.2.9
Method guide for all µPacNeo columns	0.1 – 2.5 µL/min	Nano/Cap	DI and Trap-and-Elute	5.2.10

Table 14: Summary of Default Methods

Each of the application entries listed comes with

- A link to the technical note
- A short summary
- A link to the methods from the Thermo Scientific AppsLab Library of Analytical Applications, where available

5.2.1 Nano LC-MS methods for bottom-up proteomics (TN-74152)

Vanquish Neo UHPLC system sets new performance standards for single-shot nanoLCMS bottom-up proteomics

This TN demonstrates the superior performance of the Vanquish Neo system for nanoLCMS bottom-up proteome profiling when coupled to Exploris 480 using the 75 µm I.D. × 75 cm EASY-Spray PepMap Neo column. Demonstrates system versatility and potential for new levels of proteomic depth coverage through the coupling of two 75 cm long nano-columns.

Complete methods are available for download from AppsLab.

5.2.2 Targeted Peptide Quan using nano- and capillary-flow (TN-000137)

Quantitative targeted nano- and capillary-flow LC-MS peptide analysis using the Vanquish Neo UHPLC System coupled to a triple quadrupole mass spectrometer

This TN demonstrates the robust performance of the Vanquish Neo System coupled to the TSQ Altis Triple Quadrupole MS for capillary-flow LC-MS/MS based peptide quantification compared to a standard nano-flow LC-MS/MS based method.

5.2.3 High throughput nano- and capillary- flow methods for high throughput proteome profiling (TN-000138)

Fast, sensitive, and reproducible nano- and capillary-flow LCMS methods for high–throughput proteome profiling using the Vanquish Neo UHPLC system hyphenated with the Orbitrap Exploris 480 MS

This TN demonstrates the performance of the Vanquish Neo System, the next−generation nano-, capillary- and micro-flow LC, coupled to Exploris 480 for high-throughput bottom−up proteome profiling using a 75 µm I.D. × 15 cm EASY-Spray PepMap Neo Column.

Complete methods are available for download from AppsLab.

5.2.4 High throughput microflow peptide quantification (TN-74161)

Ultra-robust micro-flow LC-MS/MS for targeted high-throughput peptide quantification using the Vanquish Neo UHPLC system

This TN demonstrates the robust performance of the Vanquish Neo system for micro-flow LC-MS/MS based peptide quantification.

Complete methods are available for download from AppsLab.

5.2.5 Vanquish Neo System-to-System reproducibility (TN-000199)

Vanquish Neo UHPLC system-to-system reproducibility ensures consistent and reliable results in nanoLC-MS proteomics

This study demonstrates how the combined power of the Vanquish Neo UHPLC system, accompanying PepMap Neo columns and the latest HRAM Orbitrap mass spectrometers deliver the level of system-to-system reproducibility required to meet the demands of large cohort and multi-center studies. In particular, the ability of the Vanquish Neo UHPLC system to deliver standardized, rugged, and reproducible analytics will help foster the adoption of nanoLC-MS for large sample cohort analysis.

5.2.6 Robust long-term continuous Vanquish UHPLC system operation (TN-000172)

Robust long-term Vanquish Neo UHPLC system operation enabling high-performance high-pressure nanoLC separations

This TN demonstrates the long-term robustness and consistent chromatographic performance of the Vanquish Neo System under nanoLC conditions for bottom-up proteome profiling using a 75 µm I.D. × 50 cm PepMap Neo Column.

5.2.7 Fast and Efficient “Zebrawash” protocol to reduce trap column carryover (TN-000816)

ZebraWash: An innovative approach in the Vanquish Neo UHPLC system to reduce trap column carryover

This TN demonstrates the superior performance of the ZebraWash procedure in Thermo Scientific™ Vanquish™ Neo UHPLC systems for rapid and effective reduction of the trap column carryover in the trap-and-elute workflow for low-flow LC-MS applications.

5.2.8 Large Volume injections (500µL) for lyophilization free LC-MS proteomics workflows (TN-001357)

Multi-draw: Enabling large volume injections for lyophilization-free LC-MS proteomics workflows on the Vanquish Neo UHPLC system.

This TN demonstrates the performance of the multi-draw functionality using the Thermo Scientific™ Vanquish™ Neo UHPLC system for large volume injections in bottom-up proteomics experiments within the trap-and-elute workflow.

Complete methods are available for download from this AppsLab link.

5.2.9 High throughput Ultra-high sensitivity low-nano flow LCMS (TN-001939)

High-sensitivity low-nano flow LC-MS methods for high-throughput sample-limited proteomics

This TN demonstrates high-throughput and high-sensitivity nano-flow LC-MS methods using a 50 µm i.d. column operated at 100 nL/min for sample-limited proteomics analysis, including single-cell proteomics (SCP).

Complete methods are available for download from this AppsLab link.

5.2.10 Complete guide to setting up and using µPAC Neo HPLC columns (Start-up guide 001891)

Getting started with µPAC Neo HPLC columns

A comprehensive guide for the correct configuration and use of the µPAC Neo series of columns together with the Vanquish Neo UHPLC system. The columns covered include:

- 50 cm µPAC Neo Low Load column
- µPAC Neo High Throughput column
- 50 cm µPAC Neo column
- 110 cm µPAC Neo column

And also, the following µPAC trapping columns:

- µPAC Neo Low Load trapping column
- µPAC trapping column

The manual offers details on:

- which µPAC column to use for which sample load, sample throughput, target flow rate range
- Expected proteome coverage
- Column installation and setup (LC and MS)
- Full method parameters
- Example results

6 Best Practices - Tips and Tricks

6.1 Best Practices

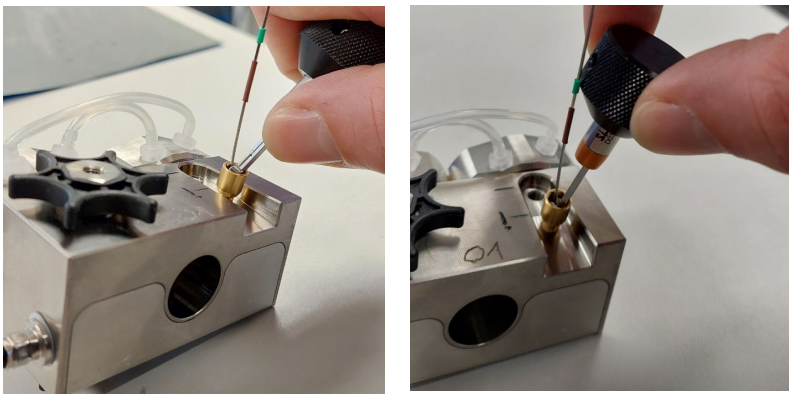
6.1.1 Using the Torque screwdriver

The torque screwdriver is designed to ensure trouble-free, leak tight (nano)Viper connectivity at 1500 bar. Hold the tool on the head and turn until a “click is heard”. The tool is supplied with three accessories (Figure 42). The Viper bit can be extended (Figure 42b) to improve the reach of the tool e.g., when tightening the autosampler fluidics. The needle seat bit should always be used for needle seat tightening.

a b c

Figure 42: Torque screwdriver: a) with viper bit, b) complete with extension adapter and viper bit, c) with needle seat bit

TIP To prolong the lifetime and torque accuracy of the tool, please refrain from continuing to turn the head after the first few “clicks” (indicating the torque limit has been reached) have been heard.



a b

Figure 43: Excessive tilting (a) of the torque screwdriver results in too much force (leverage) applied to the nanoViper fitting. Correct (b) orientation of the Torque Screwdriver ensures that the tightening is torque controlled.

