thermoscientific

Vanquish

Neo System

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

User Guide

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Contents

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.

NOTICE This document is based on Vanquish Neo Software Bundle 2.0. Some of the descriptions may deviate those used in previous versions.

1.2 Conventions

This section describes the conventions used throughout this manual.

1.2.1 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 ResourcesLow-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
- The Vanquish Neo Binary Pump N Operating Manual
- Vanquish Neo System Specification sheetsVanquish System Online configuratorVanquish User Interface (System Controller and Display) Installation Guidehttps://www.thermofisher.com/order/catalog/product/ULTIM3000RSLCNANO
- 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
- Using the proteoCHIP 12*16 autosampler holder for the Vanquish Neo UHPLC system

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.

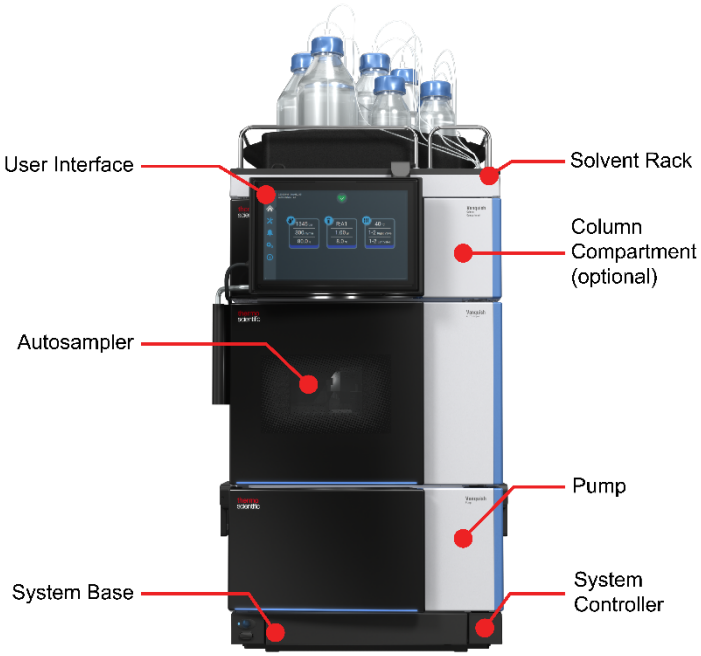


Figure 1: Vanquish Neo System Hardware Components

2.1.2 System Base

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

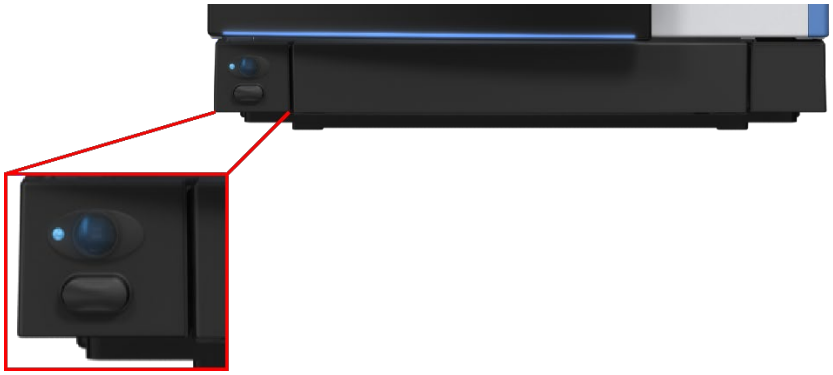


Figure 2: The Vanquish Neo System Base

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:

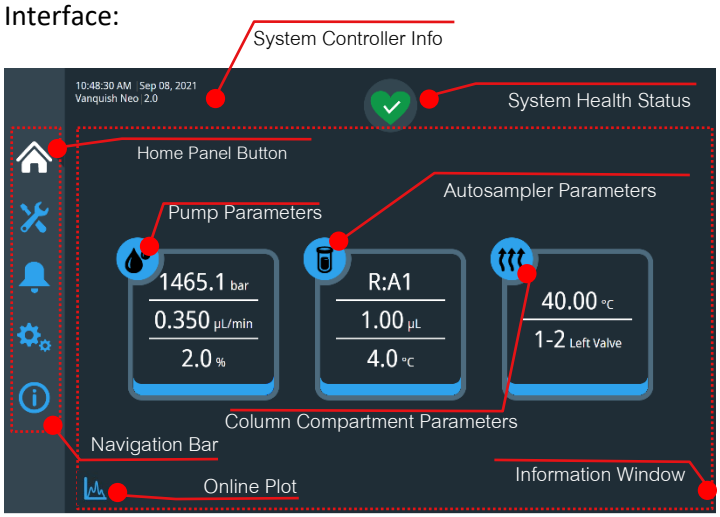


Figure 3: The Home Panel of the Vanquish User Interface

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

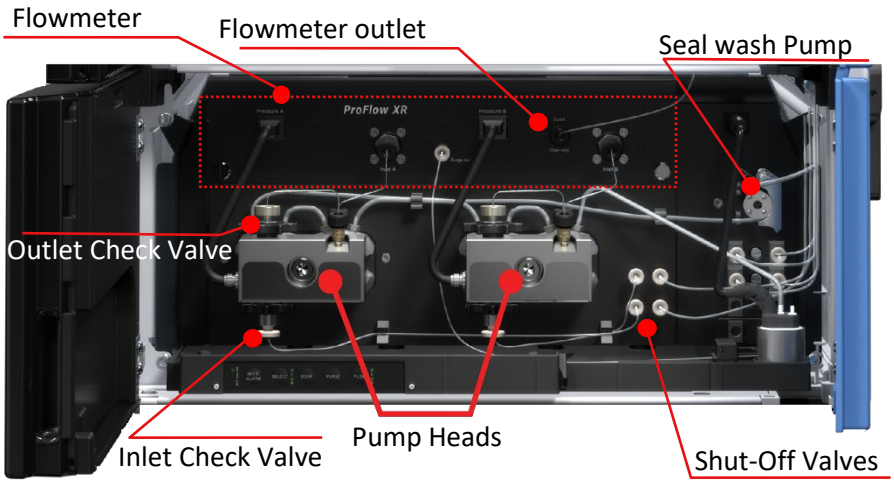


Figure 4: The Vanquish Neo Binary Pump

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 flowmeter outlet. The combined flow leaves the flowmeter 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.

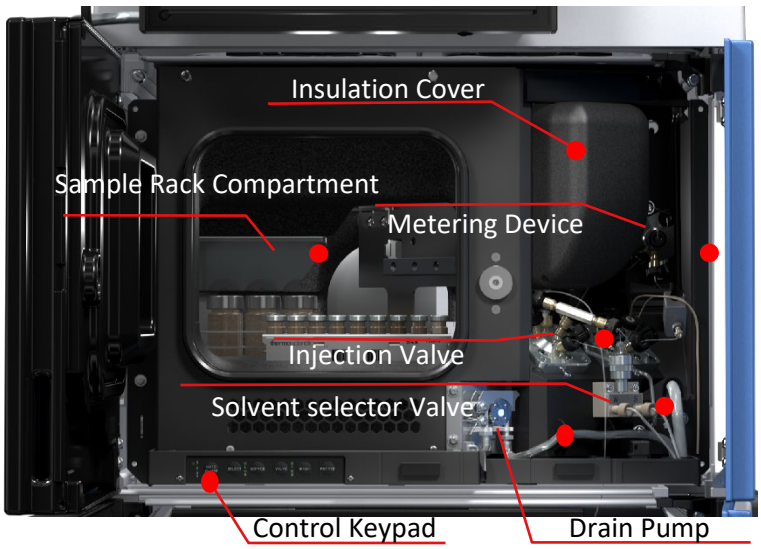


Figure 5: The Vanquish Neo Autosampler

Two 1500 bar capable proprietary 6-position 7-port valves enable the injection fluidics to be switched offline 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.

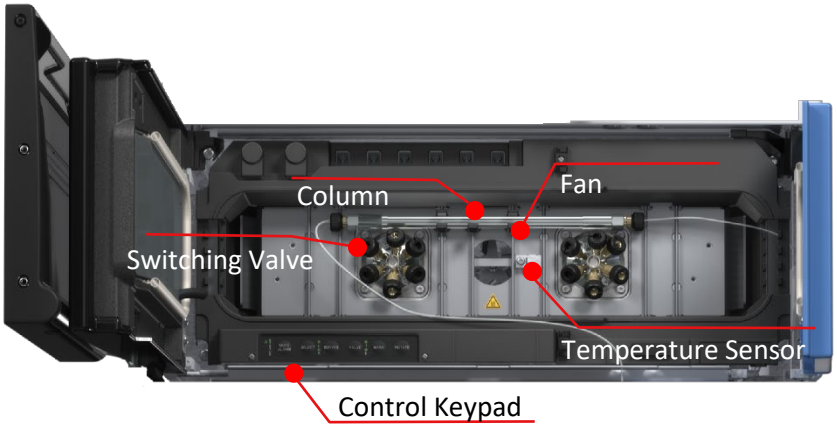


Figure 6: The Vanquish Neo Column Compartment

The Vanquish Neo Column Compartment works using advanced design forced air thermostatting to support column heating with an even airflow. This ensures that 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) is supplied 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

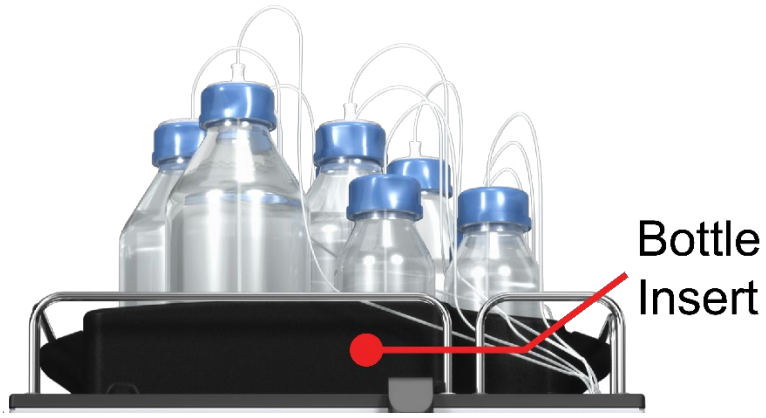


Figure 7: Solvent Rack Including Bottle Insert and Solvent Reservoirs

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

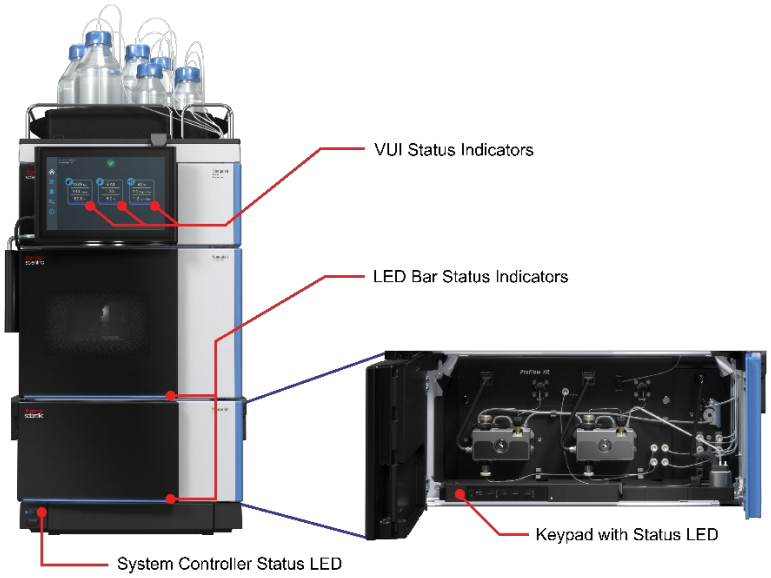


Figure 8: Vanquish Neo module status LEDs and keypads

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 colors 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 keypad 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 independent of the control PC. This chapter guides the user through the control options.

TIP The Vanquish User Interface can 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 into the web browser. The IP address 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.

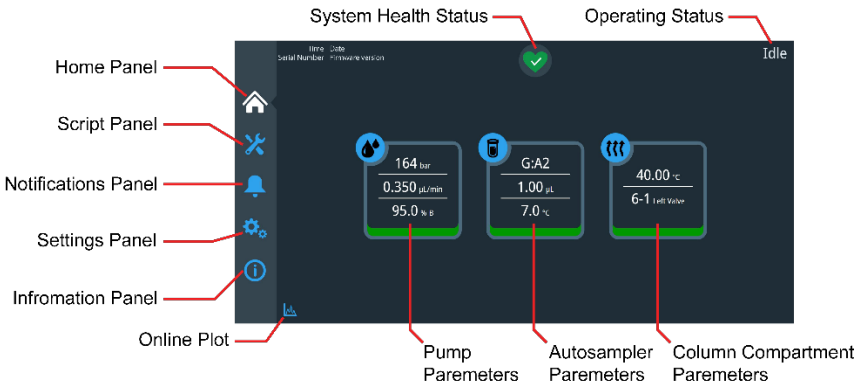


Figure 9: Vanquish User Interface (Home panel)

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 value. The direct control options available for the Vanquish Neo Binary Pump are shown in Figure 10 below.

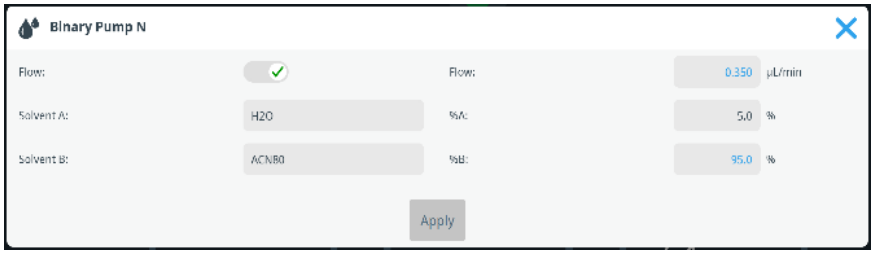


Figure 10: Vanquish Neo Binary Pump direct control panel

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 hand 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).

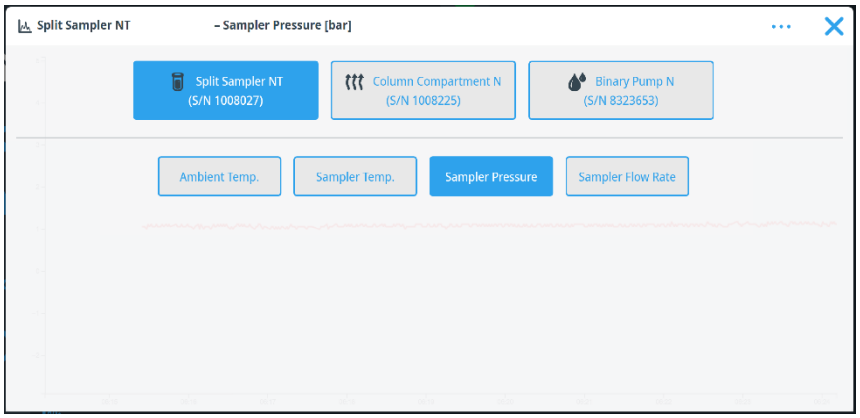


Figure 11: Online plot menu dialogue box

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 refresh rates are 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.

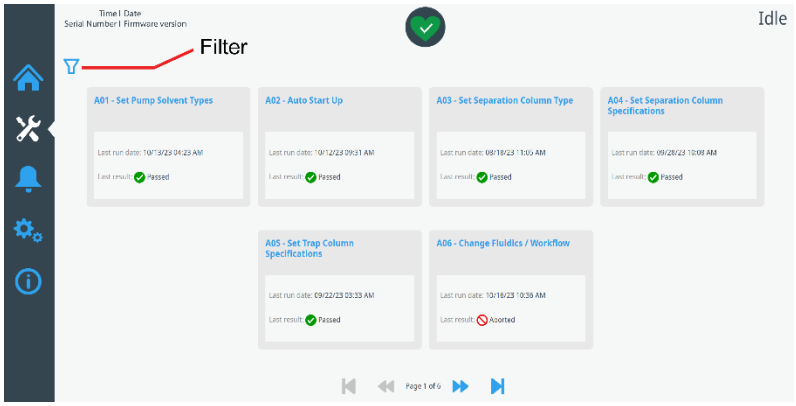


Figure 12: Page 1 of the VUI scripts

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)

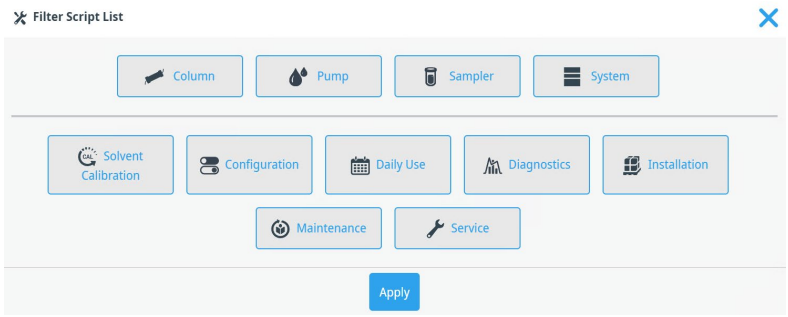


Figure 13: Script Filter List of Options

A list of the scripts available at time of publication (Vanquish Neo Bundle 2.0), 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 Column Compartment to mount a compatible VICI valve. 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.

ID	Script	Description
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. 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 flowmeter 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'.

ID	Script	Description
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” 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	Clean or Replace Needle Unit and Seat	This script provides the user with the option to i) clean the needle seat ii) replace the needle seat only iii) replace both 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

ID	Script	Description
		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.
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)

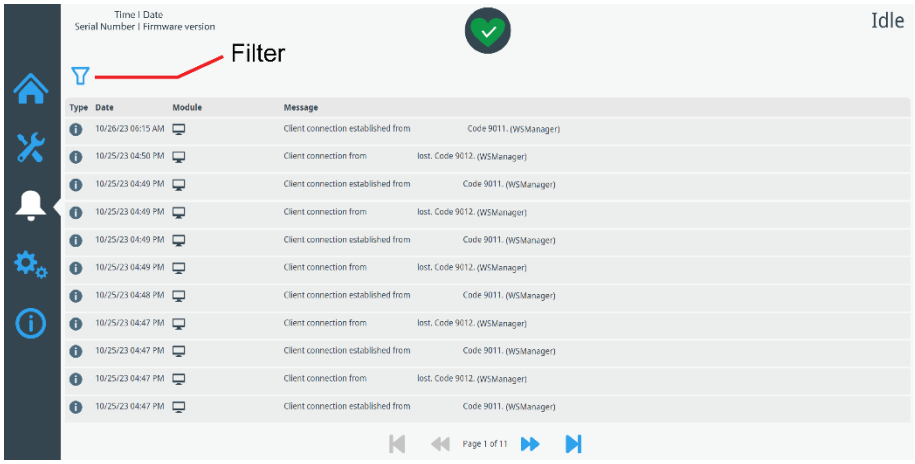


Figure 14: Notification Panel

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

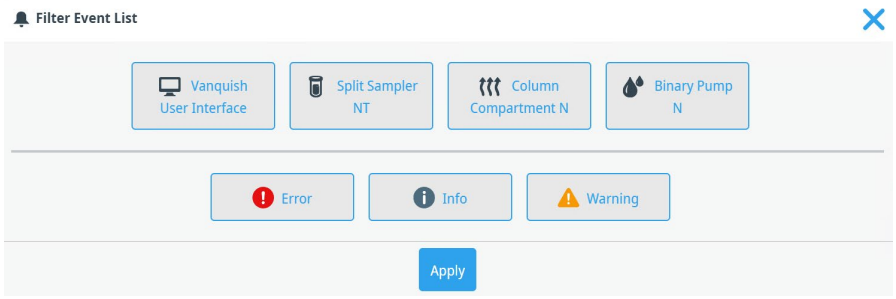


Figure 15: The filter event list dialogue box for the notification panel

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.

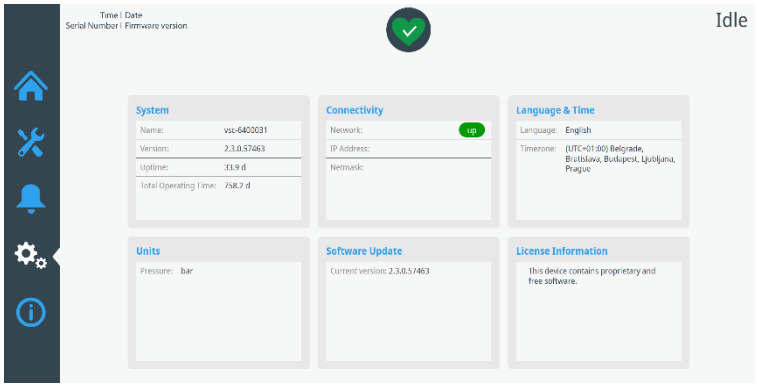


Figure 16: Settings Panel

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 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:

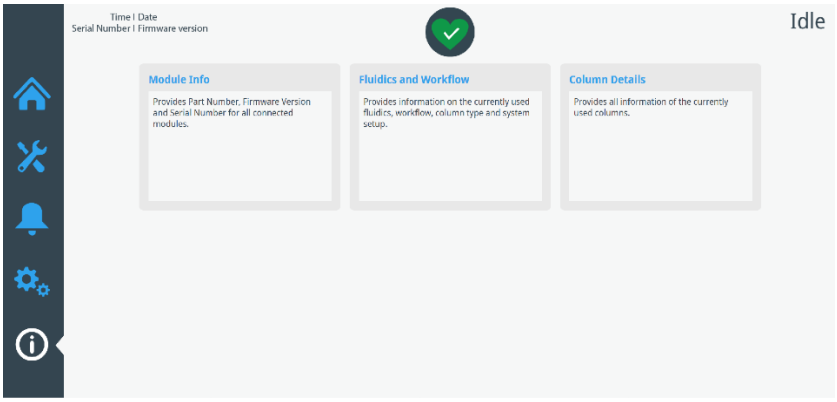


Figure 17 The VUI Information Panel

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

This chapter provides information on how to setup, configure, connect and prepare the system to run a sequence

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:

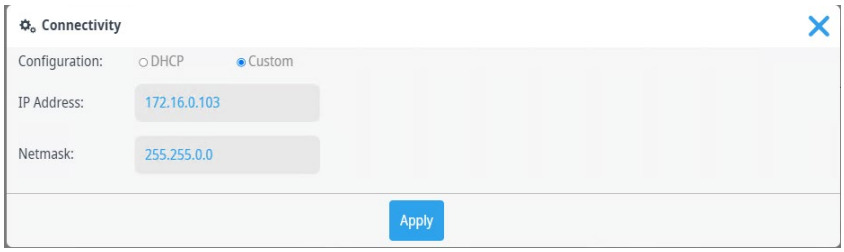


Figure 18: Manual configuration of the VSC IP address

TIP The Vanquish User Interface can 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 into the web browser. The IP address can be read out from the Settings Panel of the Vanquish User Interface (VUI).