NOTICE: Do not use the Torque screwdriver for connecting any of the **Thermo Scientific µPAC HPLC analytical or trap columns**. These columns are not operated at UHPLC pressures, and the fused silica inlet and outlet capillaries may be irreversibly damaged if the torque screwdriver is applied.

6.1.2 Vials, Caps and Plates

For the best user experience, we recommend the following:

Part Number	Description	
6PK1655	KIT VIAL 0.2 ML AMBER TPX SCREW 9MM SHORT THREAD WITH CONICAL GLASS INSERT; CAP SCREW 9MM BLACK PP WHITE SILICONE / RED PTFE SEPTA BONDED 1.0MM; 100/PACK ***One of these packs is shipped with every Vanquish Neo system***	
60180-1655	VIAL 0.2ML AMBER TPX SCREW 9MM SHORT THREAD WITH CONICAL GLASS INSERT; 100/PACK	
6PSC9STB1 (previously known as C5000-64B)	TALCUM-FREE CAP SCREW 9MM BLACK PP WHITE SILICONE / RED PTFE SEPTA BONDED 1.0MM 100/PACK ***Use these screw caps with the vials above/below, they have the lowest particle release***	
6PSV9-TR1 (previously known as 1.2-UHRSV)	VIAL 1.5 ML CLEAR SCREW 9MM SHORT THREAD TOTAL RECOVERY 100/PACK ***The best vial for working with small sample quantities in combination with vial bottom sensing***	
6PSV9-V1	1.7 ML HIGH RECOVERY GLASS SCREW TOP MICROVIALS 100/PACK ***The best vial for repeat injections (e.g QC standards)***	
60180-P201B	PP 96 WELL PLATE, 7MM **ROUND U-BASE**, 1.0ML, PP, **BARCODED** 50/PK	-
60180-P210B	96 LOW-VOLUME WELL PLATE, 5.6MM TOTAL **V-BASE**, 100UL, PP **BARCODED**	-
60180-M146 / 60180-M176	PLATE SEAL TAPE, PART ADHESIVE PET/SIL/PET, 100/PK As above, but 25/PK	-

Table 15: Recommended Sample handling products for Vanquish Neo

NOTICE The caps with talcum free septa P/N 6PSC9STB1 listed in Table 15 are the only ones compatible with the Vanquish Neo System at the time of publication. All other (talcum containing) caps shed particulates which will block the autosampler needle seat frit prematurely.

6.1.3 System check

The scripts on the Vanquish User Interface provide facile and intelligent diagnostics enabling the user to monitor and maintain the system on a regular basis.

The following sequence of scripts should be used:

- to confirm that the system is in optimal working order
- when an error occurs

	ID	Script title
1	C02	Purge Pump
2	C04	Purge Sampler
3	C01	Adjust Pump Flow Sensor Offsets
4	C05	Adjust System Pressure Sensors
5	D02	Test System Tightness
6	D01	Test System Back Pressure
7	E03	Download Service Data

Table 16: Vanquish Neo System Check Procedure

TIP Keep a manual record of the diagnostic script results (those scripts with the prefix D).
The service data can be downloaded from a PC browser connected to the VSC. The service data should be stored in case it is required for troubleshooting by Thermo Fisher Scientific service personnel.

6.1.4 Needle Seat maintenance

6.1.4.1 Needle seat cleaning and health status monitoring

The needle seat contains a 0.5 µm filter frit which act as a frontline defense against insoluble particulates, protecting all downstream fluidic components e.g., switching valves, capillaries and columns from blockage or damage. For this reason, the needle seat requires regular cleaning and / or replacement.

The health of the needle seat should be monitored on a regular basis by executing the script “C21 – Clean Or Replace Needle Unit and Seat” with the parameter “Clean Needle Seat” selected in the dropdown menu.

The script execution, which takes approximately 6 minutes includes an automatic diagnostic which reports the back pressure of the sample loop and needle along with the needle seat (Figure 44):

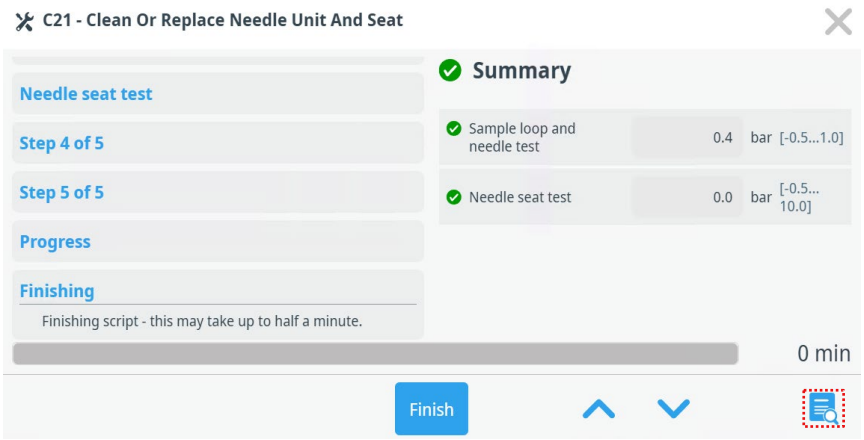


Figure 44: Example summary results for script C21

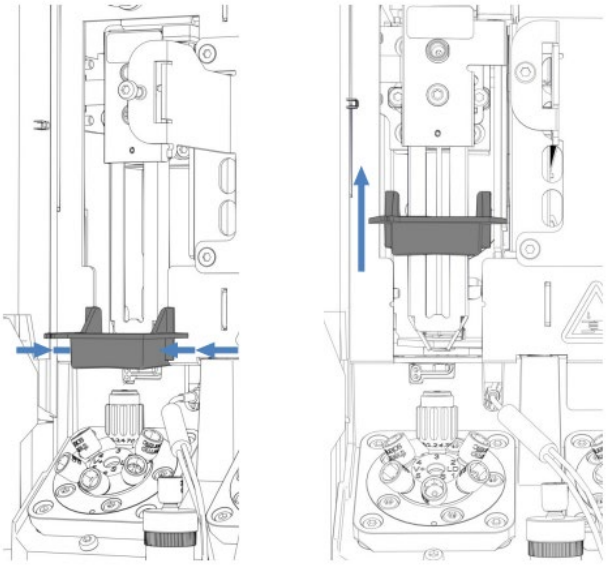
A detailed view of the results summary is also available by clicking on the icon in the bottom right-hand corner of the screen.