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).

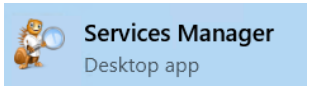


Figure 19: Service Manager application

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).

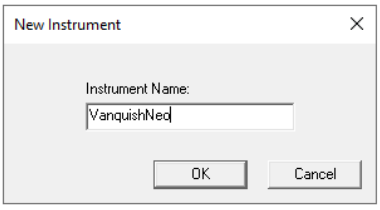


Figure 20: New instrument

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.

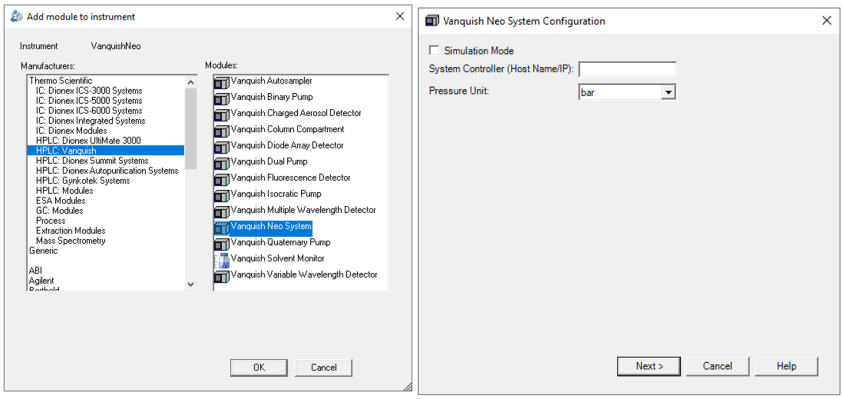


Figure 21: Add new module to the instrument and set the system controller IP address

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).

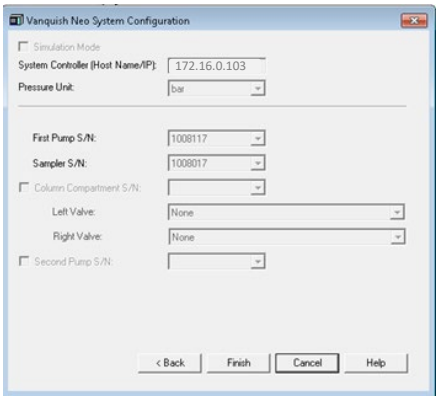


Figure 22: Vanquish Neo system and connected modules

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% (v/v) 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)

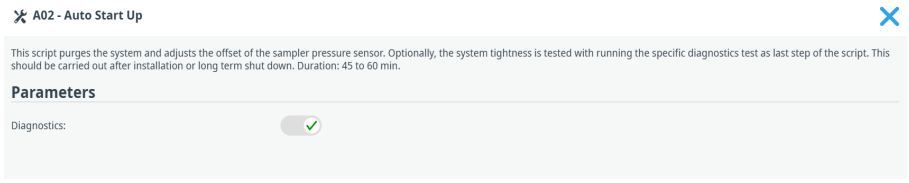


Figure 23: Dialogue box for script A02 showing the diagnostics set to “on”

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.

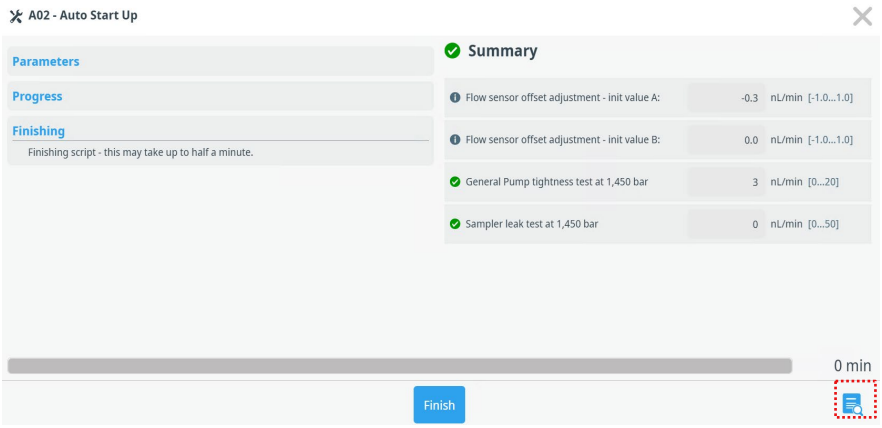


Figure 24: Example results summary table following the completion of the auto start up script

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 as it is not included in the auto start up procedure. 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.

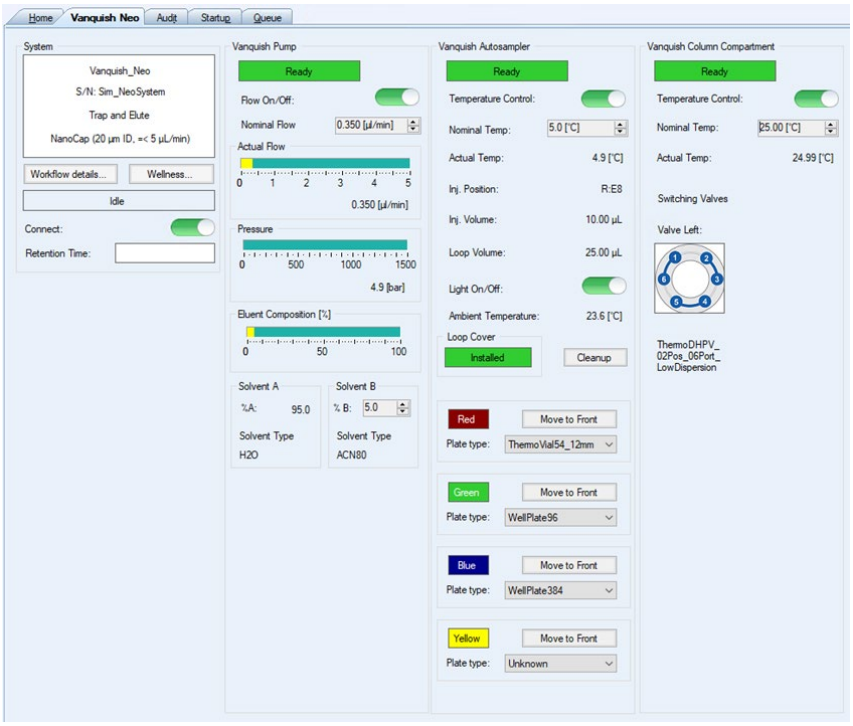


Figure 25: Vanquish Neo System ePanel

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

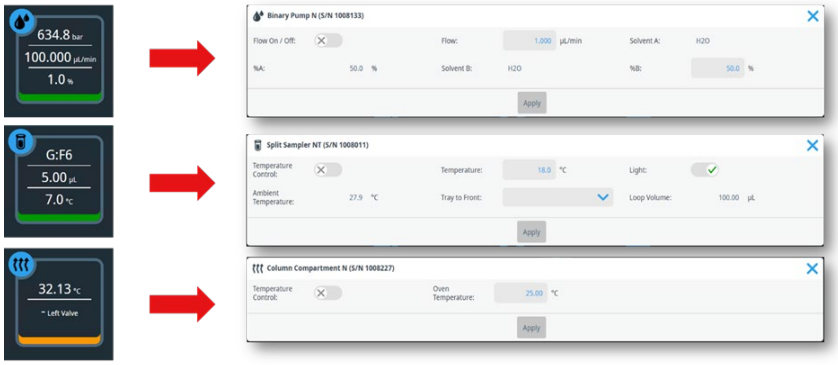


Figure 26: Direct Control Options on the VUI home screen

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 (script A04)

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

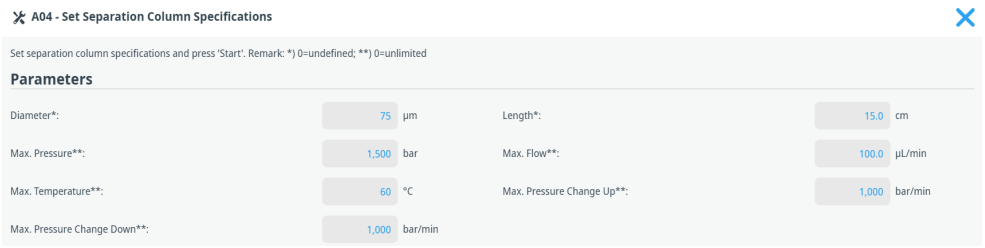


Figure 27: Example analytical column specifications for a 75 µm x 15 cm ID PepMap Neo column

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

Several 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 given below:

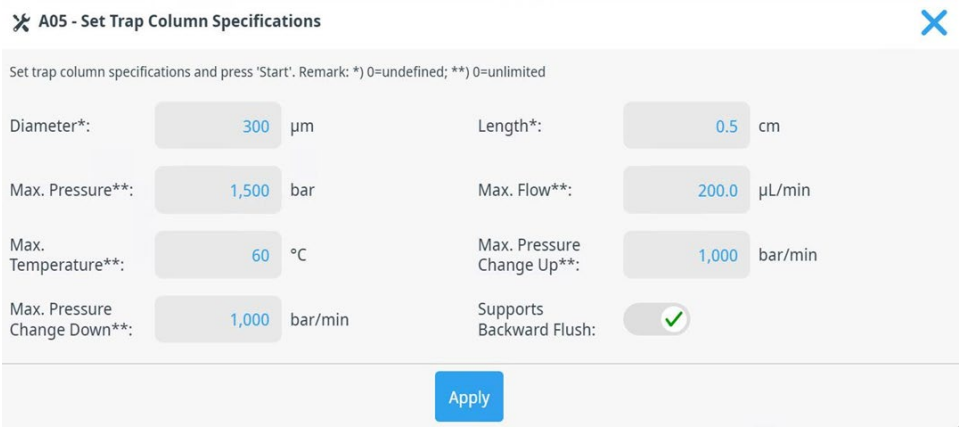


Figure 28: Default specification settings for PepMap Neo Trap Cartridges

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:

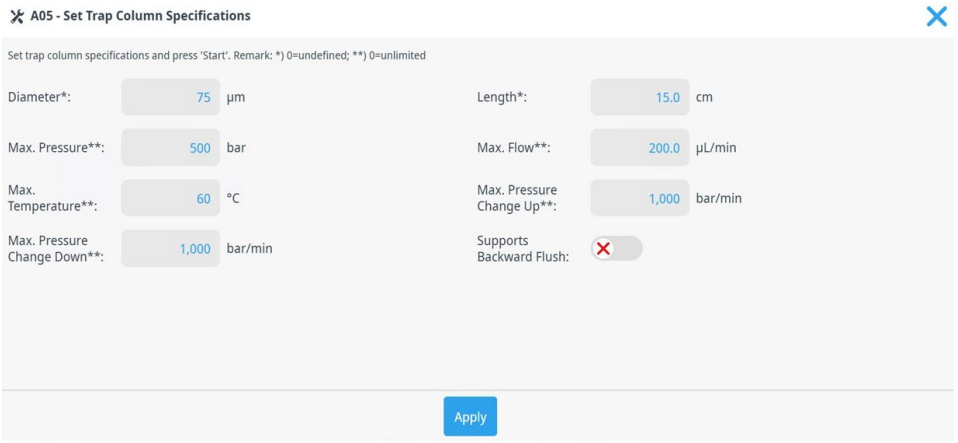


Figure 29: Default specification settings for the PepMap NanoTrap columns

NOTICE: 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 columns 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)

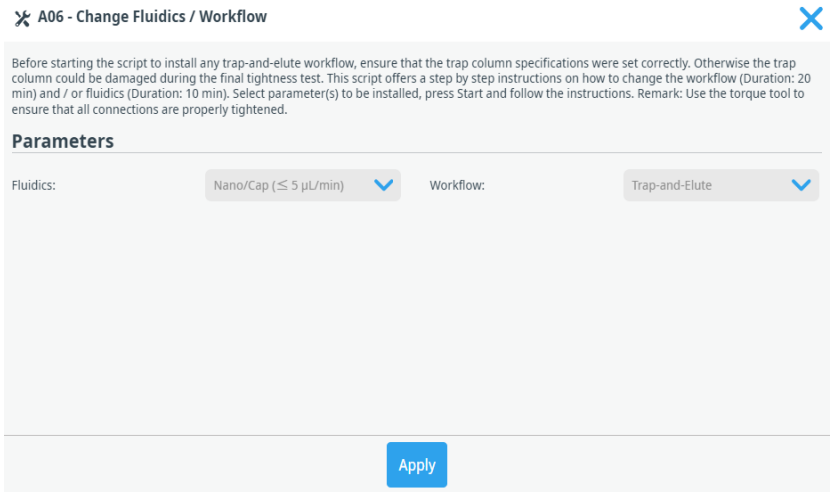


Figure 30: The change fluidics / workflow script

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 valves 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.

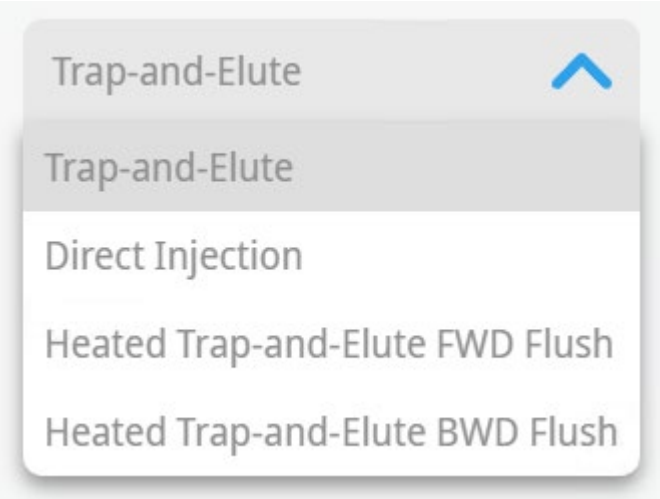


Figure 31: Workflow options in heated trap-and-elute mode

TIP: The heated trap-and-elute workflow uses 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 when the workflow type is selected on the VUI. This contrasts with the “non-heated” trap-and-elute workflow which uses the 7-port-6-position valve situated in the Vanquish Neo Autosampler. Here the choice between forward and backward flush can be changed simply by selecting the desired mode in the instrument method editor. NOTE: This assumes that the trap column can support backward flush operation and that the “supports backward flush” option has been activated in script A06 (see Figure 29).

5 Methods and Applications

This chapter details how to create instrument methods using the Vanquish Neo System as well as providing links to template methods for a number of common 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 includes all the possible options. Specific parameters are limited to specific workflows and configurations. As such, the view you get for your selected workflow may differ from the view shown in this document.

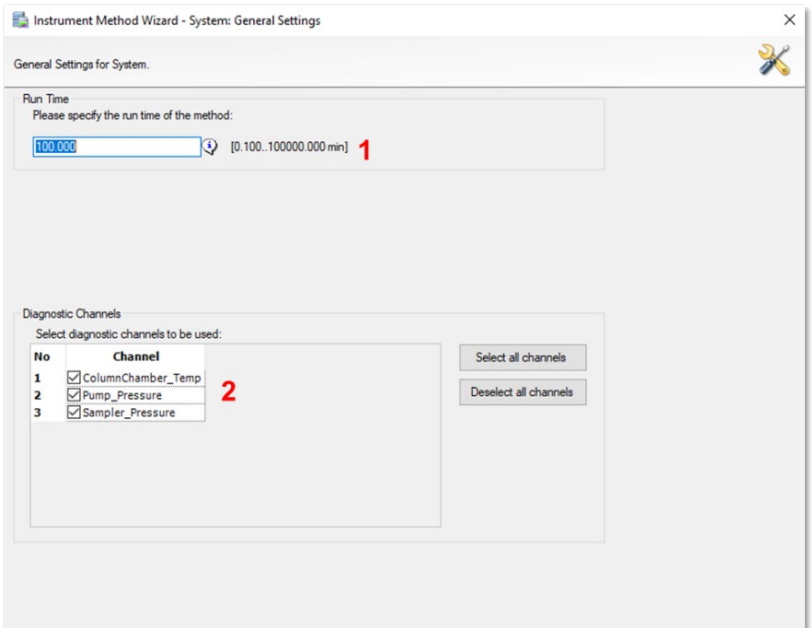


Figure 32: IMW Page 1 - System: General Settings

#	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 IMW Page 1 system general settings

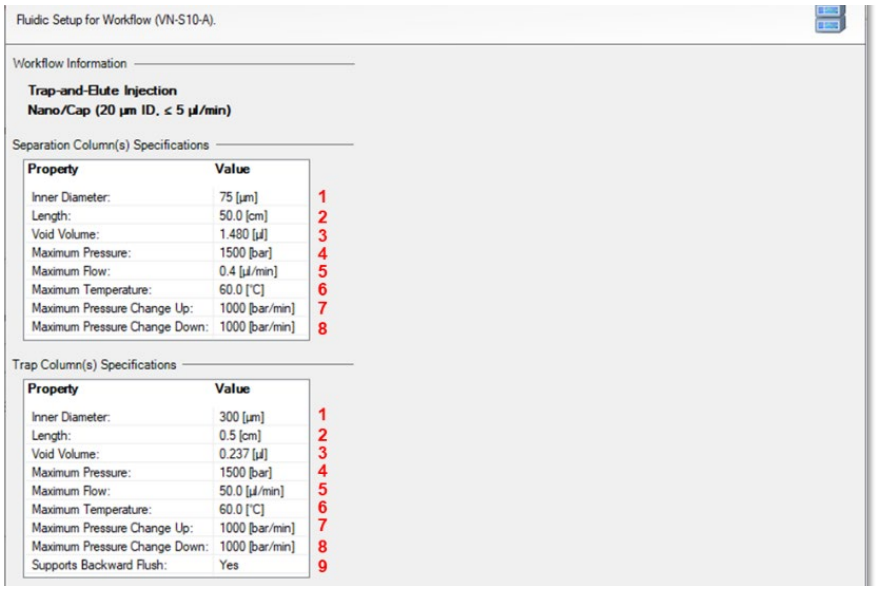


Figure 33: IMW Page 2 – Workflow: Fluidic Setup

#	Description
1	Inner diameter of the separation/trap column*.
2	Length of separation/trap column*.
3	Theoretical separation/ trap column void volume based on the assumption that this is equivalent to considering 67% of the 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: Use 1000 bar/min unless column specifications dictate otherwise.
8	Maximum pressure change down for the separation/trap column. Note: use 1000 bar/min unless column specifications dictate otherwise
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 IMW page 2 Workflow fluidic setup *see column label or specification sheet for details.*

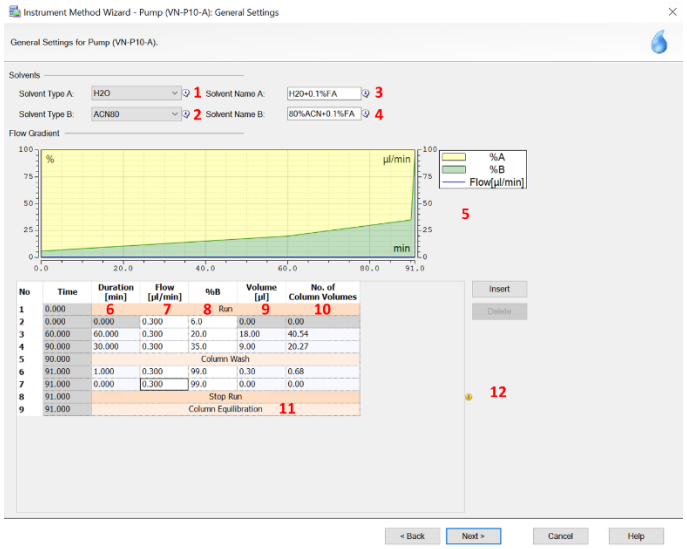


Figure 34: IMW Page 3 – Pump: General Settings

#	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 flowmeter 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 flowmeter 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.

#	Description
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 IMW Page 3 Pump General Settings

TIP It recommended to use a maximum of %99 for eluents A & B when programming instrument methods when running low-flow instruments as it helps to eliminate backflow between the A and B channels.

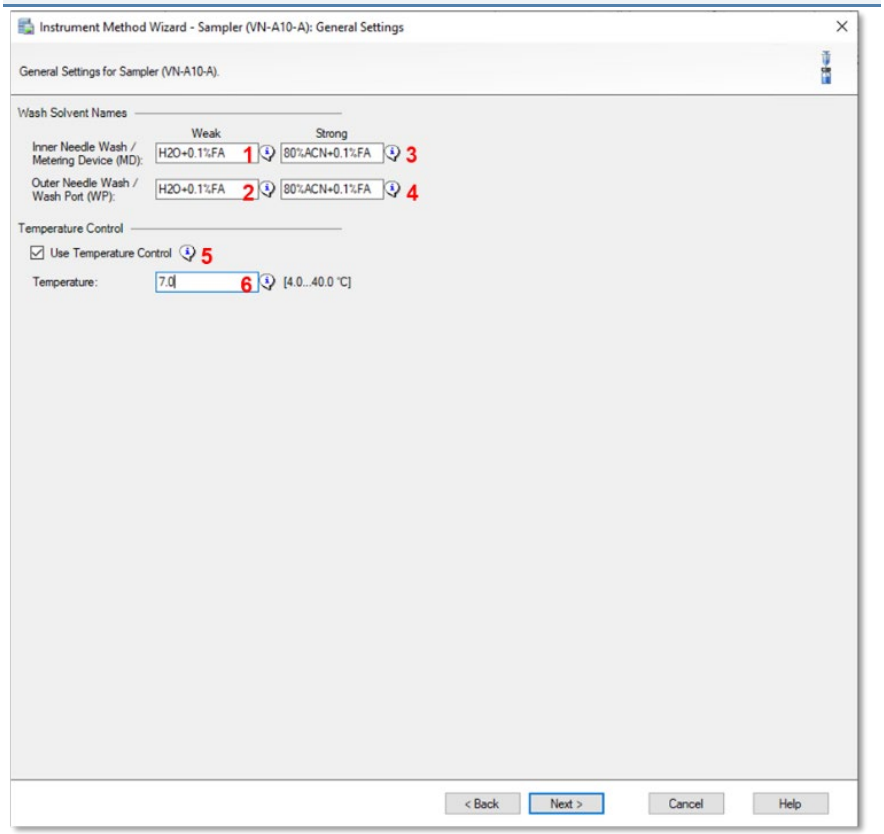


Figure 35: IMW Page 4 – Sampler: General Settings

#	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 IMW Sampler general settings

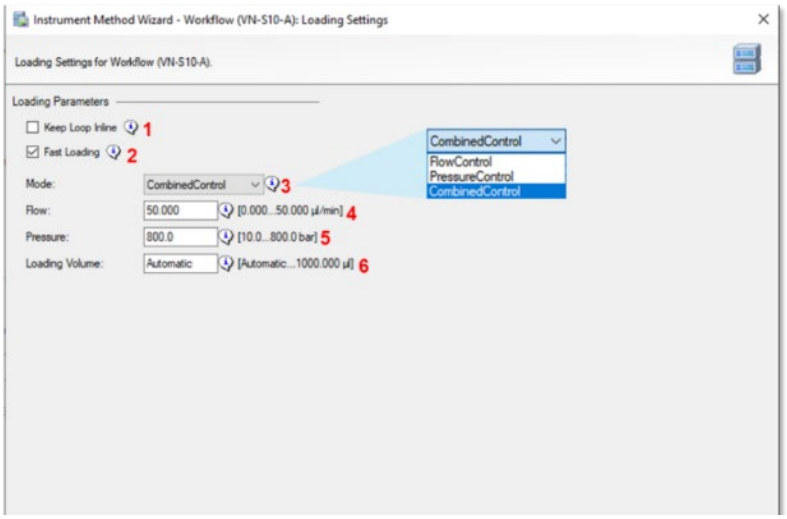


Figure 36: IMW Page 5 – Workflow: Loading Settings

#	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, precisely 5µL of eluent is used to transfer the sample plug to the separation column. A nominal loading volume can be specified, this particular volume of eluent will be used for loading.

Table 8: Key to figure 36 IMW page 5 Workflow – Loading settings

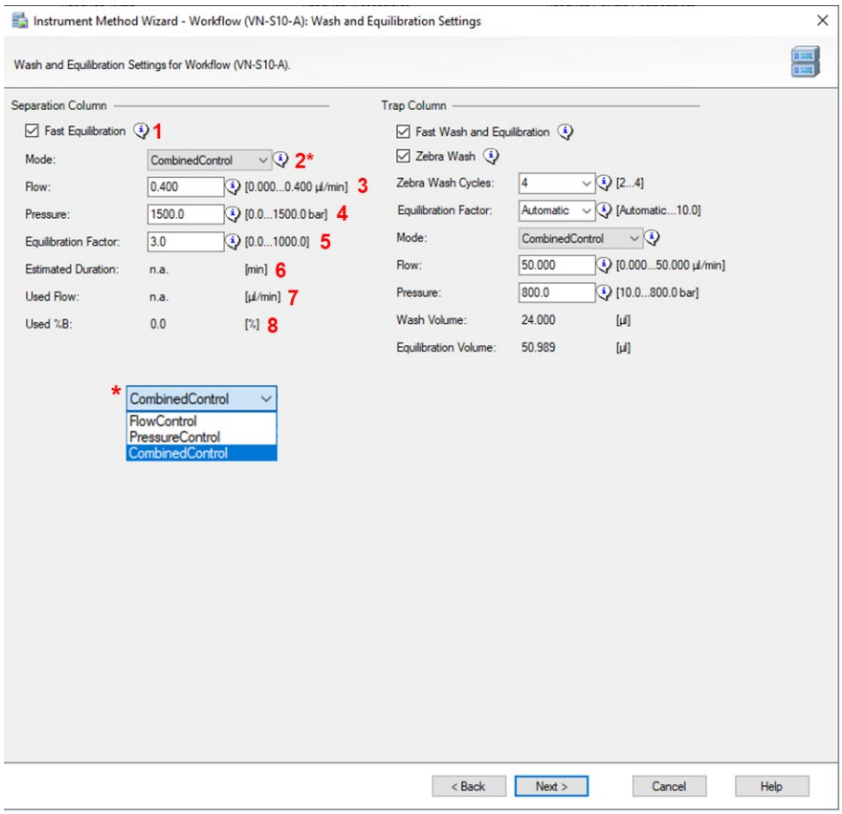


Figure 37: IMW Page 6 - Workflow: Wash and Equilibration Settings (1)

	Description
1	Specifies whether column equilibration should be accelerated reducing measurement time overhead. 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) PressureControl: The pump maintains the specified pressure (#4). 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.

	Description
5	Determines the eluent volume used during column equilibration. This equates to the void volume of the column(s) 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.
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.IMW Page 6 Wash and Equilibration Settings (1)

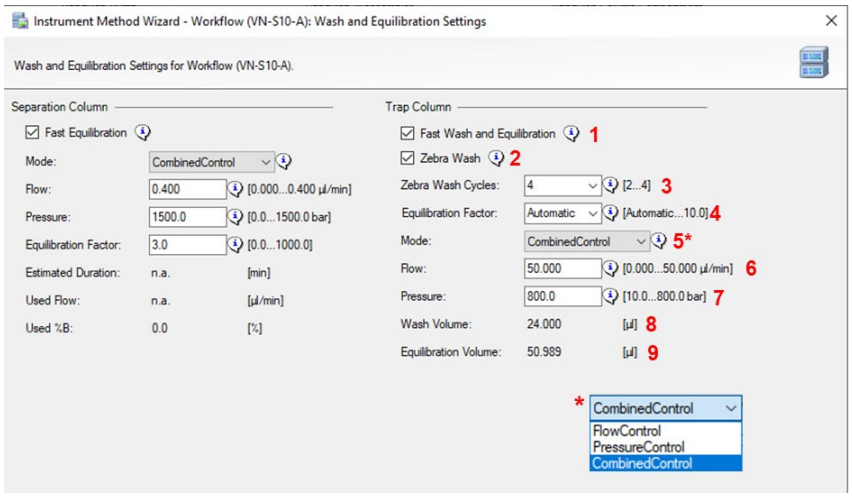


Figure 38: IMW Page 6 - Workflow: Wash and Equilibration Settings (2)

#	Description
1	Specifies whether trap column wash and equilibration should be accelerated, reducing measurement time overhead. Enabling this option will switch the trap column offline. The metering device then washes and equilibrates the trap column. If this option is disabled, the trap column is kept inline and washed and equilibrated together with the separation column by 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	If the Zebra Wash check box (see 2) is deactivated: This value 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. If the Zebra Wash check box is activated (see 2): This value 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.

#	Description
4	Determines the volume of weak liquid from the metering device used for 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 (the total volume used to wash the column). 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.
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 IMW page 6 - Wash and Equilibration Settings (2)

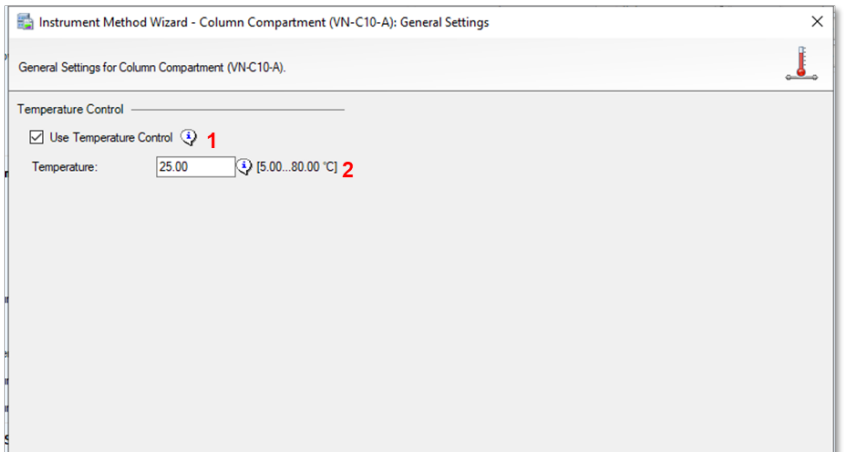


Figure 39: Page 7 - Column Compartment: General Settings
Note: This page is only shown if a Vanquish Neo Column Compartment is part of the instrument configuration.

#	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.

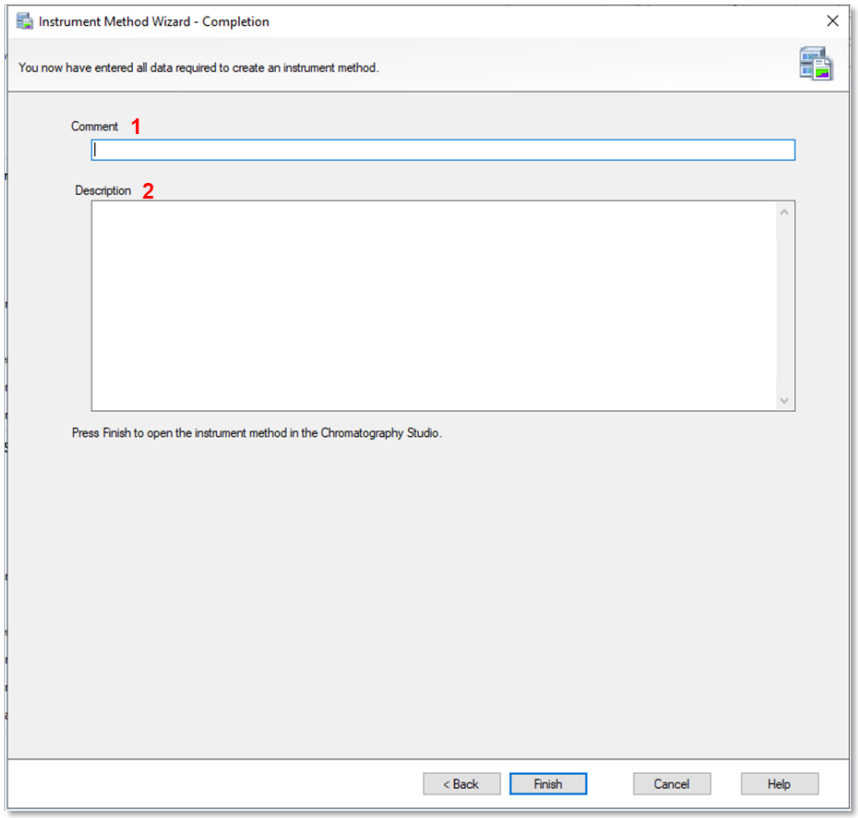


Figure 40: Page 8 – Completion

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

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

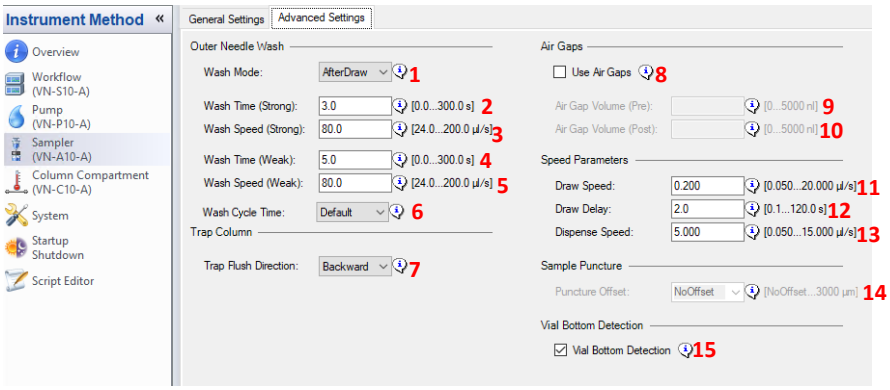


Figure 41: Instrument Method Editor – Sampler: Advanced Settings
Note: This page will be only present in the instrument method editor and not in the instrument method wizard.

#	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.

#	Description
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 13: Key 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

This chapter details advice on how to maintain the Vanquish Neo system as well providing useful tips applicable for daily operation

6.1 Best Practices

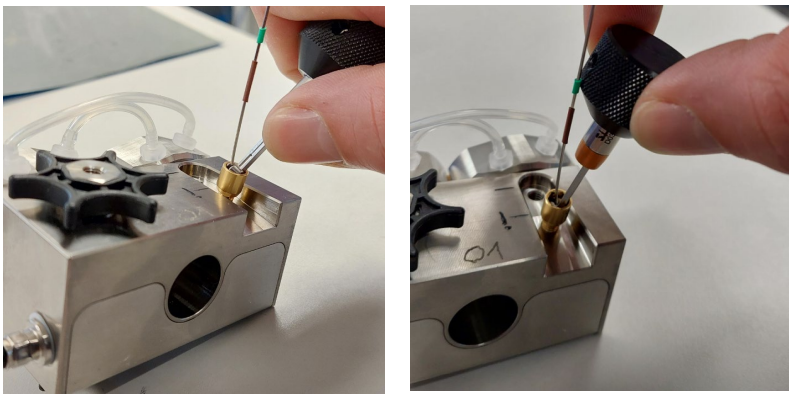
6.1.1 Using the Torque screwdriver

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 the 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*** * 6PSC9STB1T 1000/PACK, 6PSC9STB1F 5000/PACK	
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 – ***Can be used with e.g fractionation***	
60180-P210B	96 LOW-VOLUME WELL PLATE, 5.6MM TOTAL V-BASE, 100UL, PP BARCODED – ***Go to option for low volume applications***	
60180-M146 / 60180-M176	PLATE SEAL TAPE, PART ADHESIVE PET/SIL/PET, 100/PK As above, but 25/PK ***Recommended for use with both P/N 60180-P201B and P/N 60180-P210B***	
60180-P340	SureSTART™ WebSeal™ Plate+ 384-Well Microtiter Plates, Square-Rounded V-Bottom, Level 3 High Performance Applications. 145UL, 10/PK	
60180-M150	SureSTART™ WebSeal™ 384-Well Plate Sealing Mat, Square, Flat Base, Level 1 Everyday Analysis, Pre-slit, 5/PK	

Table 15: Recommended Sample handling products for Vanquish Neo

NOTICE We recommend these specific products because they have proven to only shed very low amounts of particles when they are pierced. The general issue with septa and covers is that many products on the market make use of “filler materials”. These fillers are typically silica gel (used to increase septa rigidity and covering a size range of 1-25µm) and/ or titanium dioxide (white pigment). In many cases, the situation with the septa is exacerbated by the fact that some manufacturers use “talcum powder” / a non-stick agent during manufacturing to prevent septa from sticking to one another. These particles may shed during the piercing process, landing in the sample liquid from where they can be aspirated by the autosampler needle and injected onto the needle seat filter frit causing it to block prematurely.

NOTICE The Vanquish Neo Autosampler operates according to the “split-loop” principle. Here the needle forms part of the flow path. This reduces third party surface exposure of precious samples during the transfer from sample receptacle to the column. It also greatly shortens the transition path. Since the system operates using 20µm ID capillaries in nano/cap flow configuration – Note the low dispersion needle seat filter frit is necessary to avoid premature capillary blockages. Nevertheless, using the recommended consumables listed in Table 15 will help prolong the needle seat lifetime and give the best possible customer experience.

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	D01	Test System Back Pressure
4	D02	Test System Tightness
Optional		
5	C01	Adjust Pump Flow Sensor Offsets
6	C05	Adjust System Pressure Sensors
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):

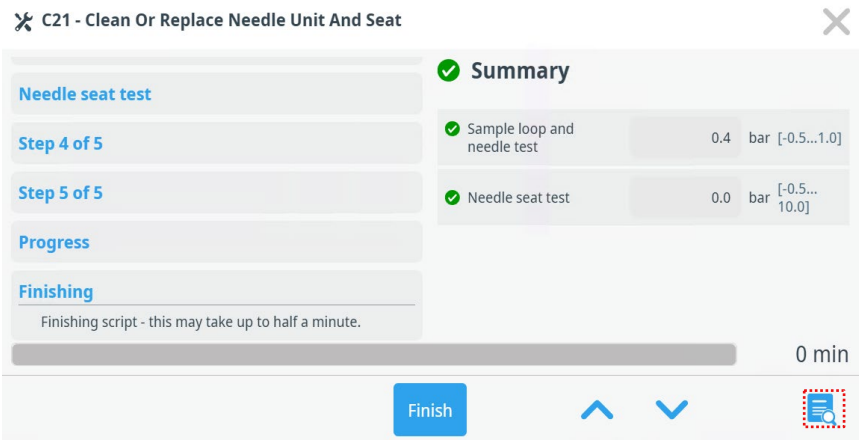


Figure 44: Example summary results for script C21

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 behavior 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

If the needle seat is irretrievably blocked (i.e. the C21 script with the option “Clean Needle Seat” selected cannot free the blockage) please execute script C21 with the option Replace Only Seat selected (Figure 45).

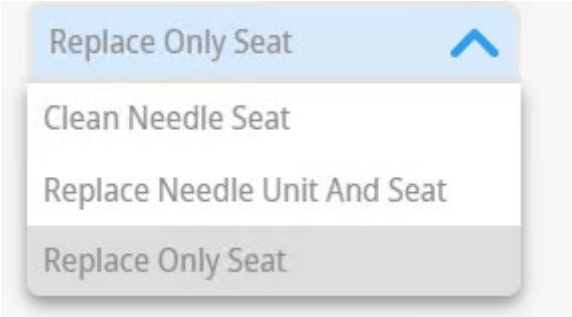


Figure 45: Script C21 Drop Down Menu with the option “Replace Only Seat” selected)

- Follow the instructions provided in the script

Please adhere to the following instructions whilst carrying out the procedure outlined in the script.

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

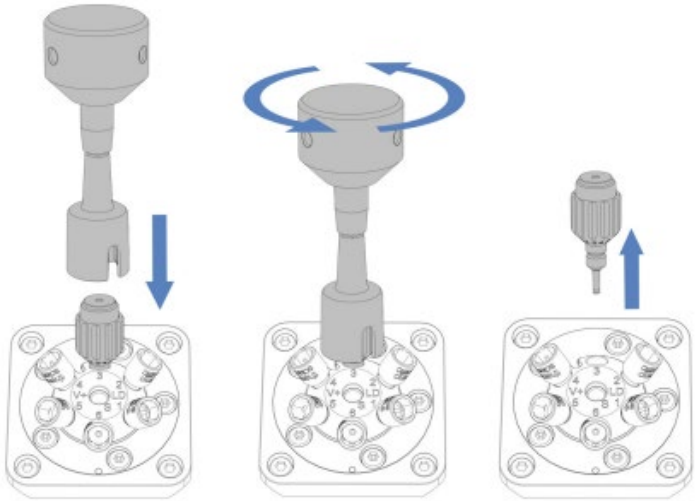


Figure 46: Removing the needle seat with the torque screwdriver

- After inserting the new needle seat, please tighten using the torque screwdriver with the needle seat bit attached. Turn it clockwise to 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).

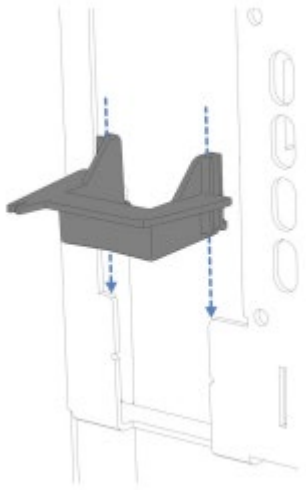


Figure 47: Installing the insulation cover mounting bracket

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.

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 for shutting down the system (script B05).

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

If 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 from the dropdown menu on the right-hand side of the dialogue box (Figure 48).

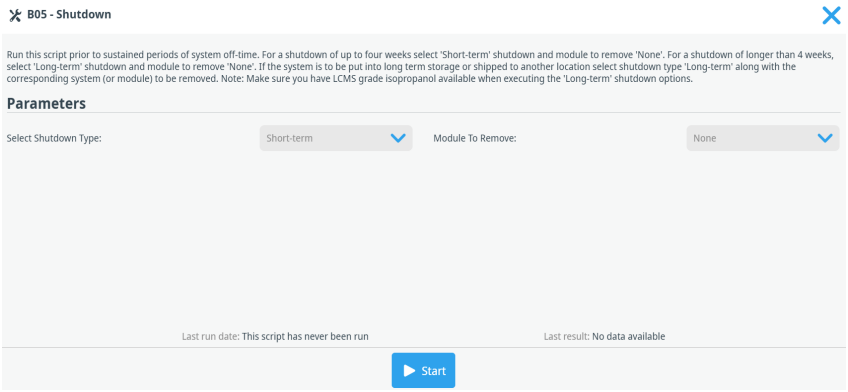


Figure 48: Script B05 – Shutdown dialogue box

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.

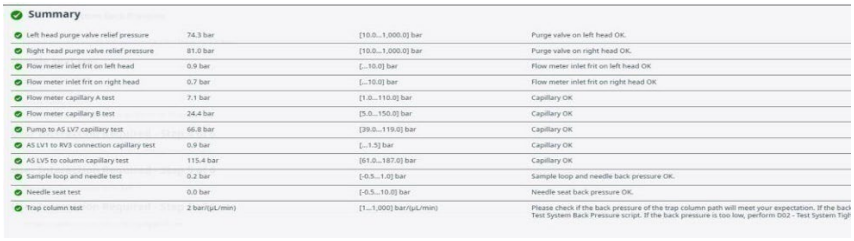


Figure 49: Typical Back Pressure Test Summary Data for the trap-and-elute workflow in nano/cap fluidic configuration.

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).