TIP For best practices on how to both monitor the performance and behaviour as well as prolong the lifetime of the needle seat please refer to Vanquish Neo Tips and Tricks Issue 1.

6.1.4.2 Replacing the needle seat

In the event that the needle seat is irretrievably blocked (i.e., can no longer be backflushed) please use the following steps for its replacement.

- Press the **SERVICE** button two times (Figure 5) on the autosampler.
- Turn off the autosampler with its main power switch.
- Remove the insulation cover.
- Free the capillary from the insulation cover mounting bracket (if the autosampler is configured for trap and elute workflows this step can be ignored).
- Squeeze the insulation cover mounting bracket and push it upward to remove it (Figure 45).



a b

Figure 45: Removing the insulation cover mounting bracket: First squeeze the bracket on each side (a) and then slide upwards (b)

- Use the torque screwdriver tool with the needle seat bit attachment (Figure 46) to remove the needle seat (F.

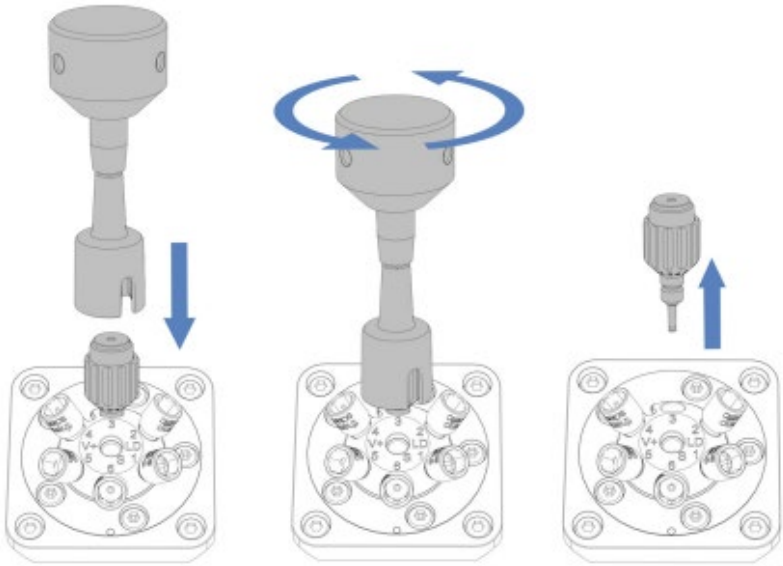


Figure 46: Removing the needle seat with the torque screwdriver

- Insert the replacement needle seat and tighten it clockwise with your fingers until it is hand tight.
- Use the torque screwdriver with the needle seat bit and turn it clockwise to tighten the needle seat until the torque limitation has been reached as indicated by a clicking sound.

NOTICE The needle seat will get damaged using other tools for either tightening or untightening. Do not use any other tool.

- Reinstall the insulation cover mounting bracket by pushing it into the guide rails and moving it down until it snaps into place (Figure 47).

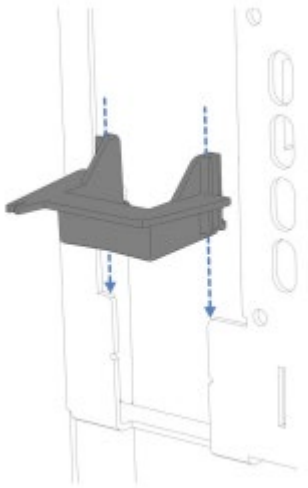


Figure 47: Installing the insulation cover mounting bracket

NOTICE Ensure that the insulation cover mounting bracket is pushed down as far as it will go. If it is not properly fitted, the autosampler needle will foul on the mounting bracket base plate as it travels out of the cooling chamber causing it to bend and forcing its replacement.

- Remount the insulation cover.
- Press the **SERVICE** button on the autosampler one time (location shown on Figure 5) to move the needle back to its home position in the needle seat.

The system is now ready for use.

NOTICE In the event that an autosampler needle is damaged or blocked and requires replacement, **both the needle and the needle seat must be exchanged.** In this case, please execute **script C21** with "Replace needle unit and seat" selected from the dropdown menu and follow the instructions provided.

6.1.5 System Shutdown

There are two types of shutdown procedure available when executing the shutdown procedure (script B05).

- Short term shutdown – for intended shutdown shorter than 4 weeks
- Long term shutdown – for intended shutdown longer than 4 weeks.

If the one of the modules or the entire system needs to be shipped (e.g., to the factory) the module to be removed should be selected accordingly from the dropdown menu on the right-hand side of the dialogue box (Figure 48).

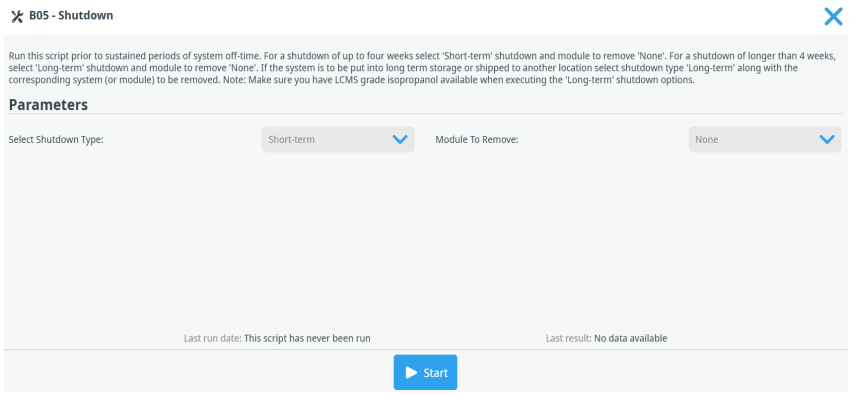


Figure 48: Script B05 – Shutdown dialogue box

NOTICE LC-MS grade isopropanol is required for long term shutdown procedures.

6.1.6 System start-up procedure following long periods of system shutdown

- Rinse and fill the solvents, rear seal wash liquid and needle wash liquid reservoirs (section 4.1.1).
- Switch on the system (section 4.1.3).
- Check / Set the Solvent types selected on the VUI match those that have been installed on the system (script A01).

The complete procedure described in Table 17 describes the remaining scripts required to re-start the instrument after long term storage or long periods of off time.