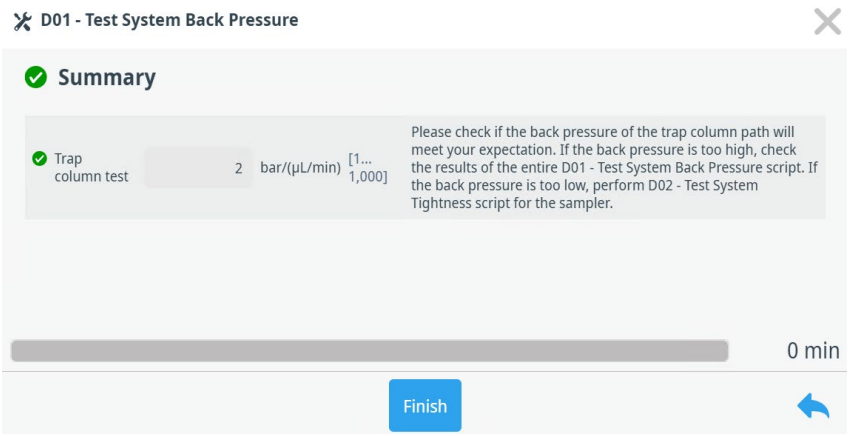


Figure 50: Diagnostic script D01 test result with the option for test only trap column back pressure set to “On”

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).

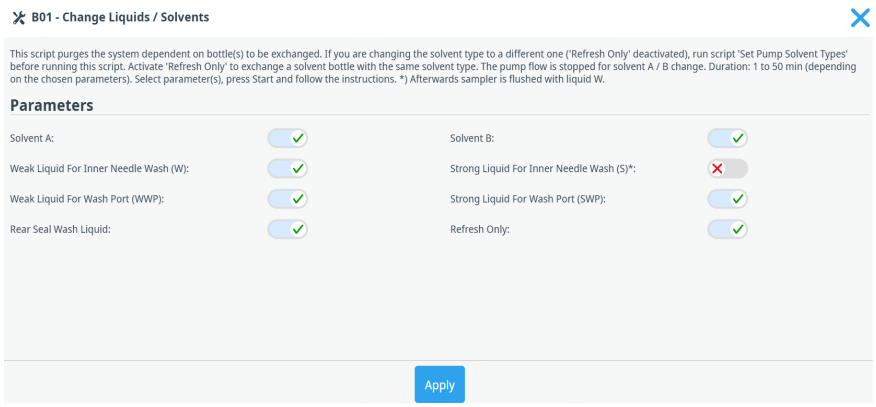


Figure 51: Recommended B01 script settings for refreshing solvents.

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 can run 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).

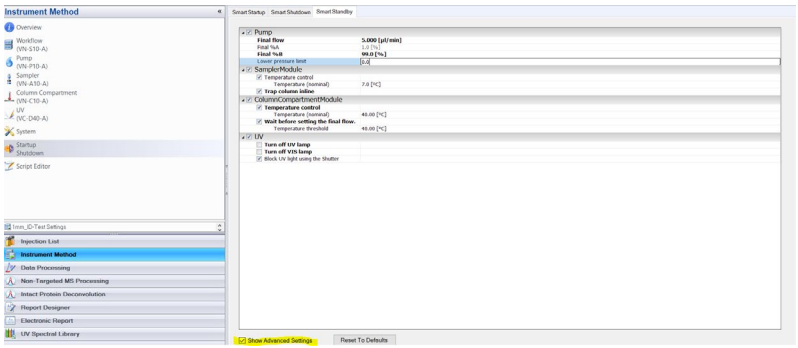


Figure 52: The Start Up, Shutdown window of the Instrument Method Editor

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).

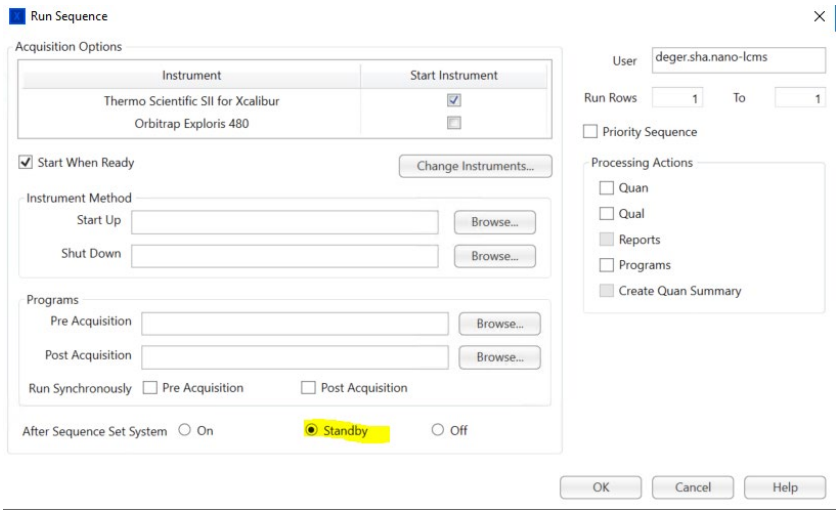


Figure 53: Xcalibur Run Sequence Settings Table for Sequence Submission with After Sequence Set System to "Standy" activated.

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 or OptaMax Plus MS ion sources.
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.

6.1.10 Deformation of the NanoViper Fitting in the Metering Head

Frequent switching between Direct Injection and Trap-and-Elute workflows, can lead to deformation and closure (blockage) of the NanoViper tip. This is due to the difference in bore size between the Metering Device and the Valve port. Similarly, if the capillary connecting the metering device to the valve port (P/N 6252.1924) in the direct injection workflow configuration is not always installed in the same orientation, the same issue can occur. To address this, it is recommended to install the Viper guard connector (P/N 6261.1130) to the front port of the metering device (Figure 54).

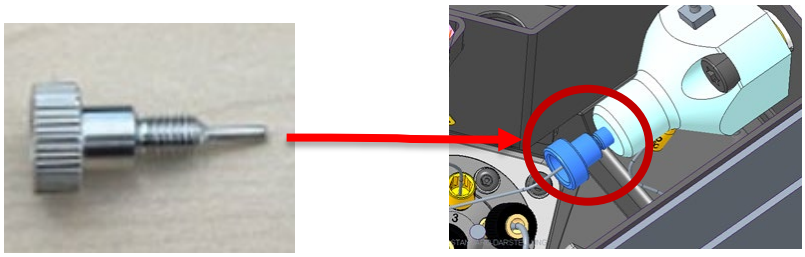


Figure 54: Connecting the Viper Guard Connector (P/N 6261.1130) to the metering device head outlet

Once installed, the Viper Guard Connector should always remain in place, irrespective of the workflow being performed.

To further prolong the lifetime of the connecting capillary required in the direct injection configuration ensure that it is always installed in the same orientation (see section 6.2.8 for details)

6.1.11 Considerations for Method Transfer from legacy instruments (EASY-nLC™ 1200 and the UlitMate3000™ RSLCnano) to the Vanquish™ Neo

This section details tips and tricks to consider when transferring methods from the Thermo Scientific legacy low-flow instruments to the Vanquish Neo System. To aid user understanding in what variables require particular attention when transferring methods, a basic understanding of the principal differences in operation between the systems is required.

Below are listed the key design and operational differences between the Vanquish Neo and the legacy instruments which play a role in method transfer (Table 19).

Attribute	UltiMate3000 RSLCnano	EASY-nLC 1200	Vanquish Neo
Programming variables for Sample Loading and Column Equilibration	Time, Flowrate	Pressure, Flowrate, Volume	Pressure, Flowrate, Volume
Autosampler Type	Pulled-Loop	Pulled-Loop	Split-Loop
Sample Aspiration directly into injection flow path	No (Microliter pickup using transfer liquid)	No (Partial loop using Air gaps for sample transfer)	Yes
Sample Loading recorded in data acquisition file	Yes	No	No
Switching Valve Type	6 or 10 port, 2 position	6 port, 2 position	Up-to 7 port 7 position
Default Loop Position during Gradient	Online	Offline	Offline*
Column Equilibration included in Gradient Table	Yes	No	No
Column Equilibration recorded in data acquisition file	Yes	No	No

*Table 18: Key operational and functional differences between the Vanquish Neo and the legacy low-flow instruments. *An option to keep the loop online is available for the microflow fluidic configuration.*

6.1.11.1 Sample Loading

UltiMate3000 RSLCnano -> Vanquish Neo

Sample preconcentration is the most used workflow on the RSLCnano system. Here, loading and column equilibration are defined according to the time at which the column compartment valve is switched to put the trap column inline or offline with respect to the analytical column. The time from the start of data acquisition, together with the flow rate employed by the loading pump, determine the loading volume. This volume can be used as a starting point for the **Loading Volume** for the Vanquish Neo based method (see section 5.1).

EASY-nLC 1200 -> Vanquish Neo

Whilst the EASY-nLC series instruments require loading and equilibration volumes to be input in the method editor, it is important to note that the loading volumes are defined differently for the EASY-nLC 1200 and the Vanquish Neo systems respectively. For the EASY-nLC, the loading volume comprises the total volume used to transfer the sample from the loop to the column prior to acquisition start.

For the Vanquish Neo system, the total volume used to transfer the sample to the column is a sum of the **injection volume plus the loading volume** (see section 6.2.7 for details.). As such, the sample pickup volume should be subtracted from the loading volume as specified in the EASY-nLC method editor to determine the sample loading volume for Vanquish Neo based methods.

TIP The Vanquish Neo system has a “default” sample loading volume labelled “automatic”. This equals **5µL** and is independent of the sample volume being injected. There may be situations in which larger loading volumes are required e.g for online detergent removal, as evidenced by broad elution peaks in the sample chromatogram. In such cases, the loading volume may need to be dramatically increased (to match the displacement volumes adopted for the UltiMate3000 RSLCnano, for example) to ensure complete detergent removal.

TIP If trap column breakthrough is observed for applications requiring large loading volumes (see above) consider switching the weak metering device wash liquid to H_2O + 0.1% TFA.

6.1.11.2 Column Equilibration

In contrast to both the RSLCnano and EASY-nLC systems, the Vanquish Neo instrument method editor adopts equilibration “factors” which dictate the column equilibration volume. For the analytical column, the total volume used for equilibration is the column void volume multiplied by the factor. The default value (factor of 3) is sufficient to enable reproducible chromatography with stable retention times for most column types and applications.

For the trap columns, the equilibration volume for Vanquish Neo based methods is based on the wash volume multiplied by the factor input by the user in the method (default factor = 2). In both cases, the total volume used for the equilibration is reported in the instrument method editor (Figure 55). As such the factors can be adjusted such that the equilibration volumes employed match those from the transferred method.

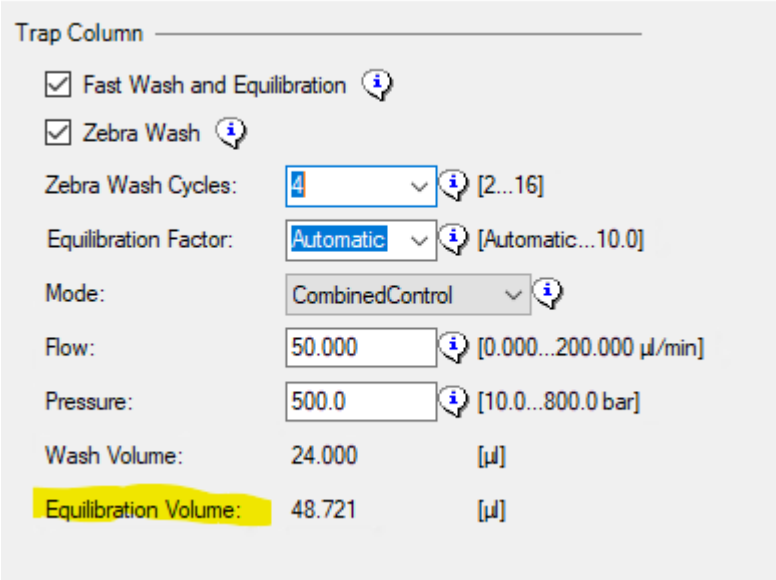


Figure 55: Example Trap Column Wash and Equilibration settings. In this example 4 zebra wash cycles and equilibration factor “automatic” are selected yielding an equilibration volume of ≈ 48µL

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 56: Error message caused by incomplete execution of script A06 – change fluidics / workflow. In this case, the user is requested to either rerun the workflow change initialize their system using script E01.

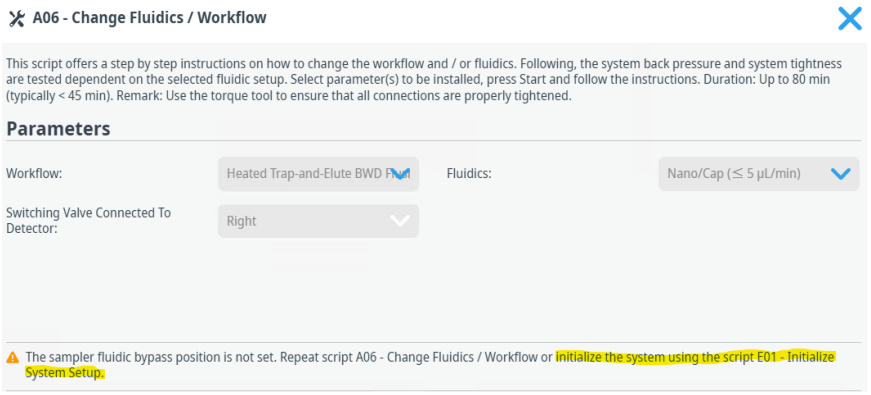


Figure 56: Error message caused by incomplete execution of script A06 – change fluidics / workflow

It should be noted that a characteristic of this failure mode is the reduced number of scripts available despite no filter being added to the selection (Figure 57).

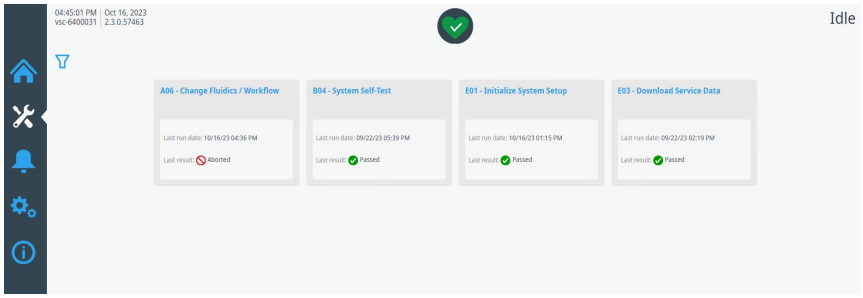


Figure 57: The four remaining scripts available when the Vanquish Neo system is no longer able to determine its fluidic workflow configuration

To return the system to an operational state, either re-run script A06 or execute script “E01 -Initialize System Setup”. If script E01 is run, a

dialogue box opens, asking the user to select the configuration currently installed on the system (Figure 58).

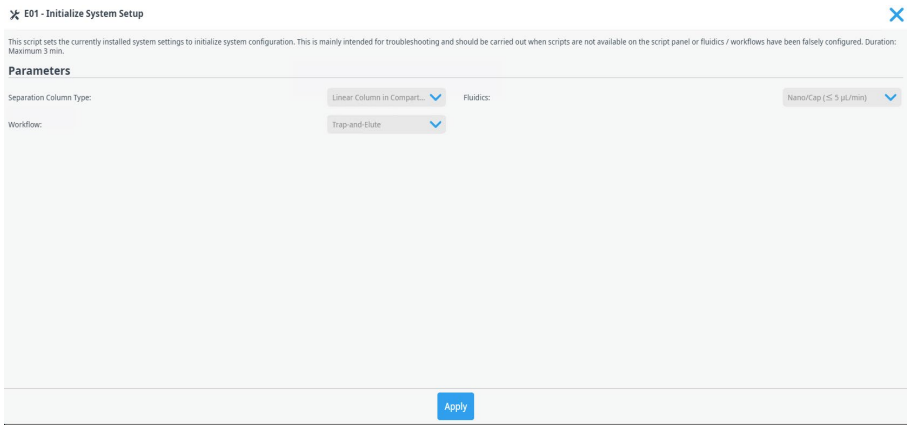


Figure 58: Dialogue box displaying fluidic and workflow configuration options which need to be defined by the user when executing the E01 script

The user is requested to ensure that the workflow set is identical with the one currently installed on the system (Figure 59).

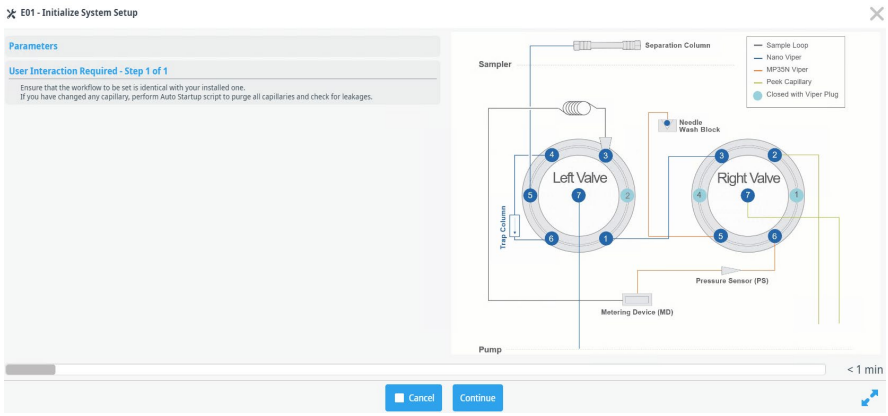


Figure 59: Dialogue box in the E01 script showing the schematic of the selected workflow configuration.

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, and that the system is purged and leak tight**. 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 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 **Error! Reference source not found.**)

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 60)

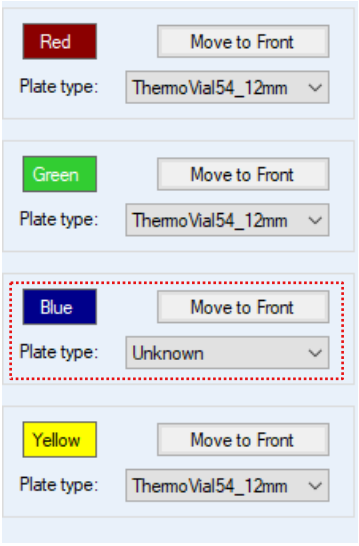


Figure 60: Autosampler plate type for the blue segment registered as “Unknown” due to the missing bar code on the plate

In this case, the plate type must be set manually by selecting the correct option from the drop-down menu (see Figure 61).

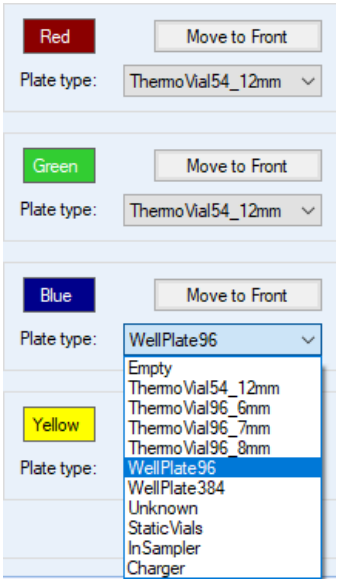


Figure 61: Selecting the correct well plate type from the dropdown menu

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.

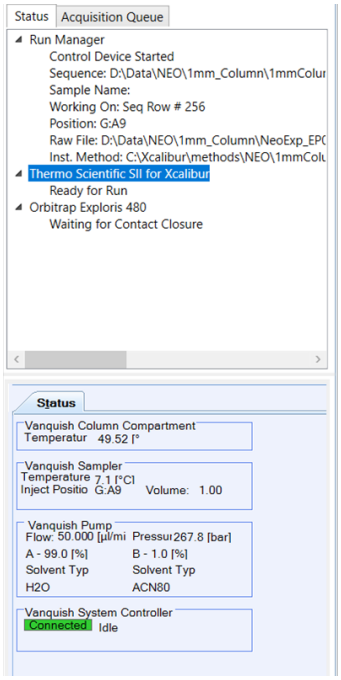


Figure 62: The Xcalibur status and mini ePanel display shown after an attempt has been made to inject from an empty vial position.

The VUI notification panel reports an aborted command.

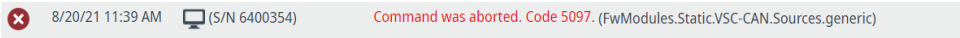


Figure 63: VUI notification panel signally an aborted command

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 64).

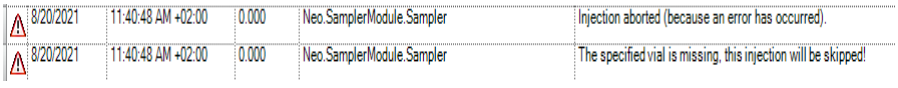


Figure 64: ePanel audit trail showing the root cause of the aborted injection

To recover from this error:

- Right click “Thermo Scientific SII for Xcalibur” in the status window (Figure 62) 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 65).

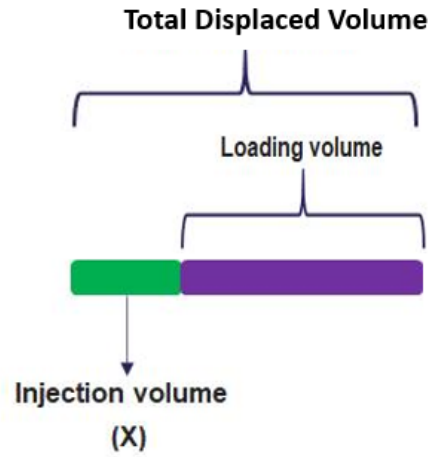


Figure 65: Schematic representation of the relationship between loading volume, injection volume and the total volume displaced during the sample loading procedure

Example of the dependencies between injection and loading volumes and their effect on the total volume displaced are given in Table 19 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 19: 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 most bottom-up proteomics applications employing reversed phase chromatography

6.2.8 Maximizing the lifetime 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 66). To prolong the lifetime of this capillary it is recommended to always install the capillary with the same orientation. Please also consider installing a Viper Guard Connector on the front outlet of the Metering Device (see section 6.1.10 for details).

Connect the end with the sharp bend to the valve

Connect the end with the shallow bend to the Viper Guard Connector

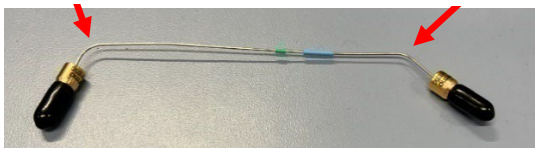


Figure 66: 0.25mm ID x 170mm MP35N Viper Capillary (P/N 6252.1924, available both as an individual item or part of capillary kit with P/N 6252.1920) connecting the Metering Device to the injection valve port 6

6.2.9 Avoiding sample loss during repeat injections

Care must be taken to select an appropriate sample vial if sample re-injection from the same vial is required (e.g for the (re-) injection of QC standards during a sequence run). The narrower the sample container, the greater the sample displacement during the needle insertion into the vial and subsequently the greater the risk of vial surface adsorption, particularly for low concentration samples (Figure 67). A list of sample receptacle for given applications is given in section 6.1.2.

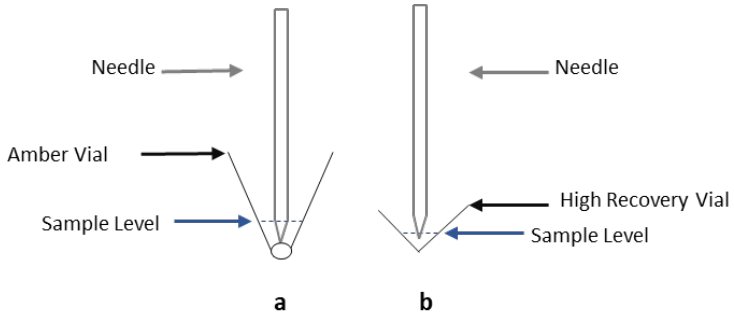


Figure 67: How vial geometry affects sample displacement during sample pickup. a) Greater sample liquid displacement caused by narrow insert vials e.g. Amber vial (P/N 60180-1655) compared to the broad based high recovery vial b) (P/N 6PSV9-V1)

TIP If possible, avoid more than 3-5 repeat injections from the same vial using one vial cap. Vial septa can only last for a limited number of injections as the integrity of the closure is increasingly compromised with each repeat injection causing more debris to be deposited inside the vial.

6.2.10 Relocating the valve from the left to the right hand side of the Column Compartment for heated trap-and-elute workflows

With the introduction of the Vanquish Neo Software bundle 1.5, the Vanquish Neo System now supports heated trap-and-elute workflows for valve operation from both the left and right hand side of the column compartment.

If you wish to relocate the valve from the left to the right hand side of the oven, please use the following procedure to perform the valve migration.

- Note the workflow details (i.e. heated trap forward vs backflush).
- Detach all the capillaries from the column compartment valve
- Turn off the power to the column compartment.
- Remove the re-install the valve according to the instructions given in the Vanquish Neo System Operating Manual (ensure the valve is installed such that port 3 Is located at the top of the valve “12 o’clock”
- Turn on the power to the column compartment
- Once the Column Compartment becomes visible on the VSC, select the required heated workflow type (backward or forward flush) using script E01.
- Re-attach all the valve capillaries and columns in compliance with the image shown in script E01.
- Run script D02 to verify system tightness

NOTICE failure to switch off the column compartment power switch prior to removal and re-installation of the valve may result in permanent valve damage.

TIP The E01 script shows the following warning message after the valve has been migrated “The workflow requires the “right or left – position of the installed valve” column compartment valve. Ensure that the column compartment valve is installed and all capillaries are connected, or initialize the system using the script E01 – initialize system setup.”

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 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 use. This script adopts the maximum pressure and flow rates according to the set column specifications (scripts A04 and A05).

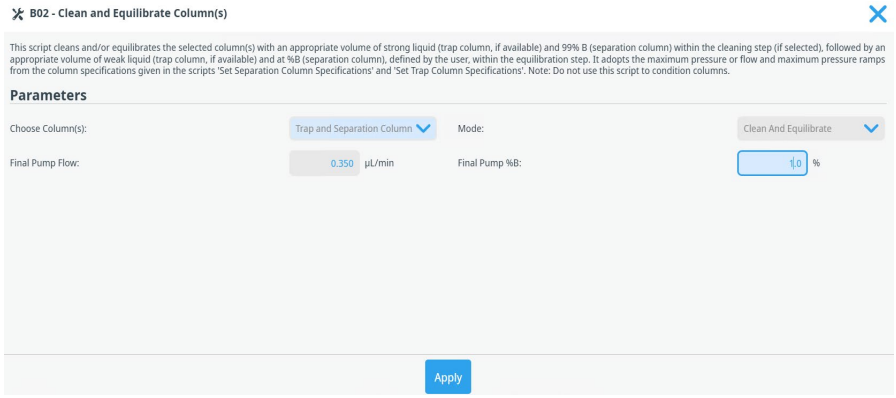


Figure 68: The B02 script options page with trap and separation column selected

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 Vanquish Neo Tandem Direct Injection Workflow

This chapter explains how to set up and operate the Vanquish Neo Tandem Direct Injection Workflow

7.1 Preparing the system for the Vanquish Neo Tandem Direct Injection Workflow

7.1.1 Introduction to the Workflow

The Vanquish Neo Tandem Direct Injection Workflow comprises a low-flow (nano, capillary and microflow) UHPLC system (Figure 69) containing two analytical pumps, an autosampler and a column compartment which houses two low-dispersion switching valves. The workflow permits the use of two identical separation columns. The workflow uses intelligent workflow tools to permit almost continuous detector utilization as the sample pick-up, loading, and washing steps on column one are parallelized with the execution of the separation on column two.

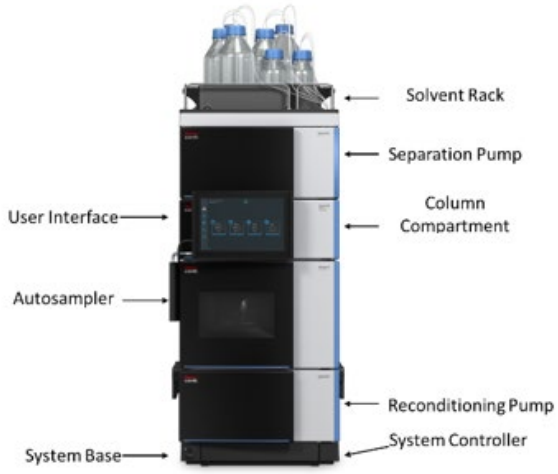


Figure 69: Vanquish Neo Tandem Direct Injection Workflow Configuration

NOTICE Both low-dispersion 1500 bar switching valves (P/N 6250.1520) must be installed in the Vanquish Neo column compartment (left and right hand side) for the Tandem Direct Injection Workflow to be activated

7.1.2 Software requirements

The workflow is supported by Xcalibur (SII) or full Chromeleon installations as follows.

7.1.2.1 Supported Data Systems

The workflow requires the following versions of the Thermo Scientific Chromeleon Chromatography Data System (CDS):

- Chromeleon 7.2.10 MUe or higher
- Chromeleon 7.3.1 or higher
- Chromeleon 7.3.2 MUa or higher

The following versions of Thermo Scientific Standard Instrument Integration (SII) for Xcalibur:

- SII for Xcalibur version 1.7 or higher with Xcalibur 4.7 or higher

7.1.2.2 Vanquish Neo Driver

The support of the Vanquish Neo Tandem Direct Injection Workflow requires Vanquish Neo driver package version 1.5 or higher. Please refer to the release notes that accompany the driver package release for details on how to install the software.

NOTICE For LC-MS installations under Xcalibur control. Please ensure that all the appropriate (and mutually compatible) peripheral software packages (Foundation, Xcalibur, Tune, SII (+ Hotfix, if using version 1.7), and then the Neo System Driver) are all correctly installed before installing the Neo Driver bundle.

7.1.3 Associated Workflow Kits

There are two kits associated with the tandem workflow, the tandem workflow kit (see section 7.1.3.1) and the tandem source kit (see section 7.1.3.2). Whilst the tandem workflow kit is mandatory for all tandem workflow installations, the tandem source kit is only required for tandem workflow operation at flow rates ≤ 1µL min. This kit is used in conjunction with the dual spray source option(see section 7.1.4.3 for details).

7.1.3.1 Tandem Workflow Kit

The Tandem Workflow Kit (P/N 6250.1030) contains the following items:

	P/N	Item Name
1	6250.5121	NanoViper Capillary IDxL 20µmx150mm (3 Pieces)
2	6250.5250	NanoViper Capillary IDxL 20µmx450mm (2 Pieces)
3	6250.5260	NanoViper Capillary IDxL 20µmx550mm (1 Piece)
4	6250.1009	Low Dispersion Y-piece 50µm w. insert (2 Pieces)
5	6040.2303	Viper Plug (2 Pieces)
6	6822.0012	Ferrule and fitting kit SR/FS-7 (2 Packages)
7	6720.0078	PEEK sleeves for fused silica tubing 360µm (2 Pieces)
8	6250.5550	NanoViper Capillary IDxL 50µmx450mm (2 Pieces)
9	6250.5124	NanoViper Capillary IDxL 50µmx150mm (5 Pieces)
10	756.161089	Cytochrome C digest test sample (1 Piece)
11	4822.5012	Flow Scheme Vanquish Neo Tandem (1 Piece)
12	5036.2005	Set Color Sticker Vanquish (1 Piece)
13	164711	Acclaim™ PepMap™ 100 C18 1 mm x 15 cm column (2 µm dp) (2 Pieces)

Table 20: Tandem Workflow Kit components

Details of the fluidic configuration options for the workflow are shown in the accompanying Flow Scheme (P/N 4822.5012) also included in the kit. Complete instructions on how to install the fluidics are given in **script A00** on the VUI.

TIP This kit does not contain every item required to setup the tandem direct injection workflow hardware as some components are included in other kits shipped with other system components ((e.g. Ship Kit VN-P10 (P/N 6251.9110) and Ship Kit VN-S10 (P/N 6250.9110).

7.1.3.2 Tandem Source Kit

The Tandem Source Kit (P/N B51004433) is only required for tandem workflows running at ≤ 1µL/min and should be used in conjunction with the dual spray source option (see section **Error! Reference source not found.**). The kit comprises the following components:

P/N	Item Name
TIP3600301 0-5	Pulled ESI Emitter (360 um OD × 30 um ID × 5 cm L) (1 Package) (CoAnn Technologies)
SC603	Sleeve for Emitter (Beige 1/32 OD to 360um, 2 cm L) (1 Package)
SC903	Sleeve for Column (Black 1/32 OD to 280um, 2 cm L) (1 Package)
13040420	P-771 Microtight Union (2 Pieces) (IDEX Health and Science)
164940	Acclaim PepMap™ 100 2 µm C18 75 µm X 15 cm (2 Pieces)

Table 21: Tandem Source Kit contents

TIP Some of the required items are already included in other kit ((e.g. Ship Kit VN-P10 (P/N 6251.9110) and Ship Kit VN-S10 (P/N 6250.9110), for that reason they are not included in Tandem Source Kit.

NOTICE The tandem source kit should be used in conjunction with the Dual Spray Source (see section **Error! Reference source not found.**).

7.1.4 Hardware Configuration Options

The Vanquish Neo Tandem Direct Injection Workflow supports **two fluidic configurations**: Nano/ Capillary-flow employs 20µm ID capillaries and is best suited to columns of up to 150µm ID, running at flow rates up to 5µL/min. The micro-flow configuration adopts 50µm ID capillaries and is ideally suited to 300µm - 1 mm ID columns operating at flow rates from 5 – 100 µL/min.

There are two ion source configurations compatible with the Tandem Direct Injection Workflow. **A Dual Spray and a Single Spray option**. The Dual Spray Source is recommended for flow rates ≤ 1µL/min and is therefore best suited to the nano/capillary fluidic configuration. The Single Spray Source options require a post column low dispersion switching valve at the junction between the two column outlets and the capillary inlet to the source. As the post column valve can cause extra-column dispersion at very low-flow (nano)rates, the single spray option is best suited to flow rates ≥ 1µL/min. The dual spray source configuration should be used exclusively with the Nanospray flex ion source complete with Sonation double barrel oven. For the single spray option, a choice of MS ion sources is available, depending on user preference (e.g. nano spray flex ion vs Easy-Spray for flow-rates up to 5µL/min) and/ or application flow rate range (e.g. OptaMax ion source

complete with low-flow metal needle for flow rates in the range 5 – 100µL/min). An overview of the different configuration options is given in Table 22 below together with a list of supported column consumables (Table 23).

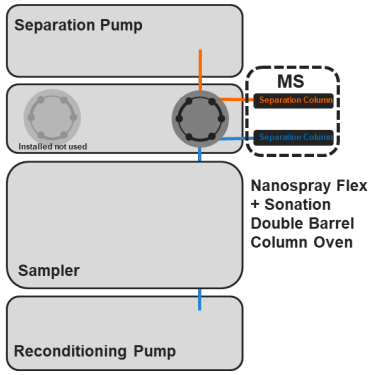
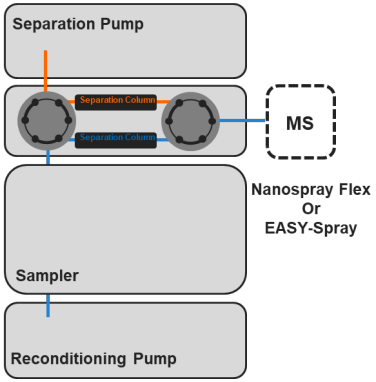
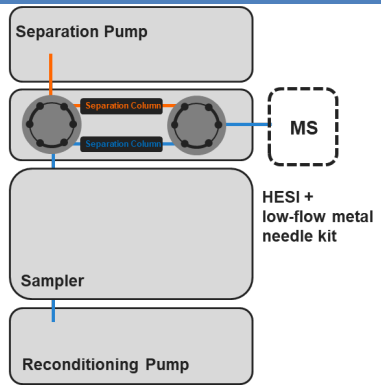
	Nano/Capillary-flow (< 5 µL/min)	Capillary-flow (1 - 5 µL/min)		Micro-flow (5 - 100 µL/min)
Tandem Workflow Configurations				
Ion sources	Nanospray Flex™ (ES072)	Nanospray Flex™ (ES072)	EASY-Spray™ (ES082)	OptaMax NG or OptaMax Plus (part of MS ship kit)
Required Add-ons (IQ & applications)	• **1x *NEW* Double Barrel Oven** with Mounting Kit NG *(P/N B51003991)* • **1x *NEW* Tandem Source Kit** *(P/N B51004433)* includes emitters and nano IQ columns (for details see Table 20 below, 100nL - 5µL/min)	• **Stainless steel emitters**, 30µm ID, 1-4µL/min *(P/N ES542)* • **Microtight adapter,** 1/16" to 1/32" *(P/N 00109-02-00055 or IDEX P/N P-881)*	• **Capillary EASY-Spray Emitter**, Bullet Type without transfer line, 15 µm i.d., 1-2 µL/min *(P/N ES994)*	• **Low-flow metal needle kit** incl. NanoViper 50umX150mm, 5-100 µL/min (P/N OPTON-30697)
Installation Qualification (IQ) Notes	IQ columns to cover the nano-flow setup are included in the Tandem Source Kit (see above) and do not need to be ordered separately. Note: The above add-ons are required for IQ and applications.	No specific IQ in cap-flow available, IQ will be performed in micro-flow. IQ columns for micro-flow are included in the Tandem Workflow Kit (See Table 20) and do not need to be ordered separately. Note: To conduct applications, the above required add-ons should be ordered separately.		IQ columns to cover micro--flow setups are included in the Tandem Workflow Kit (Table 20) and do not need to be ordered separately. Note: The above add-ons are required for IQ and applications.

Table 22: Vanquish Neo Tandem Direct Injection Workflow Source and Fluidic Configuration Options – For Nano/Capillary Flow fluidic configurations use 20µm ID capillaries. For Micro-flow fluidic configurations use 50µm ID capillaries

Supported consumables and column examples			
	Nano/Capillary-flow (< 5 µL/min)	**Capillary-flow (1 - 5 µL/min)**	**Micro-flow (5 - 100 µL/min)**
Column formats	Single nanoViper™or pulled-tip	Double nanoViper™, µPAC™	Double nanoViper™, SST
Dimensions	50, 75, 150 µm ID	150 µm ID, µPAC HT	300 µm, 1.0 mm ID
Column examples	**PepMap™ RSLC C18**, 2µm, **50 µm x 150 mm**, 1200 bar (P/N 164943) **PepMap RSLC C18**, 2µm, **75 µm x 150 mm**, 1200 bar (P/N 164940) **PepMap RSLC C18**, 2µm, **75 µm x 500 mm**, 1200 bar (P/N 164942) ***NEW*** **PepMap Neo C18**, 2µm, **75 µm x 750 mm,** 1500 bar (P/N SNV75750PN) ***NEW*** **PepMap Neo C18**, 2µm, **150 µm x 150 mm**, 1500 bar (P/N SNV150150PN)	**MabPac™ RP** 4µm **150µm x 150mm**, 600 bar, DNV (P/N 164947) **PepMap Neo C18**, 2µm, **75µm x 150 mm**, 1500 bar, DNV (P/N DNV75150PN) ***NEW*** **PepMap Neo C18**, 2µm, **150µm x 150 mm**, 1500 bar, DNV (P/N DNV150150PN) **High Throughput µPAC™ Neo** HPLC Column, **55 mm**, 450 bar, DNV (P/N COL-CAPHTNEOB)	**PepMap RSLC C18**, 2µm, **0.3 mm x 50 mm**, 800 bar (P/N 164560) **PepMap RSLC C18**, 2µm, **0.3 mm x 150 mm**, 800 bar (P/N 164537) **PepMap RSLC C18**, 2µm, **1.0 mm x 50 mm**, 800 bar (P/N 164454) **PepMap RSLC C18**, 2µm, **1.0 mm x 150 mm**, 1200 bar (P/N164711)

Table 23: Example Selection of Columns Supported by the Vanquish Neo Tandem Direct Injection Workflow

7.1.4.1 Single Spray Source Configuration

In this configuration, the two separation columns are situated between the two low-dispersion valves in the column compartment with a single outlet to the detector (see Figure 70). The single source configuration is supported for nano/cap (using 20µm I.D. capillaries) and micro-flow (using 50µm I.D capillaries) fluidic regimes.

Mass spectrometer acquisition is triggered by using contact closure cable (P/N 6000.1004) attached to the Autosampler Relay 2 (See Figure 70).

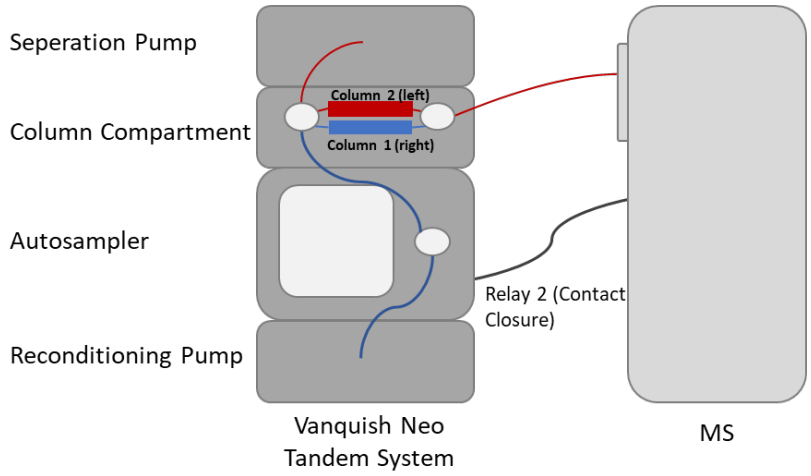


Figure 70: Schematic depicting the single spray source options. In this example "switching valve connected to detector -> right" has been selected

Details on how to install the tandem workflow are given in 0

TIP The single spray configuration depicted in

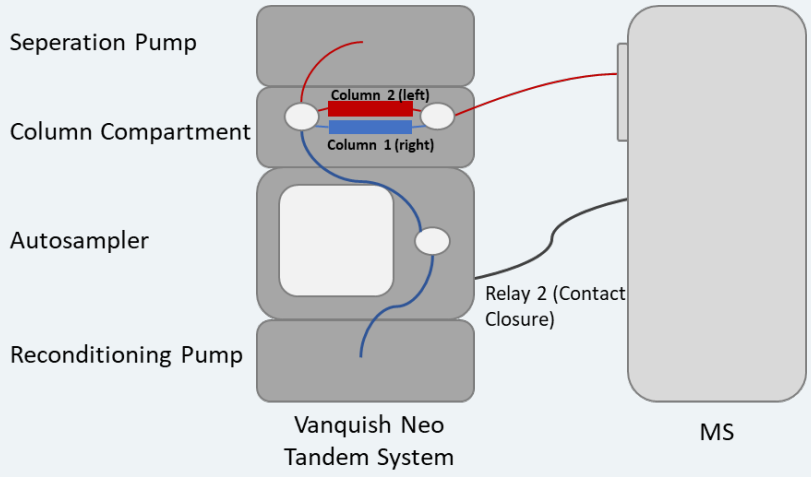


Figure 70 shows the detector (MS) located on the right hand side of the LC stack. Here the option “Switching Valve Connected to Detector -> Right” was selected in the A00 script during workflow setup. If the detector (MS) is located on the left hand side of the LC stack, then selecting the option “Switching Valve Connected to Detector -> Left” guides the user to install all the fluidics in a mirror image format compared to that shown above.

7.1.4.2 Source Options for the Single Spray Source Configuration

The following MS ion sources are supported for the capillary flow rate range (1-5µL/min):

- Nanospray Flex™ Ion Source with metal ion emitters (P/N ES542)
- EASY-Spray™ Ion source complete with bullet emitter (ES994)

The following MS ion sources are supported for the micro-flow range (5-100µL/min)

- OPTAMAX NG™ or OPTAMAX PLUS™ complete with the low-flow metal needle kit (OPTON-30697)

A complete list of options is given in Table 22

7.1.4.3 Dual Spray Source Configuration

In this configuration, the analytical columns are installed in the Sonation double barrel oven (Sonation GmbH, Biberach, Germany) located on the ion source rather than in the column compartment. Here the column outlets interface directly with the ionization emitter. This can be via a union at the column outlet or with the column complete with an integrated emitter (packed emitter column).

Only one of the valves located in the Vanquish Neo column compartment is in use for the workflow. Whether the left or right compartment valve depends on whether the LC stack is situated on the left or right hand side of the detector (mass spectrometer).

NOTICE. Although only one column compartment valve is in operation for this workflow, BOTH must be installed for the Vanquish Neo Tandem Direct Injection Workflow to be selectable on the VUI.