	ID	Script description
1	A01	Set Pump Solvent Types
2	A02	Auto Start Up
3	D01	Test System Back Pressure
4	A03	Set Separation Column Type
5	A04	Set Separation Column Specifications
6	A05	Optional: Set Trap Column Specifications
7	A06	Optional: Change Fluidics/Workflow

Table 17: Vanquish Neo System Startup procedure

6.1.7 Monitoring Trap Column Performance

In trap-and-elute mode, the trap cartridge or column receives maximum exposure to the sample matrix. This generally leads to an increase in back pressure over time as the trap column matures. Fast loading trap-and-elute based protocols (for details on method editor settings see Figure 36,) may become compromised by excessively slow trap column loading due to increased flow resistance. **In the worst case, this can lead to a complete halt of the flow and an analytical run which never reaches completion.**

To ensure trouble-free trap column operation, please observe the following:

6.1.7.1 Every time a new trap column is installed

- Run the “D01 -Test System Back Pressure” script each time a new trap column is installed.

- Set the “test only trap column back pressure” toggle to “off”.
- Keep a record of the diagnostic results. Typical example data are given for the nano/cap fluidic configuration with a PepMap trap cartridge in Figure 49 below. Note that a PepMap Neo trap cartridge back pressure of 2 bar/µL/min is typical when the trap is newly installed.

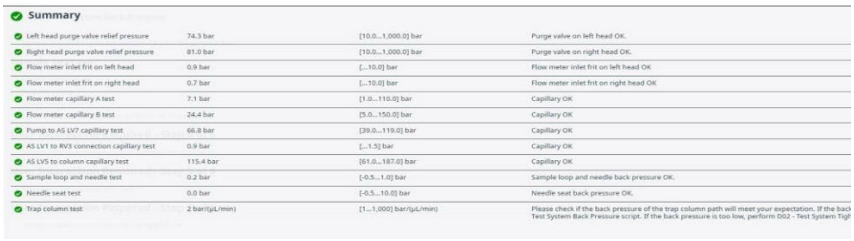


Figure 49: Typical Back Pressure Test Summary Data for the trap-and-elute workflow in nano/cap fluidic configuration.

6.1.7.2 *Test Only Trap Column Back Pressure*

The trap column back pressure can be checked “on the fly” using the D01 script with the “test only trap column back pressure” toggle to “on”.

The test takes ≤ 5 minutes and the pump flow across the analytical column is not interrupted during the execution of this script. An example test result (detailed view) is shown below (Figure 50).

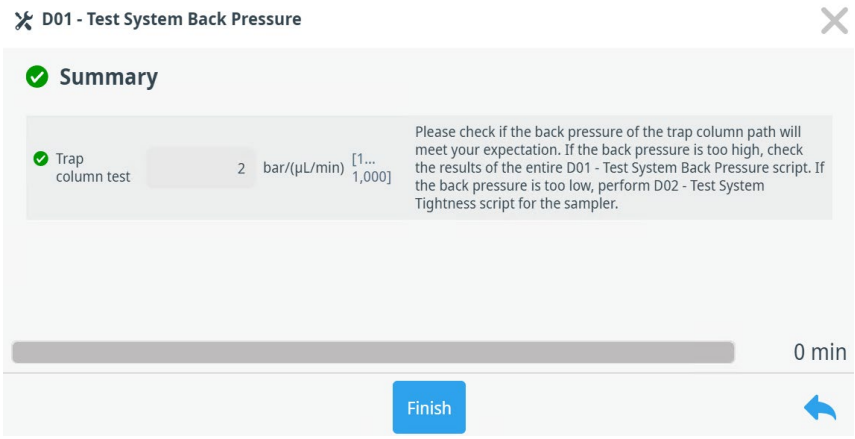


Figure 50: Diagnostic script D01 test result with the option for test only trap column back pressure set to “On”

NOTICE The change in back pressure resulting from the trap column throughout its lifetime can vary from column to column and is dependent on multiple factors including sample type, matrix and purity. The user is responsible for monitoring and recording the back pressure profile of the trap column throughout its life cycle and deciding on when the column should be exchanged.

6.1.8 Changing / Refreshing Solvents (script B01)

6.1.8.1 Refreshing solvents

If solvents are being exchanged for the same solvent type, then the "refresh only" toggle should be set to "on" (Figure 51).

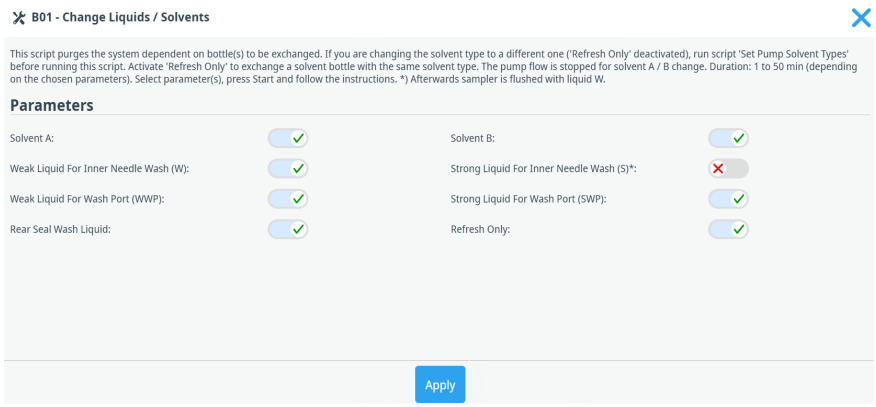


Figure 51: Recommended B01 script settings for refreshing solvents.

Note: Activating the "Strong liquid for inner needle wash (S)*" toggle greatly extends the duration of the script. This is because the metering device must always be re-equilibrated with weak wash liquid (20 purge iterations) after the strong liquid purge, to ensure all strong wash liquid is completely removed. It is good practice to activate this option at least once per quarter to help maintain the cleanliness of the metering device.

TIP The Vanquish Neo rear seal wash liquid is being consumed the whole time that the instrument is switched on. The **rear seal wash liquid consumption** is approximately **280 mL per 2-week period.** This should be taken into consideration when filling the rear seal wash bottle.

6.1.8.2 *Changing solvents*

If solvents are to be changed to a different solvent type, please execute the B01 script with the “refresh only” toggle set to “off”.

TIP If solvent A or Solvent B is being switched to a different solvent type, the correct solvent type(s) should be selected in script A01 prior to running the B01 script.

6.1.9 Smart Standby – options for microflow applications

The Vanquish Neo system is capable of running at up to 100µL/minute, far beyond the flow rates typically associated with conventional NanoLC systems. The instrument method editor contains options for reducing the flow rate at the end of a sequence to both prevent unnecessary solvent waste and to avoid running the analytical pump dry.

The options for “Smart Standby” are located under the “Startup Shutdown” tab in the instrument method editor (Figure 52).