Mass spectrometer acquisition is triggered using a contact closure cable (P/N 6000.1004) attached to the Autosampler Relay 2. The voltage switch between the two columns on the Dual Spray Source is triggered by using a data cable HPLC-controller (a part of the Double Barrel Oven complete kit, P/N SON 004.801.02) via connection to Column Compartment Relay 1 (See Figure 71). The details of the fluidic configuration are shown in the Flow Scheme included with the Vanquish Neo Tandem ship kit (P/N 4822.5012).

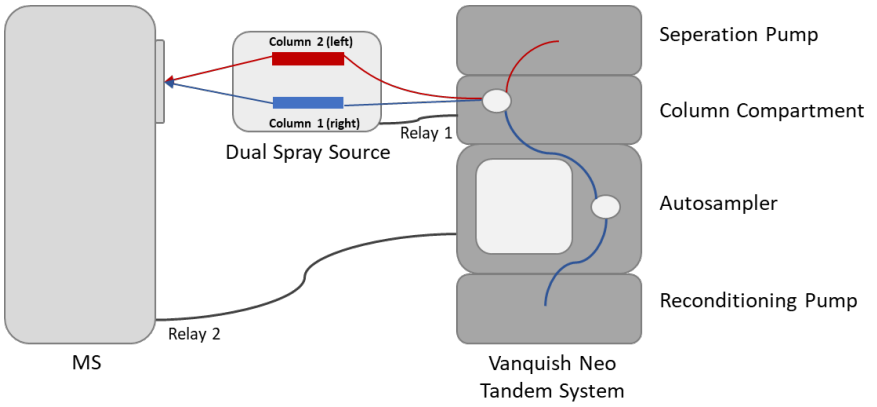


Figure 71:Schematic depicting the dual spray source option. In this example the option "switching valve connected to detector -> left" has been selected.

Details on the configuration part numbers. Including unions and emitters are given in Table 22.

NOTICE. The Nanospray Flex™ ion source complete with the Double-Barrel Column Oven (Sonation GmbH, Biberach, Germany) is required to run this workflow configuration.

TIP. Whilst the high pressure liquid junction fittings on the Double Barrel Oven have been designed for seamless operate with Viper fittings, they are also fully compatible with self-packed emitter fused silica columns, if these columns have been fitted with the **ferrule fitting kit P/N 6822.0012** included in the tandem workflow kit (see Table 20)

7.1.5 Installing the Tandem Direct Injection Workflow

7.1.5.1 Installing the instrument hardware

1) Place the Neo System (VN-S10-A-01) on a suitable bench. Please consider the total weight (approx. 103 kg) and height of the system stack when choosing the installation location (see Figure 72).

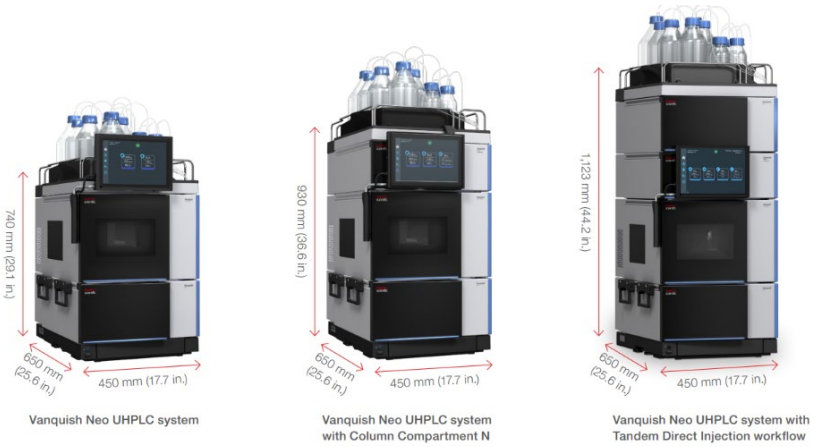


Figure 72: Comparative heights of the respective Vanquish Neo system configurations

2) Install the Column Compartment on top of the Autosampler as instructed according to the Vanquish Neo System Operating Manual

3) Place the second Binary Pump N (VN-P10-A-01) on top of the column compartment and attach the solvent rack to the top. (Figure 72).

> **NOTICE** Do not install the Viper Guard Connector in the Outlet of the Flow Meter on the Upper Pump for Vanquish Neo Tandem Workflow Operation. It is required when the Binary Pump N is in operation with Vanquish analytical flow modules, or third party peripherals or in “stand-alone” configuration. Optionally, it can instead be installed in the head of the metering device (see section 6.1.10)

4) Connect the USB and system interlink cables to all the modules. (see section 5.8 in the Vanquish Neo System Operating Manual.

5) Connect the solvent lines to the upper (separation) pump (see section 5.6.5 of the Neo Binary Pump Operating Manual)

6) Install both valves in the Column Compartment (see Vanquish Neo System Operating Manual section 5.5.2)

NOTICE Ensure that the column compartment is powered OFF before installing the valves. Failure to do so may result in permanent valve damage.

TIP Both valves must be installed to operate the Vanquish Neo Tandem Direct Injection Workflow.

7) Prepare the solvents (see sections 4.1.1 and 4.1.2)

8) Power up the system (see section 4.1.3).

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.

9) **Follow this step ONLY if you are upgrading an existing Vanquish Neo installation to a Tandem workflow configuration** (upgrading from VUI driver version 2.3 to 2.4 or above) otherwise jump to step 10.

   a. Run Script E01 on the VUI (for general details on scripts see section 3.2 and Table 1).

   b. Ensure that the pump serial numbers (S/N) for the lower (recondition) and upper (separation) pump are correctly assigned in Script E01. See Figure 73.

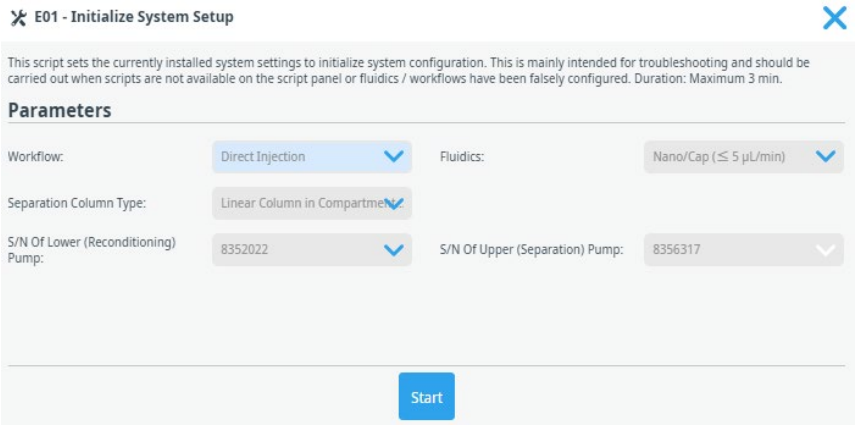


Figure 73: E01 script – Assign the correct lower pump module S/N from the dropdown menu. The upper pump module serial number will be automatically populated accordingly.

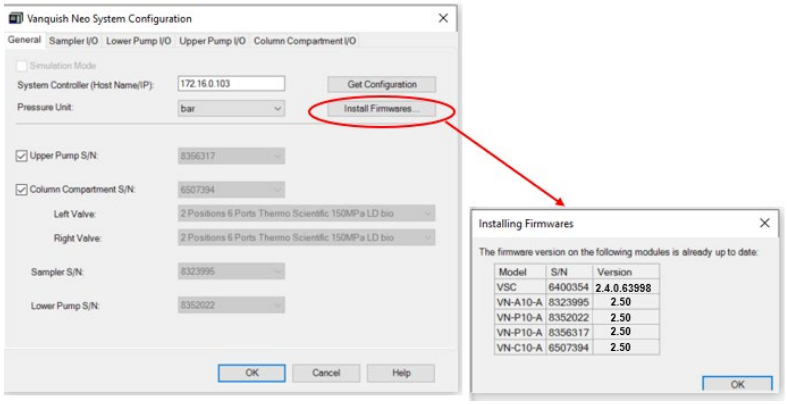
TIP All module S/Ns are shown on labels situated on the lower right-hand side of the front panel behind the module compartment door

7.1.5.2 Installing / updating the instrument firmware

10) If a Vanquish Neo LC is already configured on the instrument control PC, please delete the configuration, close the software and then uninstall the existing Vanquish Neo driver from the control PC.

11) Install the Vanquish Neo driver version 1.5 or higher on the instrument control PC and create a new instrument in the instrument configuration manager. (Refer to section 4.1.4 and 4.1.5 and the driver release notes for details).

Update the Vanquish Neo System firmware (



12) Figure 74).

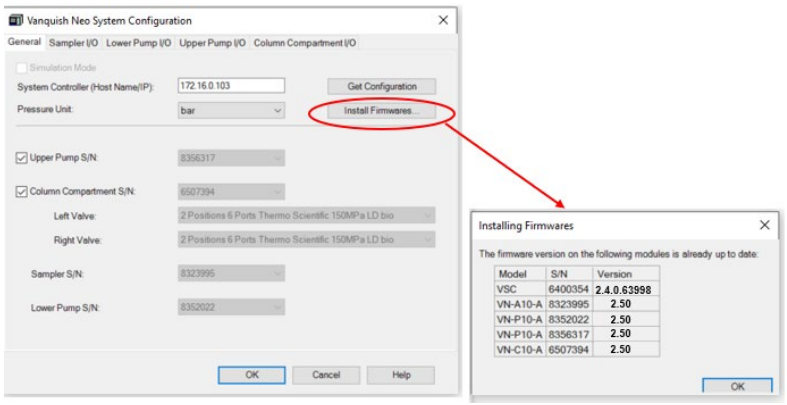


Figure 74: Vanquish Neo System Configuration and Firmware Update Tool

NOTE The serial number assignments for the lower and upper pumps must be consistent with those in the physical assembly. If this is not the case, please save and close the configuration dialogue box and run

either script A00 or script E01 to correctly assign the pumps (see Figure 73 for details).

13) Connect the MS contact closure (start) to Autosampler Relay 2.

14) For details on how to setup and operate the tandem direct injection workflow using a **single spray ion source** mode, continue onto section 7.1.7. For details on setting up and operating the workflow using the **dual spray ion source** please refer to section 7.1.8.

7.1.6 Tandem Direct Injection Relevant Changes applicable to the VUI

7.1.6.1 Home Panel

The home panel displays each of the four system modules (each represented by a module box). The position (and function) of the two pump modules can be determined by the arrow on the respective pump icon:

- The ↑ denotes the upper (separation) pump
- The ↓ arrow denotes the lower (reconditioning) pump

The column oven module box displays the valves positions. When a sequence is running, the active separation column is displayed in the top right corner (see Figure 76)

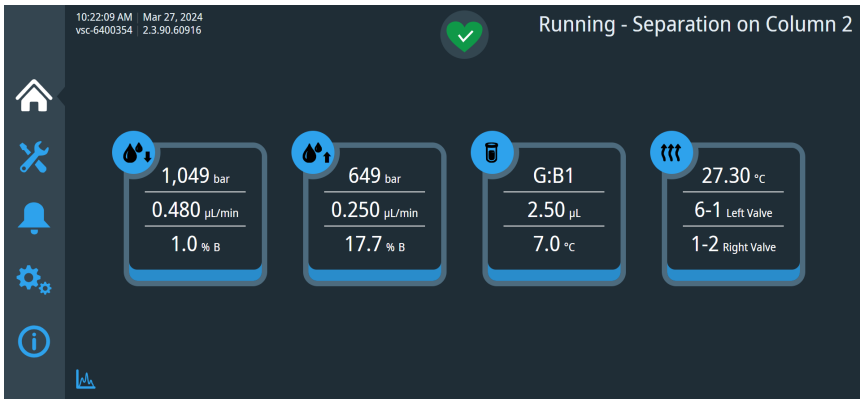


Figure 75: VUI Home Screen during tandem direct injection operation

7.1.6.2 Script Panel

The following scripts have been included for operation with the tandem Direct Injection Workflow

ID	Script	Description
A00	Change Fludics / Workflow: Tandem	This script allows the operator to change the workflow from Direct Injection to Tandem Direct Injection. This script should executed out when all modules are in place, both valves have been installed in the TCC and after the USB & CAN connections have been connected.

Table 24: List of New Scripts Available for Tandem Workflow with Description for the Vanquish User Interface

NOTICE Some scripts have been updated to ensure compatibility with the Tandem Workflow are updated, such as: A01, A02, B01, B02, B06, C01, C02, C04, C10, D01, D02, E01, M31, M32, M32a, M33 and M34 (refer section 3.2.2 for details about scripts).

7.1.7 Configuring Single Spray Tandem Direct Injection Workflows

7.1.7.1 Configuring single-spray Microflow workflows

1) Select script A00 “Change Fluidics / Workflow: Tandem” to change the workflow to Tandem Direct Injection (Figure 76).

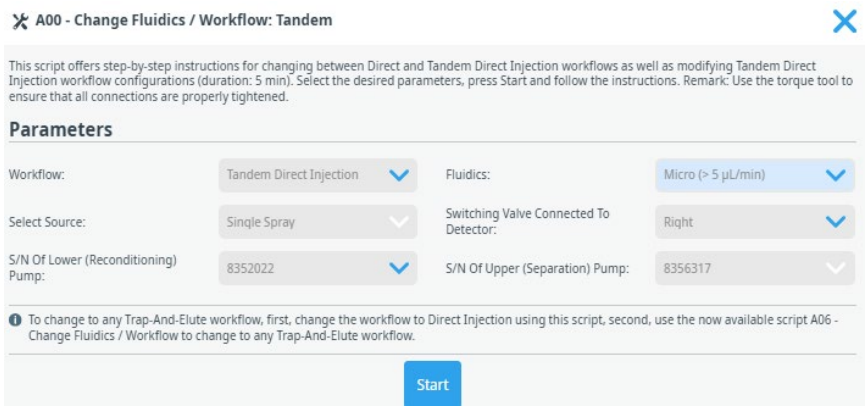


Figure 76: Dialogue box for script A00 when configuring the system to Tandem Direct Injection single spray micro-flow fluidics configuration. In this example the switching valve connected to detector “right” has been selected.

TIP If the system is an upgrade from a Vanquish Neo to a Vanquish Neo Tandem workflow configuration and is currently configured as a Trap-and-Elute workflow, please use **script A06** to switch to the Direct Injection workflow, then select script A00 to migrate to tandem direct injection. Conversely, once the system has been configured in Tandem Direct Injection Configuration the A00 script must first be used to configure a Direct Injection Workflow before the A06 script can be used to convert to a Trap-and-Elute Workflow

2) Once "Tandem Direct Injection" has been selected, several fields become available.

   i. Select Source "Single Spray" -this configuration utilizes both Column Compartment switching valves.

   ii. Confirm that the pump S/N's are correctly assigned.

TIP The pump serial number assignment is allocated by selecting the correct option for the lower (re-conditioning pump). The upper (separation) pump is auto populated.

   iii. Select "Fluidics" to choose between nano/cap- (20µm I.D. capillaries) and micro- (50µm I.D) fluidic capillary configurations.

TIP Once the "Fluidics" drop down is set to "micro" the "select Source" field in the A00 script (see i) above) becomes locked on "Single Spray". To re-enable the "Dual Spray" option, the "Nano/Cap" fluidics option must first be selected from the dropdown menu.

   iv. Select if the left or right switching valve should be connected to the detector.

   v. The script guides the user through the workflow configuration changes. Schematics showing the respective fluidic configuration options are given in Figure 77 and Figure 84below.

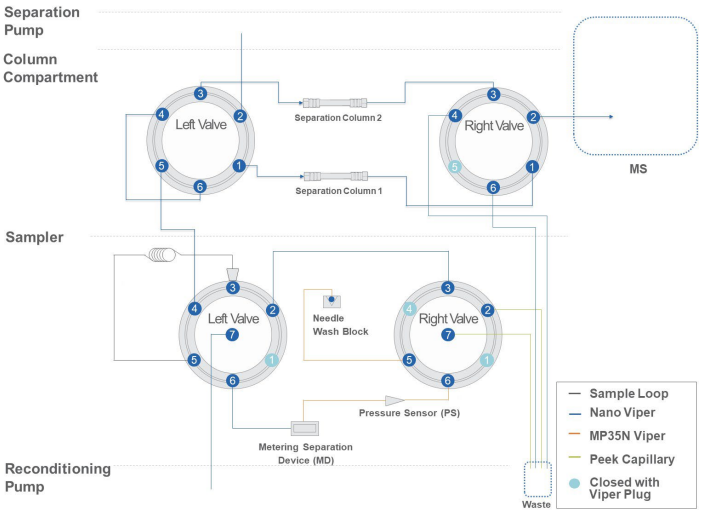


Figure 77: Fluidic schematics for the single spray microflow fluidic configuration with the right switching valve connected to the detector

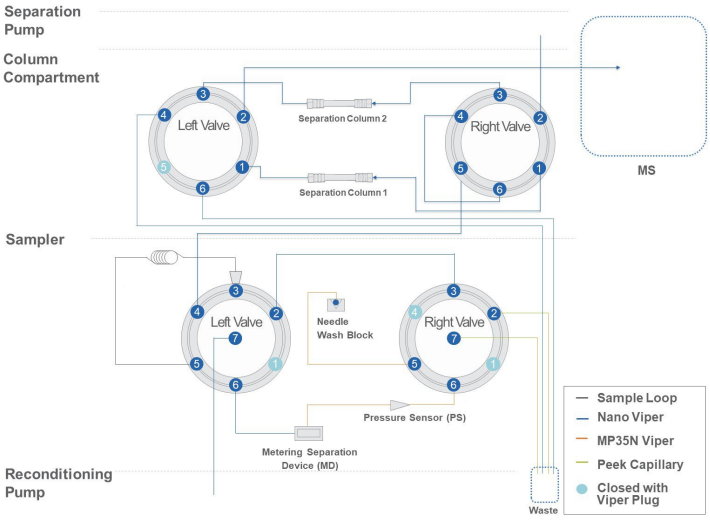


Figure 78: Fluidic schematics for the single spray microflow fluidic configuration with the left switching valve connected to the detector

TIP The schematic above (Figure 78), which is also displayed on the VUI, shows the MS detector on the right-hand side, despite the left-hand valve being connected to the detector (at valve position 2). It should be noted that this is purely for display purposes. The user should only opt for this configuration if the MS detector inlet is located towards the left-hand side of the column compartment as this enables the shortest

possible connection to the (MS) detector, resulting in minimum possible post column dispersion. Similarly, if the MS is located on the right-hand side of the column oven, the option (switching valve to detector => right) should be selected (see Figure 77).

7.1.7.2 Configuring single-spray Nano-Cap workflows

There are subtle differences in the column to valve connectivity for the nano-cap fluidic configuration compared to the micro-flow variant (see section 7.1.7.1). For the **nano-Cap single spray** fluidic configuration, only **one** (pre-column) capillary connection is required between the two column compartment valves, where-as **two** (one pre and one post) are necessary for the **micro-flow** configuration.

The discrepancy lies in the fact that both the nano and the capillary-flow columns are fitted with nanoViper fittings at both ends. This is not possible for the micro-flow columns due to the connectors on the column housing.

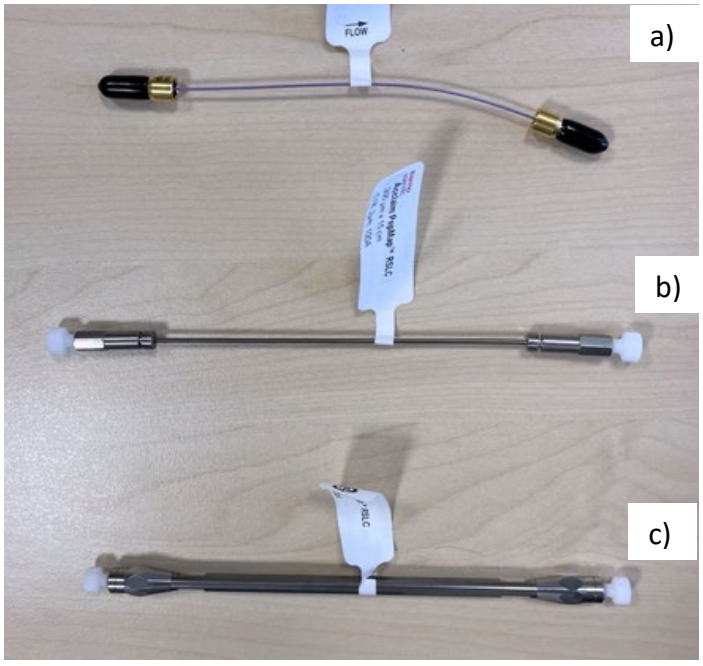


Figure 79: Comparison between a capillary column,)150µm x 150 mm DNV PepMap column a), and two micro-flow columns (300µm x 150 mm PepMap column, b) (1mm x 150mm PepMap column c).

TIP A Viper connector is required to interface the capillary column with the nanoViper capillary connected to the pre-column valve. The A00 script guides the user through all the steps required to configure the respective workflows. All capillaries and connectors are suppled with the Vanquish Neo System Ship Kit and Tandem Direct Injection Workflow kit.

7.1.7.3 Configuring Ion Sources for Single Spray Tandem Direct Injection Workflows

See sections 7.1.4.2 and Table 22 for a complete list of options.

7.1.8 Configuring Dual Spray nano-Cap Tandem Direct Injections Workflows

Use the A00 script to change to:

- Workflow ->Tandem Direct injection
- Fluidics -> Nano/Cap
- Select Source -> Dual Spray

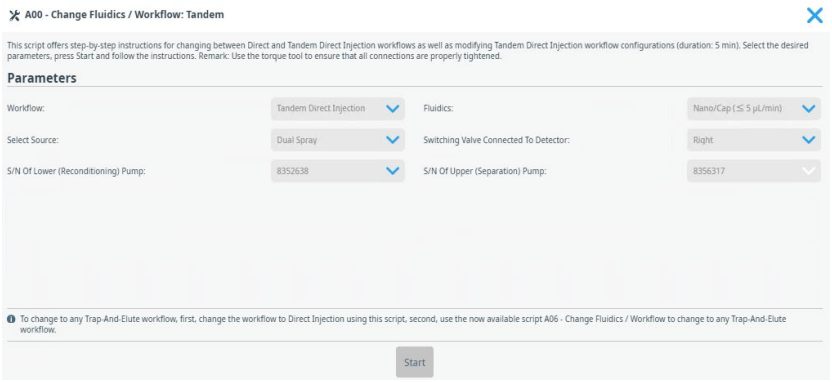


Figure 80: Dialogue box for script A00 when configuring the system to Tandem Direct Injection Dual Spray Nano/Cap workflow configuration. In this example the switching valve connected to detector "right" has been selected

TIP Choose the "switching Valve Connected to Detector -> Right / Left" according to the position of the LC stack relative to the Detector (MS) inlet to ensure the shortest possible distance between the outlet of the valve to the MS ion source.