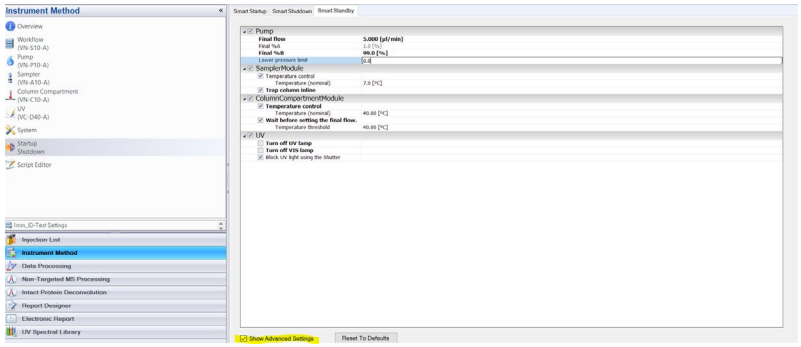


Figure 52: The Start Up, Shutdown window of the Instrument Method Editor

Set a suitable final pump flow rate and final % B which is compatible with the column and workflow. The “Show Advanced Settings” toggle must be activated for all the options shown in the figure above to appear.

For the settings to be activated, the Xcalibur “run sequence” settings should be toggled to “standby” when submitting the sequence (Figure 53).

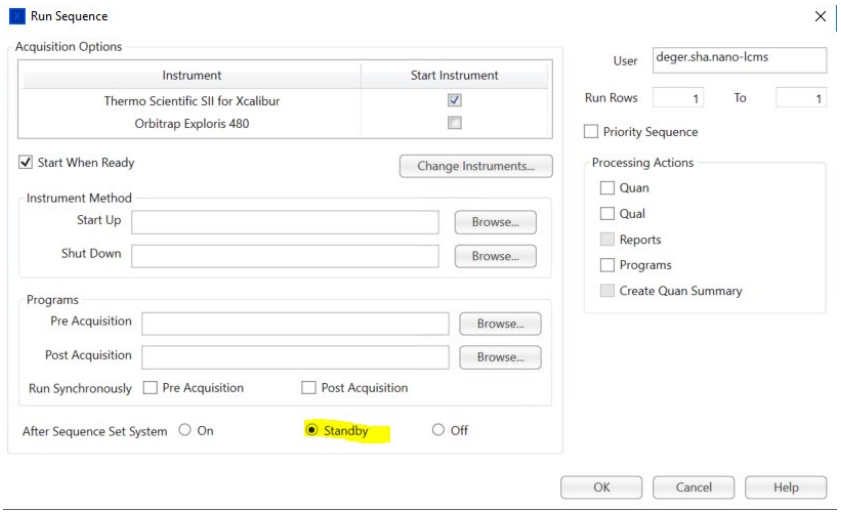


Figure 53: Xcalibur Run Sequence Settings Table for Sequence Submission with After Sequence Set System to "Standy" activated.

TIP Activating the "standby function at sequence end" will set the MS to standby as well as executing the standby protocol in the LC instrument method editor.
For this reason**,** this function is only suited for applications which adopt the HESI IonMax MS source.
It is not recommended to use the "standby after sequence" settings for applications which make use of nano or capillary flow ESI emitters **(e.g., EASY-Spray Columns or P/N ES542 metal emitters).** A nano/capillary flow ESI emitter should always have flow and voltage set to "ON" whilst the emitter is situated in front of the MS ion optics. Switching off the flow or spray under these conditions will reduce the lifetime of the emitter and can lead to blockage, contamination, or spray quality issues.

6.2 Tips and Tricks

6.2.1 Initialize System Setup – Script E01

Circumstances may arise where the Vanquish Neo System is no longer able to determine in what fluidic configuration it currently finds itself in. An example of this is shown in Figure 54: Error message caused by incomplete execution of script A06 – change fluidics / workflowIn this case, the user is requested to initialize their system.

Figure 54: Error message caused by incomplete execution of script A06 – change fluidics / workflow

It should be noted that a characteristic of this failure mode is the dramatically reduced number of scripts available despite no filter being added to the selection (Figure 55).

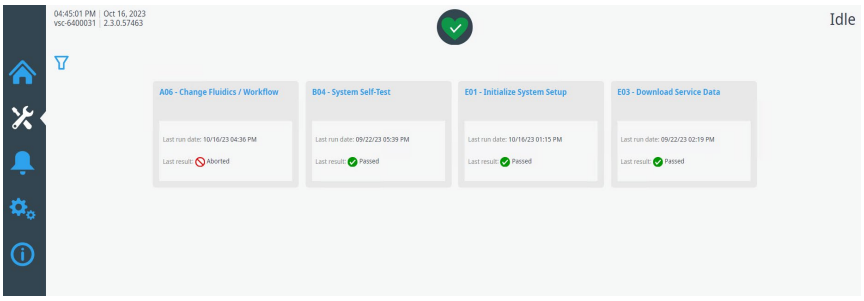


Figure 55: The four scripts available when the Vanquish Neo system is no longer able to determine its fluidic workflow configuration

To return the system to an operational state, execute script “E01 - Initialize System Setup”. A dialogue box opens, asking the user to select the configuration currently installed on the system (Figure 56).

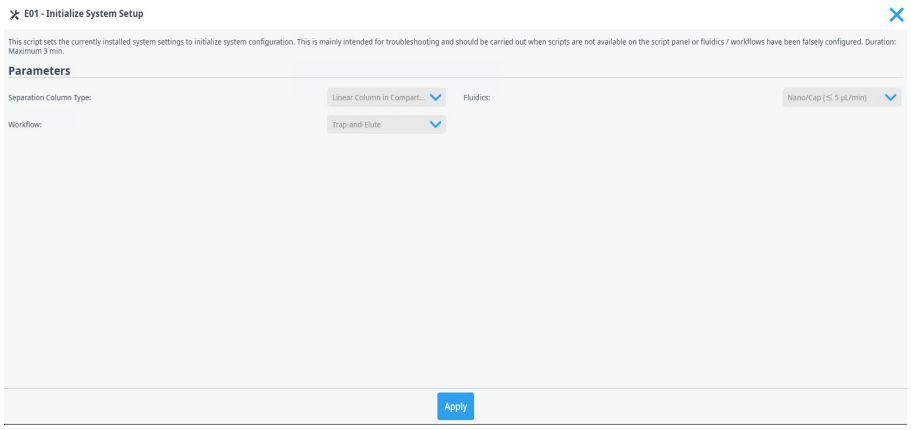


Figure 56: Dialogue box displaying fluidic and workflow configuration options which need to be defined by the user when executing the E01 script

The user is requested to ensure that the workflow set is identical with the one currently installed on the system (Figure 57).

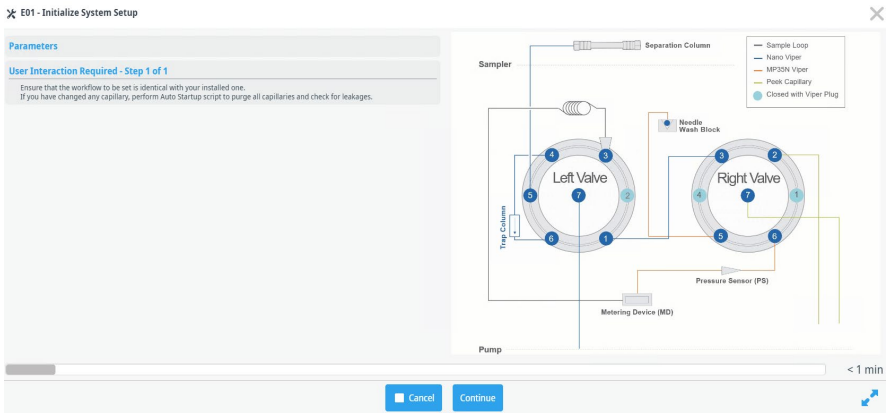


Figure 57: Dialogue box in the E01 script showing the schematic of the selected workflow configuration.

The user will see that system operation has been restored as the 30+ script options become available again.

NOTICE: When activating the E01 script, there are no **diagnostic or sanity checks carried out by the system**. The system will assume that the fluidic configuration is **correctly configured**. For this reason, this script should only be used to recover from configuration errors. For all workflow changes please use the A06 script only.

TIP: If an error or cancellation occurs during the A06 script execution, use the E01 script to set the **original** configuration and then re-start the A06 script to migrate to the new desired configuration.

6.2.2 Executing a "blank injection" using Xcalibur / SII

A "blank injection" is one where the autosampler does not execute any of the sample injection protocol. This means that the needle does not move to the vial, and therefore no sample is drawn prior to the gradient run.

To run a "blank" in this manner – simply set the injection volume to "0 µL" in the sample table in the Xcalibur console.

TIP This functionality is unique to the Vanquish Neo system in combination with SII versions 1.5.1, 1.7 or greater. **This will not work with any of the Vanquish analytical flow systems when operated with Xcalibur / SII.**

6.2.3 Recovering from an error during a sample run

The following describes the steps required in the event of an unexpected error during a sample run resulting in a run-interruption.

- Check the source of the error on the notification panel of the VUI and alleviate the symptoms. E.g., if a leak or blockage have been detected, fix the leak root cause, and clean and dry the spillage. Ensure the leak sensor is also free from spilled liquid.
- Run the relevant diagnostic script a second time to ensure the error has been resolved.
- Run script B04 – System Self-Test to recover from the error.
- Run script B03 – Clean Up System. This will execute a wash and equilibration of the autosampler fluidics.
- Run script B02 – Clean and Equilibrate Columns.
    - If the system is running a trap-and-elute workflow, select both the trap and separation column from the dropdown menu.

- Set the “Final Pump Flow” and “Final Pump B” to the start conditions you wish to use for your next sample run.

After completing this procedure:

- The autosampler fluidics will have been washed and equilibrated such that the system is ready to receive the next injection.
- The column(s) will be washed and equilibrated.
- The system will be “idle” at analytical start conditions.

TIP The “Final Pump B” flow and composition do not apply to the trap column. The trap column will be washed and equilibrated using the strong and week wash solvents. The flow across the trap will be “0” at the end of the equilibration procedure.

6.2.4 Inserting and removing sample plates during a running sequence

The sampler keypad is not operational and the VSC is not accessible whilst the sequence is running (denoted by a solid blue LED light along the bottom of the autosampler door.).

If it is necessary to add or remove sample trays during a running sequence, this should only be attempted when the LED light is solid. A running blue LED indicates that the autosampler is active /busy. In some workflow scenarios e.g. Trap-and-Elute, the blue light is continuously running, the autosampler may still be accessed, but only when it is not “busy”. For example – after the high pressure wash procedure is complete, approximately 5 minutes after the start of the injection procedure (denoted by the two high pressure peaks in Figure 67)

CAUTION A blue running light indicates that the autosampler is actively executing either an injection, or a wash and equilibration protocol during which, for example, the autosampler needle is in motion. Opening the autosampler door during this time poses a safety hazard.

6.2.5 Configuring well plates with no bar code

If a well plate is inserted into the autosampler which does not have a bar code attached, the plate type “unknown” will be displayed in the plate type field of the Vanquish Neo tab on the ePanel (see Figure 58)

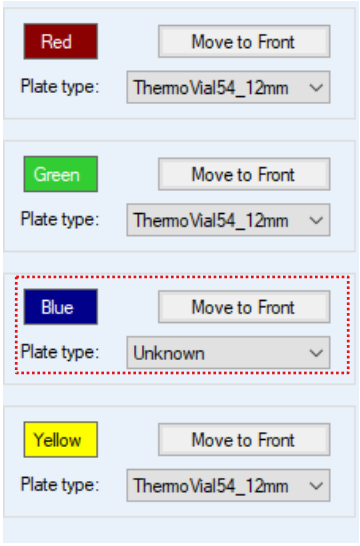


Figure 58: Autosampler plate type for the blue segment registered as “Unknown” due to the missing bar code on the plate

In this case, the plate type must be set manually by selecting the correct option from the drop down menu (see Figure 59).

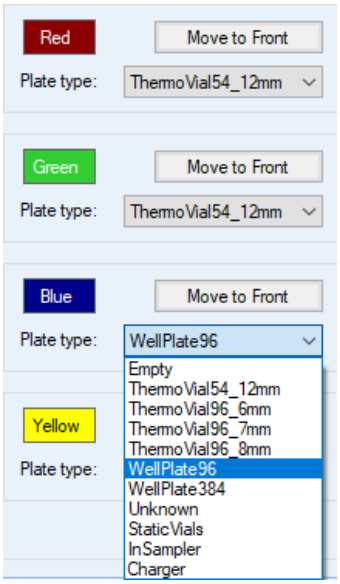


Figure 59: Selecting the correct well plate type from the dropdown menu

6.2.6 Recognizing and recovering from a missing vial error

If an attempt is made to inject from an empty vial/well plate position, the Xcalibur sequence will switch to an “frozen” state. without any further response.

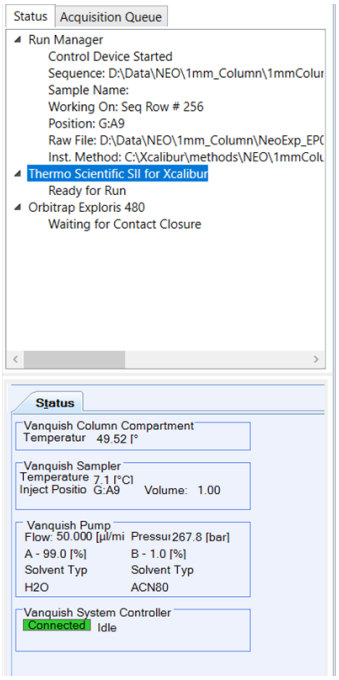


Figure 60: The Xcalibur status and mini ePanel display shown after an attempt has been made to inject from an empty vial position.