The script guides the user through the configuration change. Fluidic schematics are shown in Figure 81and Figure 82 below.

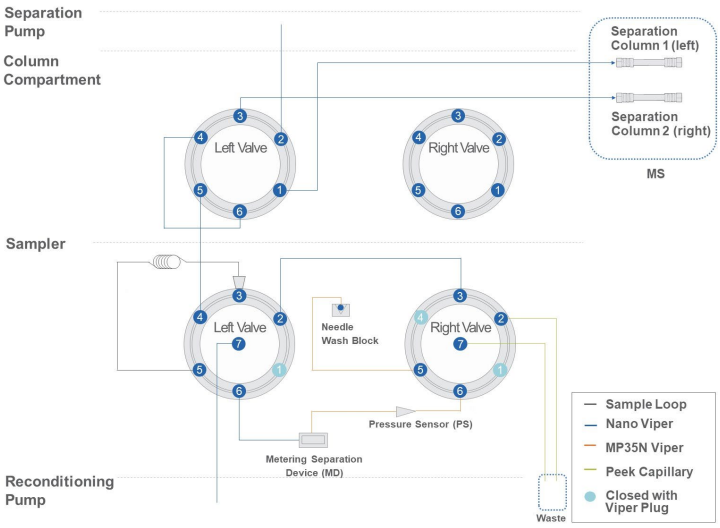


Figure 81: Fluidic schematics for the dual spray nano-cap fluidic configuration with the left switching valve connected to the detector

TIP The schematic above (Figure 81), displayed on the VUI, shows the MS detector on the right-hand side, despite the left-hand valve being connected to the dual spray source (via capillaries connected to valve positions 1 and 3). This is purely for display purposes. The user should only opt for this configuration if the MS detector inlet is located on the left-hand side of the column compartment as this enables the shortest possible connection to the (MS) detector, resulting in minimum possible post column dispersion. Similarly, if the MS is located on the right-hand side of the column oven, the option (switching valve to detector => right) should be selected (see Figure 82).

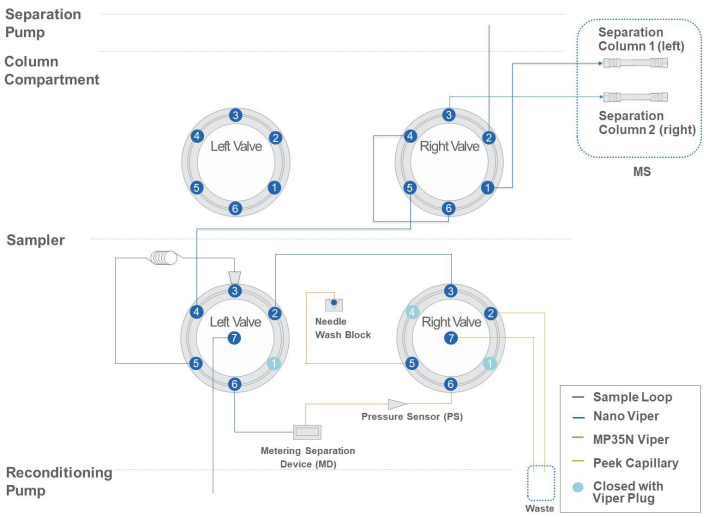
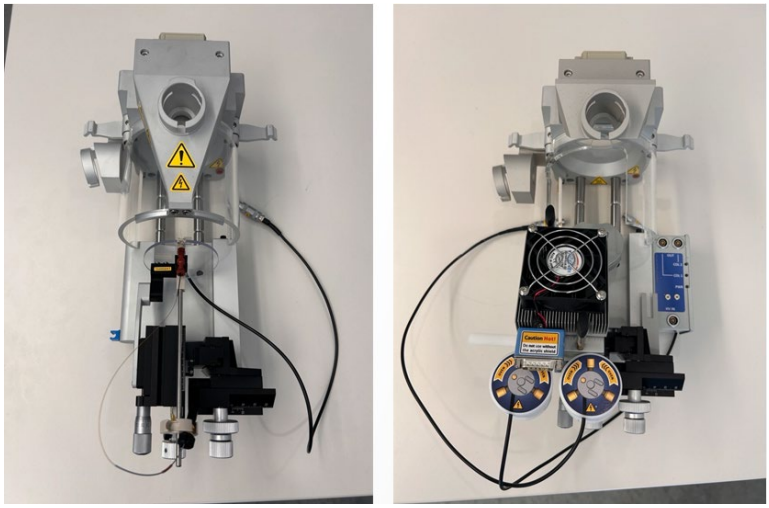


Figure 82: Fluidic schematics for the dual spray nano-cap fluidic configuration with the right switching valve connected to the dual spray source via capillary connections at valve positions 1 and 3

7.1.9 Setting up the Double Barrel Oven on the NanoSpray Flex Ion Source

The double barrel oven kit (Sonation Lab Solutions) is required to run the Dual Spray Tandem Direct Injection Workflow with the NanoSpray Flex NG Ion Source (see Figure 83). For detailed instructions on how to install the kit, please refer to the Sonation Lab Solutions User Manual.



a b

Figure 83: NanoSpray Flex NG Ion source a) in standard configuration b) with the Sonation double barrel oven installed

NOTICE Do not use oven temperatures above 50°C or flow rates above 8.2 uL/min.

Always check if the liquid connections have been made correctly and if there are any leaks once the source has been installed.

7.1.10 Starting the System in Tandem Workflow Configuration

The desired tandem direct injection workflow configuration must be set using script A00 prior to starting the system.

- For details on setting up single spray tandem direct injection workflows:
  - In microflow fluidic configuration, please see section 7.1.7.
  - In nano-cap fluidic configuration, please see section 7.1.7.2
- For details on setting up the dual spray nano-cap direct injection workflow, please see section 7.1.8

The summary page of the A00 script lists further steps which should be performed to ensure successful tandem workflow operation (see Figure 84).

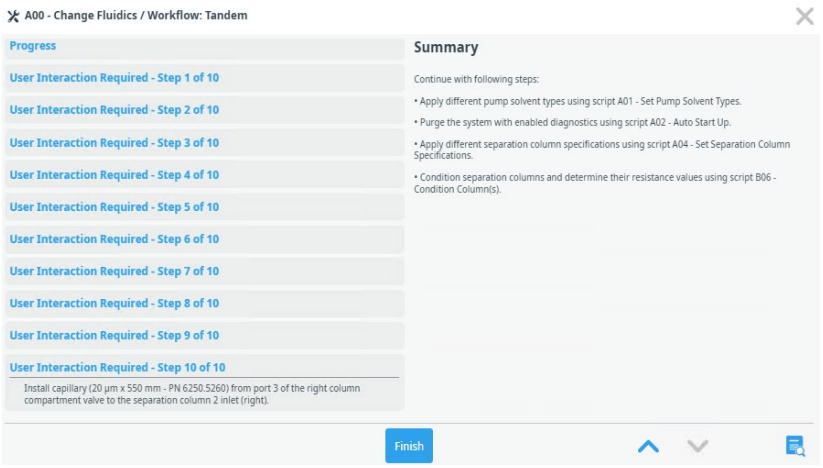


Figure 84: Summary dialog box at the end of Script A00 listing the next steps to follow

TIP The instructions should be documented prior to clicking “finish” as they subsequently disappear. Alternatively, please refer to the right hand side summary of Figure 84 when proceeding with the next steps

1) Set the Pump Solvent Types using script A01. In the tandem workflow configuration, there is only one dropdown menu for solvent type “A” for both the upper (separation) and lower (reconditioning) pump. This limitation ensures no disturbances to the chromatography during the pump handover which occurs between sample loading and gradient delivery. In contrast, the “B” solvents can be set independently for the separation and reconditioning pumps. (see Figure 85)

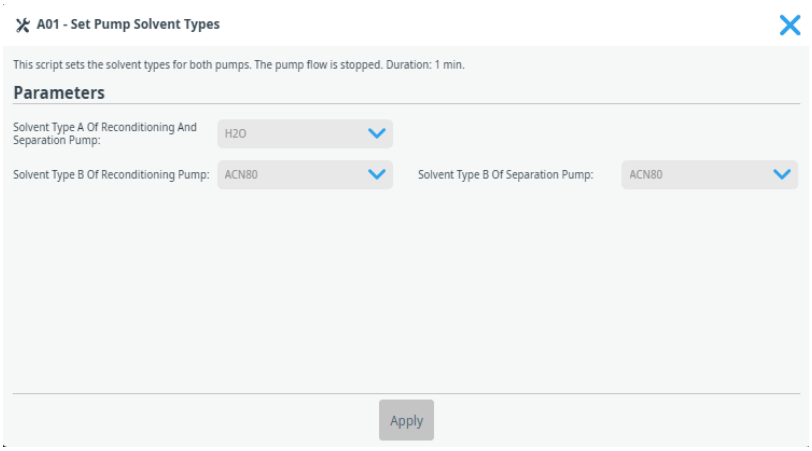


Figure 85: Script A01 dialogue box in tandem workflow configuration

TIP Manual interactions are required during execution that differs from Direct Injection and Trap and Elute Workflows.

2) Run script A02 “Auto Start Up” with the diagnostics set to “On”.

This script performs extensive purging of the LC system hardware components. At the beginning of the script, the user is instructed to install a waste line (flow meter purge capillary) to enable flushing of the flow meter in the separation (upper) pump. (see Figure 86 and Figure 87).

TIP The flow meter purge capillary (waste capillary) Figure 87 is included in the Ship kit of the Binary Pump N Module

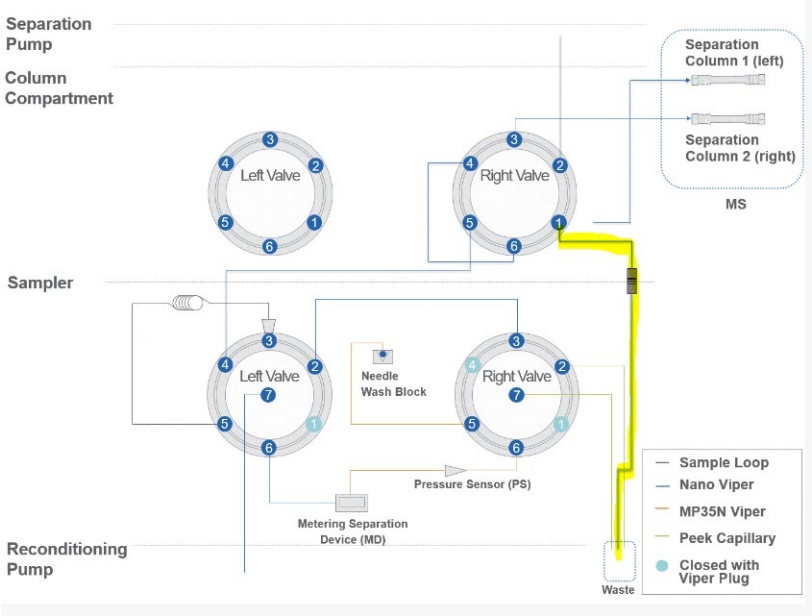


Figure 86: Fluidic schematic instructing the user on connect handling of the waste line (P/N 6079.2425) to permit flow meter purging of the upper (separation) pump. This instruction is repeated in every script in which the upper pump flow meter purge is executed (e.g. scripts A02 and C02)

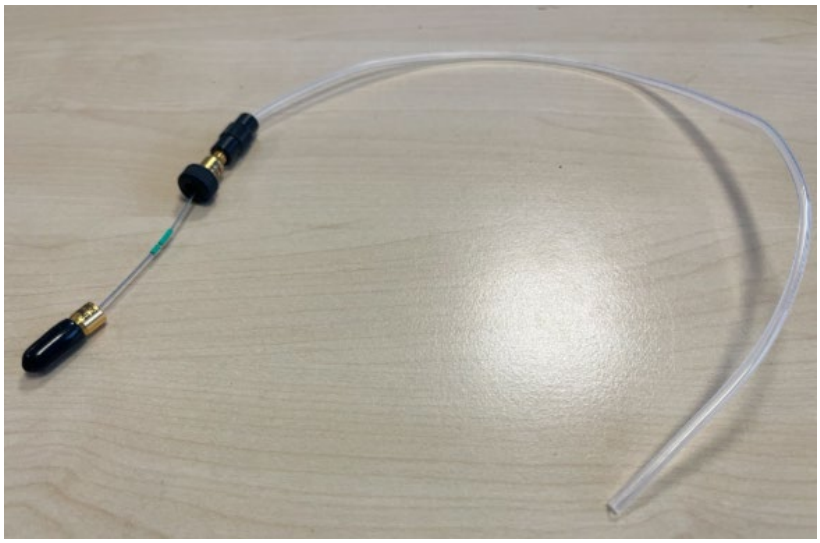


Figure 87: Waste line capillary which permits flow meter purging on the separation pump (P/N 6079.2425). The capillary is shipped with 2.5 meters of FEP (Fluorinated Ethylene Propylene) waste tubing which should be cut to the appropriate length prior to first use

With the option “diagnostics -> on” selected, the system capillaries are purged, after which back pressure and lead tightness tests are performed.

TIP The A02 script takes approximately 2 hours to complete. After the waste capillary has been installed and the user has clicked on “continue” the LC system commences with the purge of the hardware components. This takes approximately **one hour** during which time no further user interaction is required. After this initial period, the user needs to return to and remain at the system as extensive user interaction is required whilst the back pressure and tightness test diagnostics are performed.

3) Run script A04, “Set Separation Column Specifications”.

Note that only identical analytical columns are supported and therefore the same set of specifications will apply to both columns.

4) Run script B06 “Condition Column(s)” (See Figure 88).

In the tandem direct injection workflow configuration, this script

- Washes and Equilibrates the Columns
- Determines the column resistance of each individual column

NOTICE: The column resistance must be measured using the start gradient and flow composition of the intended application as well as the application specific column temperature.

Set the "Final Pump Flow", "Final Pump B%" and "Column Temperature" in script B06 according to the gradient start conditions before executing.

NOTICE: For applications run using the Dual Spray (DBO) source. Make sure to set the application specific temperature on the Dual Spray Source (CO) Control software before running the B06 script.

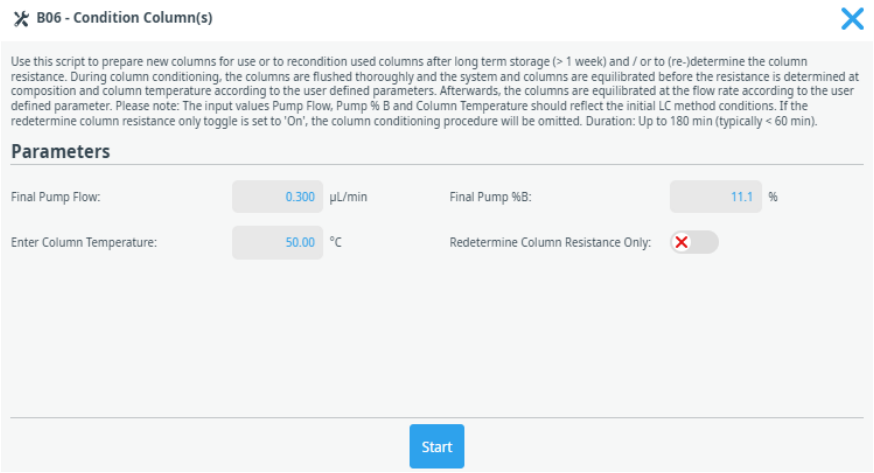


Figure 88: Dialogue box for script B06. The parameters "Final pump flow" "Final Pump %B" and "Column Temperature" must correspond to the initial gradient, temperature and flow settings of the intended application as these are used to determine the column resistance values

NOTICE: The toggle option "re-determine column resistance" is intended to be applied to columns that have already been in use, and where column conditioning is no longer necessary. Use this option if you

i) Change the temperature of your application
ii) Encounter upper pressure limit exceeded errors during the application which are caused by an increase in column backpressure,
iii) (ex)change to other previously used column(s).

7.2 Direct LC Control Options for the TDI Workflow

7.2.1 ePanel and mini-ePanel

The Vanquish Neo Tandem Workflow LC stack can be controlled either via the system ePanel in the Chromeleon console or via the direct control option in Xcalibur (Figure 89).

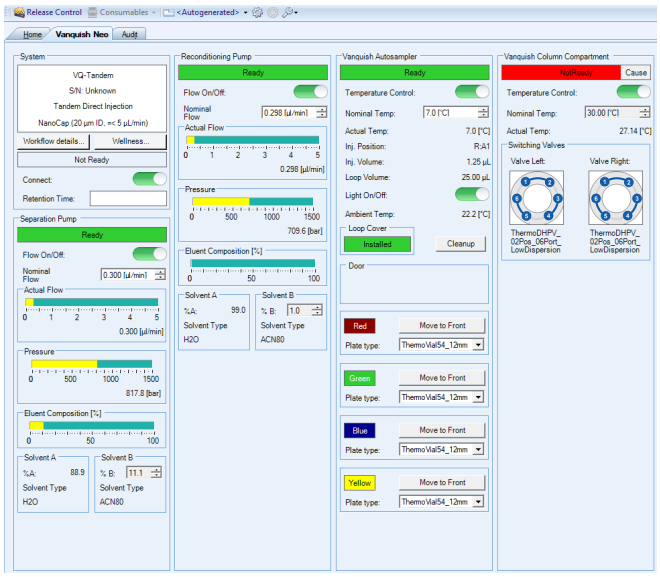


Figure 89: Vanquish Neo System ePanel

The ePanel can be accessed by clicking on the “direct control” box displayed on the mini ePanel available in the Xcalibur sequence setup view (Figure 90). The mini ePanel also shows the status and activities of each of the system modules.

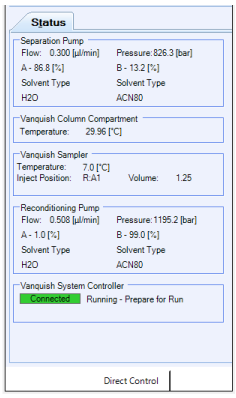


Figure 90: mini ePanel in Xcalibur

7.2.2 Control Options on the VUI Home Screen

Alternatively, each of the modules can be controlled individually via the VUI by clicking on the respective module box on the VUI home screen.

Figure 91: Vanquish Neo Direct Control Options in Tandem Direct Injection Workflow Configuration

7.2.3 Column Control and Electrospray Check Options available from the Column Compartment Module Box

Column compartment plays a central role in the Tandem Direct Injection Workflow. Examples of core functionality include:

- Housing and control of the two low dispersion switching valves
- Tempering both separation columns (in single spray workflow configuration)
- Triggering the voltage switching between the two Liquid Junction Units in the Double Barrel Oven to ensure analyte ionization from the correct column at the correct time

Whilst these operations are performed automatically during a sequence run, governed by the tandem workflow method, external control is possible when the LC stack is in “Idle” mode.

TIP The user is blocked from manually controlling any of the system modules whilst a sample sequence or a script are running.

7.2.3.1 Switching the Separation Pump Flow between Columns

Manually switch the separation pump flow between the two columns using the dropdown menu in the “Select -> Separation Pump Flow to” dialogue box. This can be executed e.g. to i) manually wash or equilibrate a specific column or ii) To determine which column will be used at the start of a sequence run (see 9.1.4).

TIP The dialogue box captions reflect the specific tandem workflow configuration (single or dual spray – see Figure 92 and Figure 93).

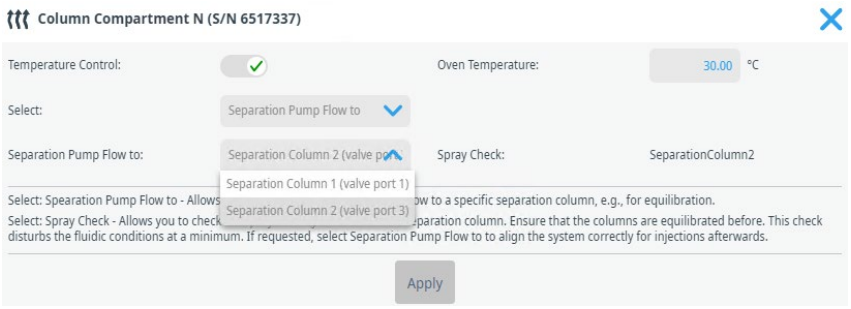


Figure 92: Switching the Separation Pump Flow between Columns (1 and 2) in Single Spray Tandem Direct Injection Workflow Configuration

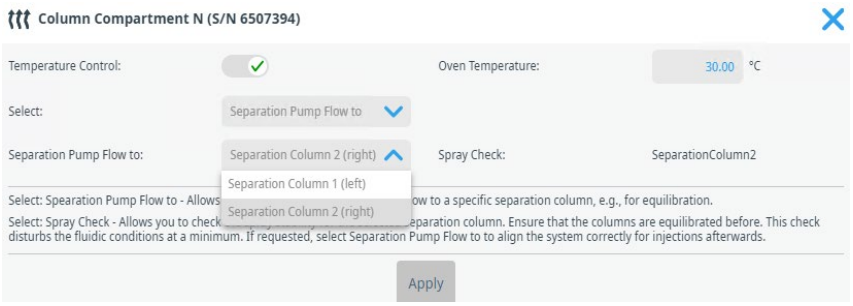


Figure 93: Switching the Separation Pump Flow between Columns (left and right) in Dual Spray Tandem Direct Injection Workflow Configuration

7.2.3.2 Electrospray Ionization Spray Stability Check

In Tandem Direct Injection Workflow configuration, the User has the option to check the electrospray signal stability on the Mass

Spectrometer Electrospray Ion Source. With the “Select -> Spray Check” option activated; the user can decide to switch either column in-line with the ion source. The display text for Single Spray and Dual Spray configuration dropdown menus reflects the respective spray configuration (see Figure 94 and Figure 95).