The VUI notification panel reports an aborted command.

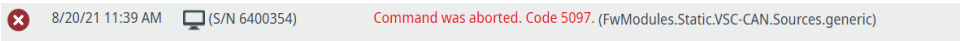


Figure 61: VUI notification panel signally an aborted command

In order to discover the root cause of the error, the user must examine the audit trail in the ePanel which is the only place where the missing vial issue can be identified (Figure 62).

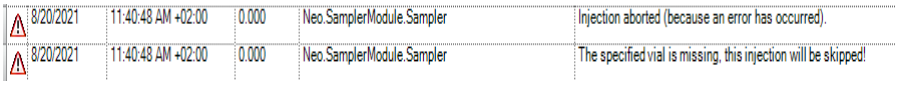


Figure 62: ePanel audit trail showing the root cause of the aborted injection

To recover from this error:

- Right click “Thermo Scientific SII for Xcalibur” in the status window (Figure 60) and select “Turn device off” and then “Turn device on”.
- Wait for the status to change to “Ready To Download”
- Correct the missing vial / well plate error by assigning the correct position or inserting the vial or well plate.

- **TIP**: If the sequence still fails to execute upon re-start via Xcalibur, reboot the instrument control PC.

6.2.7 Understanding and optimizing the sample loading volume

The “loading volume” on the Vanquish Neo System constitutes the volume of liquid displaced from the loop after the injection volume (as specified in the sample table) has been considered. As such the loading volume is independent of the injection volume. The total volume displaced from the sample loop during sample loading part is therefore the sum of the injection volume and the loading volume (see Figure 63).

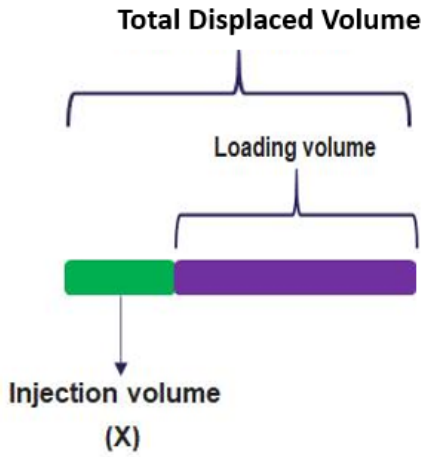


Figure 63: Schematic representation of the relationship between loading volume, injection volume and the total volume displaced during the sample loading procedure

Example of the dependencies between injection and loading volumes and their effect on the total volume displaced are given in Table 18 below.

Injection Volume (µL)	Total Volume displaced during Sample transfer using Automatic Loading (= 5µL) (µL)	Total Volume Displaced with Manual Loading Volume of 10 µL (µL)
1	6 (1+5)	11 (1+10)
2	7 (2+5)	12 (2+10)
3	8 (3 + 5)	13 (3+10)
4	9 (4 + 5)	14 (4+10)
5	10 (5+5)	15 (5+10)

Table 18: Relationship between Displacement Volume and Injection and Loading Volumes for the Vanquish Neo System – The injection volume + loading volume components are shown in parenthesis

TIP The automatic loading volume is 5 µL. This is setting should be sufficient for the majority of bottom up proteomics applications employing reversed phase chromatography

6.2.8 Maximizing the life time of the autosampler metering device direct injection capillary

A workflow change from direct injection to trap-and-elute and vice versa requires the removal (and re-installation) of a viper capillary that connects the metering device to the injection valve at position 6 in the direct injection workflow configuration (Figure 64). To prolong the lifetime of this capillary it is recommended to always install the capillary with the same orientation.

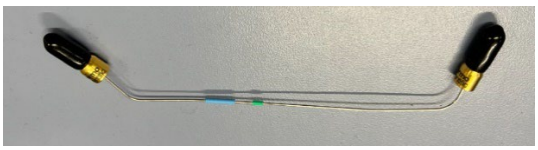


Figure 64: 0.25mm ID x 170mm MP35N Viper Capillary (part of capillary kit with P/N 6252.1920) connecting the Metering Device to the injection valve port 6

6.3 Column Care and Use

6.3.1 Preparing Columns for use

The column conditioning script (**B06**) is used either to prepare columns for first use or to prepare columns for use after long periods of storage (> 1 week).

The column conditioning script uses pressure / flow ramps which gradually compress the column packing material. The column(s) are flushed and then equilibrated using the analytical pump. Please refer to the full script description in Table 1.

TIP This script should not be confused with the script **B02** – clean and equilibrate columns which should only be used for columns which have already been conditioned (see section 6.3.2 for details).

NOTICE It is essential that the column specifications (scripts A04 and A05) are set correctly **before running this script**. Failure to do so could result in irreversible damage to the columns.

6.3.2 Clean and Equilibrate Columns (B02)

The B02 script should be used to clean and equilibrate columns which are already in regular use. This script adopts the maximum pressure and flow rates according to the set column specifications (scripts A04 and A05).

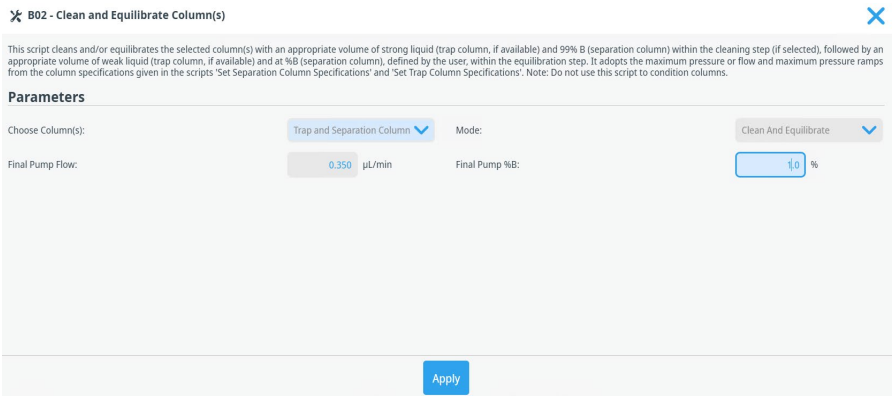


Figure 65: The B02 script options page with trap and separation column selected

TIP The final pump flow and final pump % B value options only apply to the separation column. The trap column is always flushed with strong wash liquid and equilibrated with weak wash liquid. Furthermore, the flow rate across the trap column is always “0” upon completion of the script as the trap column is in-line with the metering device which is no longer active at the end of the script.

7 Appendix

7.1 Example Reference data from the Vanquish Neo System Installation Qualification

7.1.1 The Vanquish Neo System Installation Qualification

Every Vanquish Neo installation is qualified using the Vanquish Neo System Installation Qualification. The data recorded and the column(s) supplied for the installation should be stored carefully and can serve as a valuable reference / base for a number of troubleshooting activities if or when they become necessary.

The IQ method can also be used for LC QC purposes, to quickly prove correct system functionality. Example IQ data are also shown in Figure 5.2 of the Installation Qualification document.

7.1.2 Example Pressure Profiles for Direct Injection and Trap and Elute Workflows

The autosampler pressure profile is recorded with each MS data file. The pressure profile has a distinctive “finger print” for methods where the autosampler loop and needle are washed and equilibrated offline (most common form) and is specific to either direct injection (Figure 66) and trap-and-elute (Figure 67) respectively.

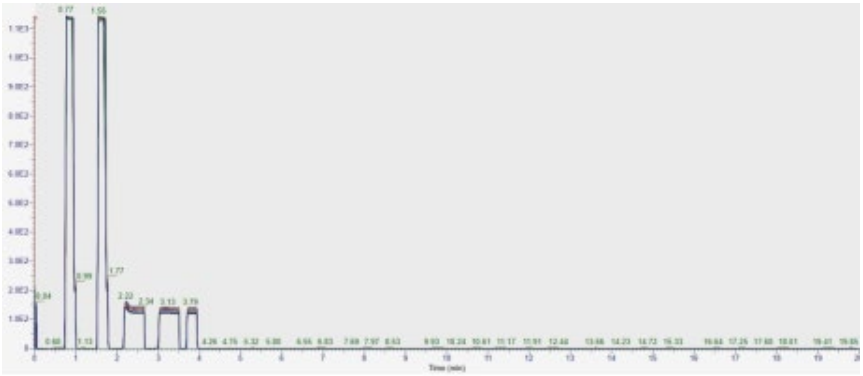


Figure 66: Sampler Pressure Profile for Direct Injection

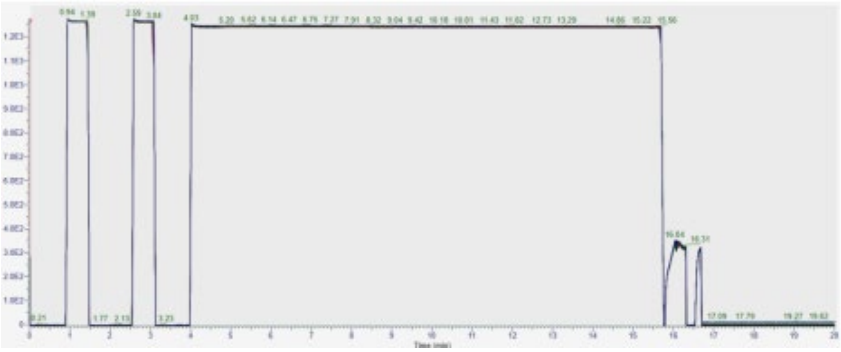


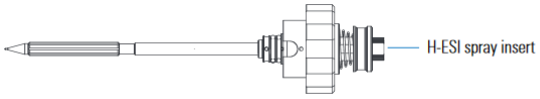
Figure 67: Sampler Pressure Profile for Trap-and-Elute

Regular monitoring of these pressure profiles can serve as a useful passive diagnostic tool, confirming correct Vanquish Neo autosampler functionality.

7.2 The Ion Max and Ion Max NG Ion sources for heated electrospray ionization

7.2.1 Heated Electrospray Ionization (HESI-II) Probe and H-ESI Spray Insert

For micro- and capillary-flow applications with columns typically ≥ 300 µm i.d. and flow rates ≥ 5 µL/min, the Ion Max Source and accompanying ionization probe can be used when adapted for low flow rates. Two electrospray ionization source housing types are available for the Thermo Fisher Mass Spectrometers, the Ion Max and Ion Max NG source. The source type depends on the mass spectrometer type. Each has their own electrospray ionization probe (see *Figure 68*).



Heated Electrospray Ionization (HESI-II) Probe for the Ion MaX source
P/N OPTON-20037 Kit

MS Compatibility
LTQ™ and Velos™ Series
Orbitrap™ Series
Exactive™ series
Legacy TSQ™ series (Quantum Access Max, Vantage, Ultra *etc*.)
H-ESI Spray Insert for Ion Max NG Source
P/N 80000-60321

Figure 68: Ionization probes for the Ion Max and Ion Max NG sources.

7.2.2 Low-flow metal needle kit for microflow applications

A low-flow (50 µm I.D.) metal needle is required for micro-flow flow based low-flow experiments to give the best chromatographic performance. Both PEEK and fused silica capillaries are available to interface the source with the column outlet. A compatibility matrix for the different low-flow options is shown in Figure 69.

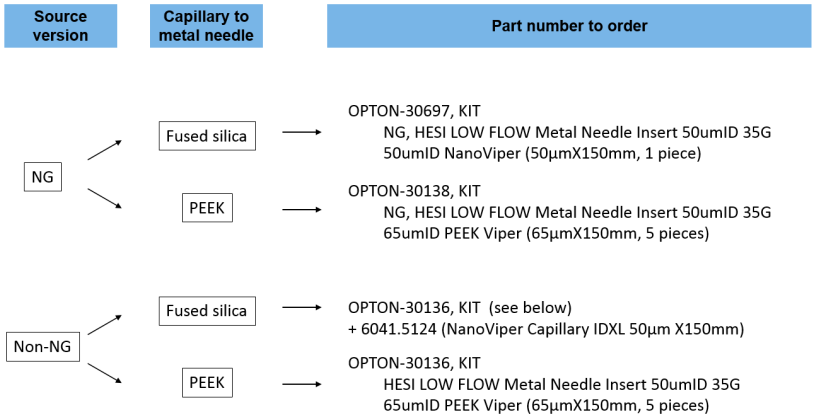


Figure 69: Ionization probes for the Ion Max and Ion Max NG sources

7.3 The Use of TFA and FA in solvent buffers

The separation of peptides by reversed phase (RP) chromatography is carried out in the presence of an ion-pairing agent, which serves a double function. First, these (typically) weak acids bring the pH of the solvents down to pH 2-3, causing most peptides to have an overall positive charge. Secondly, the negative counter-ion of the acid will serve as an ion-pairing agent for the positively charged peptides to create an overall neutral analyte that is more efficiently separated on the RP column. The double function of the ion-pairing agent results in an efficient separation with minimal quantities of these added to the solvents.

Trifluoro acetic acid (TFA) and formic acid (FA) are most commoly used used. In most LC-MS applications, FA is preferred as its use minimizes ion-suppression effects. TFA is a stronger ion-pairing agent and results in better chromatography but can result in ionization suppression. The use of TFA is generally restricted to the loading buffer or when increased retention (compared to FA) is necessary even if it occurs at a cost of MS signal intensity.

www.thermofisher.com

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