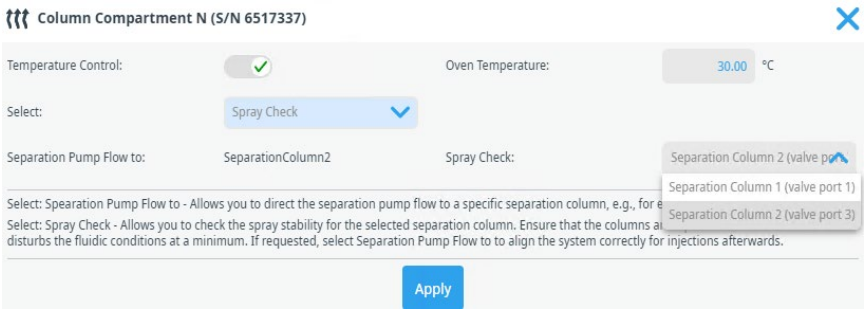


Figure 94: Vanquish Neo Direct Control Options in Single Spray Tandem Direct Injection Workflow Configuration – Here the Spray check option is linked to the column (1 or 2) in-line with the single spray source as defined by the post column valve connection.

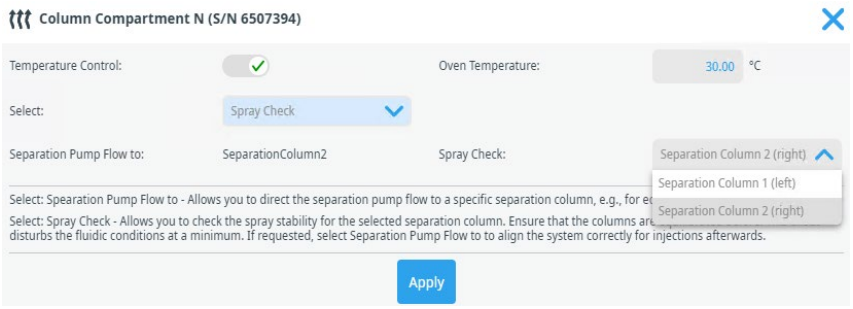


Figure 95: Vanquish Neo Direct Control Options in Dual Spray Tandem Direct Injection Workflow Configuration – The User selects either the column (left or right) located in the DBO source

8 Tandem Direct Injection Workflow Methods and Applications

This chapter details how to create instrument methods as well as providing links to technotes which include template methods available for download

8.1 Programming TDI Instrument Methods

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

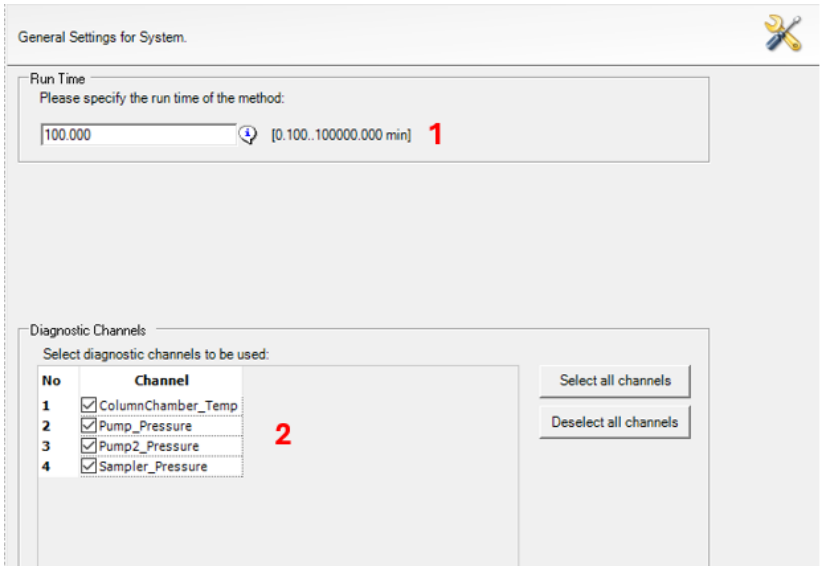


Figure 96: IMW Page 1 - System: General Settings for the Tandem Workflow

#	Description
1	Specifies the total run time for the LC method.
2	Lists the available diagnostic channels. *Note: The list of channels depends on the configured modules.*

Table 25: Key to Figure 96 IMW -Page 1 - System: General Settings

TIP Due to the interdependencies between different tasks in the workflow, it is helpful to start with a high run time on this page (greater than is intended for the desired application). The actual run time for the method will be determined by the separation gradient duration which is set on page two of the IMW

Page 2 of the IMW details the separation column specifications. All the properties are identical for both columns, except for the column resistance. Column resistances are measured and recorded for each column during **B06 script** execution (Figure 88 in section 7.1.10,)

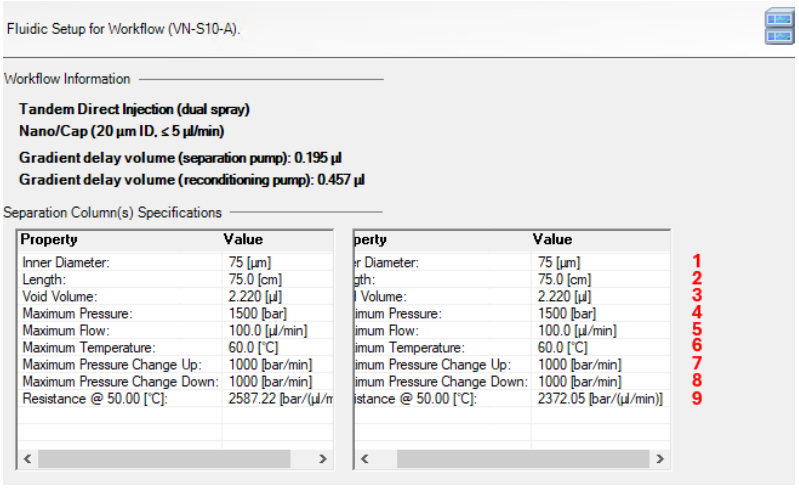


Figure 97: IMW Page 2 – Fluidic Setup for the Tandem Workflow

#	Description
1	Inner diameter of the separation columns*.
2	Length of the separation columns*
3	Theoretical column void volume (calculated by the Driver) based on the assumption that this is equivalent to 67% of the open tube volume.
4	Maximum pressure rating of the separation column*.
5	Maximum flowrate rating of the separation column. Note: Use 100µL/min if no other flowrate is given
6	Maximum temperature rating of the separation column*.
7	Maximum pressure change up for the separation column. Note: Use 1000 bar/min unless column specifications dictate otherwise.
8	Maximum pressure change down for the separation column. Note: use 1000 bar/min unless column specifications dictate otherwise
9	Separation column resistance values measured at the given temperature for the respective column.

*Table 26: Key to Figure 97 IMW page 2 Workflow fluidic setup *see column label or specification sheet for details*

NOTICE The column resistance measured and displayed on page 2 of the IMW is specific to the temperature at which it was recorded as well as the solvent type. The temperature at which the resistance was measured is displayed in the IMW / IME and displayed in units bar/(µL/min).

NOTICE In dual spray configuration, the Vanquish Neo instrument has no control over the column temperature in the DBO. Therefore, the user **must ensure that the temperature of the DBO oven is identical to the one recorded in the IMW/ IME**. If a different temperature is intended for the method execution, the desired temperature should be set in **both the Sonation DBO temperature control software** and the **B06 script**.

The “Basic Tandem Settings” are used to set all the boundary conditions for the for method. The settings are divided into three categories:

- Separation Gradient Parameters
- Injection Parameters
- Wash and Equilibration

The bottom of the page comprises a method scheduling graphic (Method Execution Timings). This displays the timing and duration of the respective workflow tasks and is updated in real time, enabling visualization of the interdependencies between scheduled tasks (e.g. pressure alignment between the reconditioning and separation pumps). Individual properties adopted for each task can be visualized by hovering the mouse over the bar.

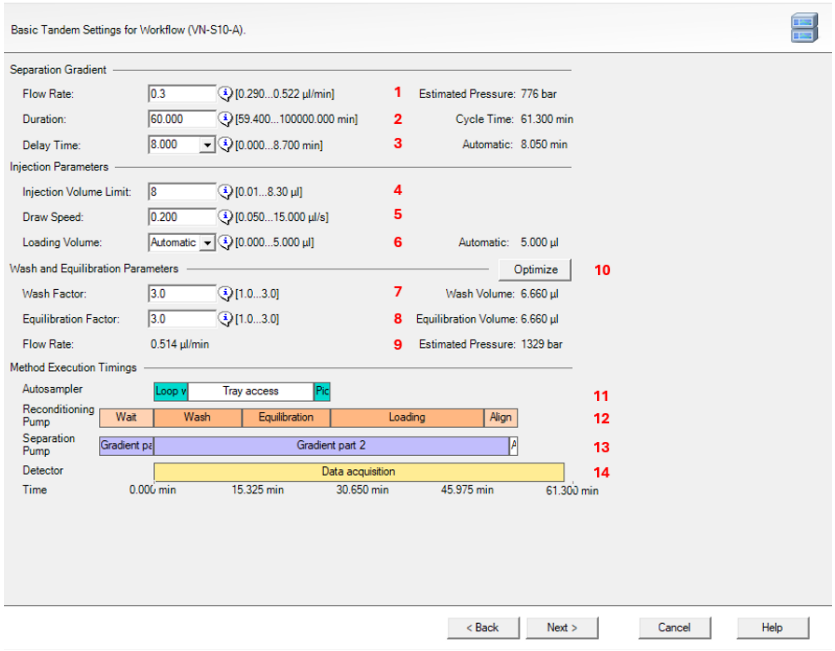
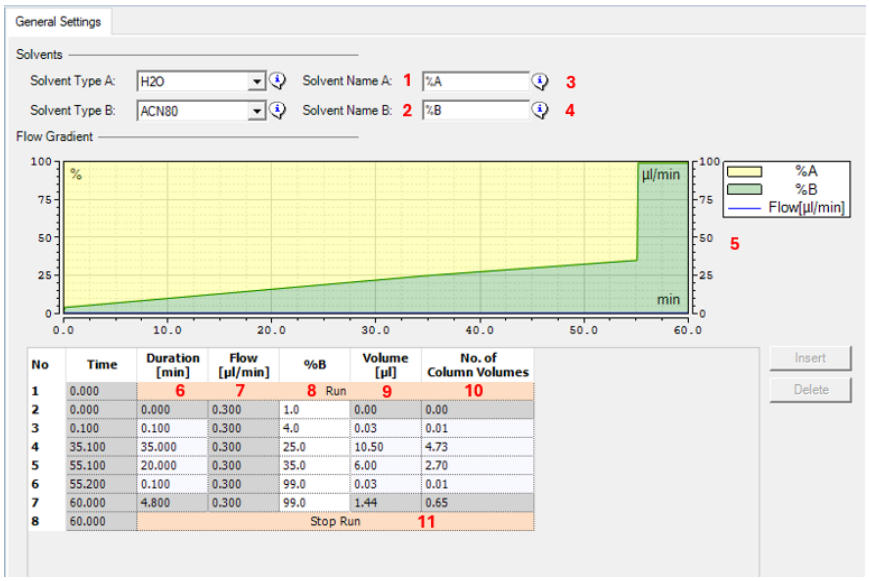


Figure 98: IMW Page 3 – Basic Settings for Tandem Workflow

TIP A range of workflow compatible limits is given in parenthesis for each specific setting (e.g. flow rate or injection volume). Interdependencies between different tasks mean that changes to one of the method parameters can have a knock-on effect on the range of permissible settings for the others. These changes are reflected in real time when and as they occur. NOTE: Input values which fall outside of the respective limits after changes are made are immediately flagged in red.

#	Description
1	Specifies the gradient flowrate and the resulting back pressure experienced by the separation pump based on the calculated column resistance.
2	Specifies the gradient duration which in turn corresponds to the length of time data is acquired. Note: The MS method should be set to the same length. The value “Cycle time” is the entire run-to-run time. It is the sum of the gradient duration and the time taken for any alignment steps.
3	The delay time is the time needed for the gradient to reach the end of the column. The automatic delay time is calculated based on the theoretical column void volume, the volume of the fluidic connections and the gradient flow rate. The delay time can also be specified manually by the user.
4	Specifies the maximum injection volume that can be used for the method.
5	Defines the speed at which the sample is drawn up by the syringe.
6	Specifies the eluent loading volume used to transfer sample onto the column. When “automatic” loading volume is selected, 5µL of eluent is used to transfer the complete sample to the separation column.
7	Specifies the volume of strong solvent (B) used to wash the column. The total volume is the factor multiplied by the column void volume plus the dead volume.
8	Specifies the volume of weak solvent (A) used to equilibrate the column. The total volume is the factor multiplied by the column volume plus the dead volume.
9	The flow rate and estimated back pressure experienced by the reconditioning pump based on the column resistance values (see Table 26, item 9).
10	The “Optimize” button generates wash and equilibration parameters which result in a reconditioning pump flow rate that aligns with the separation pump.
11	Dynamic method scheduling displaying the execution timing of the different autosampler tasks (e.g. sample pick up). Note: The term “tray access” denotes the time window during which samples can be added / removed from the system without interfering with the workflow operation.
12	Dynamic method scheduling display for the execution timings of the reconditioning pump tasks (including wash, equilibration and sample loading)
13	Dynamic method schedule displaying the execution timings of the separation pump tasks. Note: If the gradient delay time is > 0, the gradient will be displayed in 2 parts (see item “3” in Table 27).
14	Dynamic method scheduling display for the execution timings for detector data acquisition

Table 27: Key to Figure 98 IMW -Page 3 – Basic Tandem Settings for Workflow



No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0.000					
2	0.000	0.000	0.300	1.0	0.00	0.00
3	0.100	0.100	0.300	4.0	0.03	0.01
4	35.100	35.000	0.300	25.0	10.50	4.73
5	55.100	20.000	0.300	35.0	6.00	2.70
6	55.200	0.100	0.300	99.0	0.03	0.01
7	60.000	4.800	0.300	99.0	1.44	0.65
8	60.000					

Figure 99: IMW Page 4 - General Settings for the separation pump

#	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 also be added.
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 also be added.
3	Text field for entering a custom description of solvent A
4	Text field for entering a custom description of solvent B
5	Flow gradient plot.
6	Start Time and Duration of each 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 according to the column void volume.
11	Data acquisition for the current run ceases at this point.

Table 28: Key to Figure 99 IMW -Page 4 – Basic Tandem Settings for Workflow

NOTICE: Progressing from the Basic Tandem Settings Page to the Gradient Settings Page has an immediate impact on the minimum gradient duration which can be selected on the basic tandem settings page (Figure 98) of the IMW (accessible by pressing on the 'back' button of the IME).
After returning to the basic tandem settings page via the 'back' button in the IMW, the minimum possible separation gradient duration is fixed to the time shown in the gradient table. If the user decides that on reflection, a shorter gradient time is required, a completely new method must be created from scratch using the IMW.

TIP: Only the white highlighted fields in the gradient table can be edited. To insert a new line to the gradient table, first click on one of the line entries to activate the "insert" dialogue box. Please note that the "greyed out" spaces in the gradient table cannot edited. They can also not be deleted.

The column wash settings are programmed on the reconditioning pump general settings page (Figure 100)

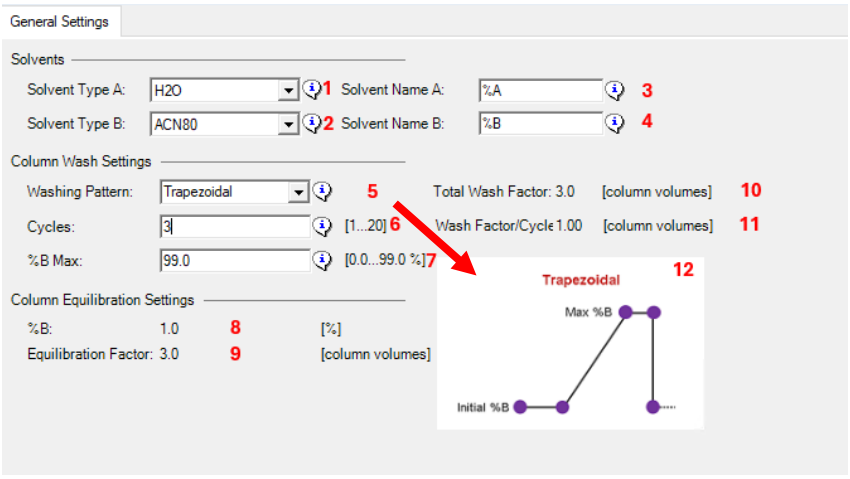


Figure 100: IMW Page 5 - General Settings for the Reconditioning Pump

#	Description
1	Specifies the calibrated solvent type for reconditioning pump A. Note: In Tandem workflow operation, Pump A (as selected on the VSC) is always the same for the separation and reconditioning pumps.
2	Specifies the calibrated solvent type for reconditioning pump B. Note: The B solvent type may differ from that on the separation pump B channel.
3	Text field for entering a custom description of solvent A
4	Text field for a custom description of solvent B
5	Column wash pattern. There are four options available from the dropdown menu (see Figure 101 for details)
6	Select the number of wash cycles (number of times the wash pattern should be repeated)
7	Select the maximum % B that should be used for the strong wash component of the wash pattern.
8	Solvent composition (based on solvent B%) used to equilibrate the columns.
9	Equilibration factor (read only parameter taken from the value defined on the Tandem Basic Settings page – see Figure 98)
10	Total wash factor taken from the value defined on the Tandem Basic Settings page see Figure 98).

#	Description
11	Wash factor per Wash Cycle – A read only field which is populated based on the number of wash cycles selected, the reconditioning pump flow rate and the column void volume.
12	Graphic depicting the washing pattern selected (item 5 in Table 29)

Table 29: Key to Figure 100 IMW -Page 5 – General Settings for the Reconditioning Pump

There are four column washing pattern available to choose from in the drop down menu. These are: isocratic, rectangular, trapezoidal, and triangular (see Figure 101 below).

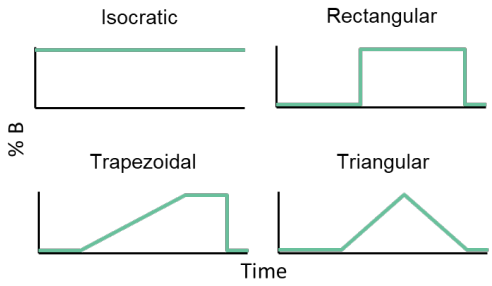


Figure 101: Schematics representing the available column washing patterns

For each washing pattern, the user can define the maximum % B which should be used in the wash and the number of washing cycles.

TIP: Choose the washing pattern and the number of wash cycles that is best suited to your column and application. For proteomics applications employing reverse phase chromatography columns and solvents, we recommend starting with the Trapezoidal wash option.

On the next page (Figure 102) enter or adjust your autosampler settings.

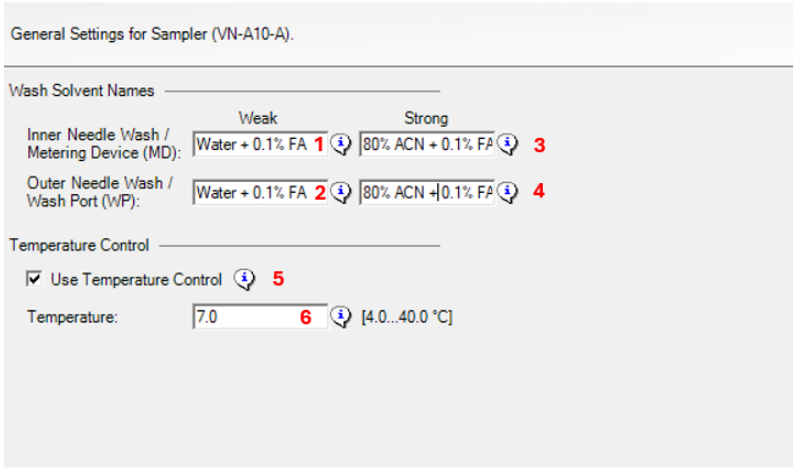


Figure 102: IMW Page 6 - General Settings for the Sampler

#	Description
1	Text field for entering a user defined description for the weak liquid for the inner needle wash used by 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 is also used for sample loading onto the column. (Default: WeakSolvent)
2	Text field for entering a user defined 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 a user defined description for the strong liquid for the inner needle wash used for intense purging of the metering device. 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 a user defined 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 30: Key to Figure 102 IMW Sampler general settings

TIP: Advanced settings for the autosampler (e.g. air gaps, needle wash procedure) can be adjusted once the wizard has been completed.

Enter the column compartment temperature Figure 103. Click next to continue

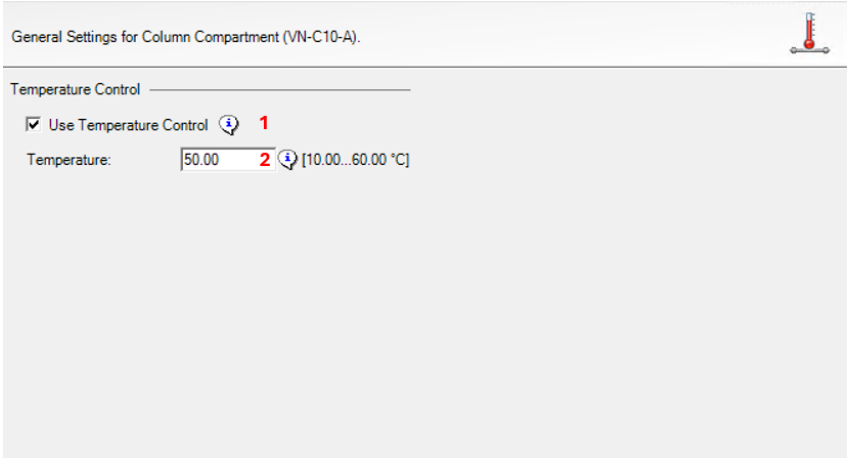


Figure 103: IMW Page 7 - General Settings for Column Compartment

#	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 31: Key to Figure 103 IMW Sampler general settings

CAUTION: If you want to change the column compartment temperature, the column resistance needs to be re-determined at the new temperature and saved to the method.

NOTICE: Ensure that use temperature control function is activated if the separation columns are in the Column Compartment (Single Spray Workflows).

The final page of the instrument method wizard is as follows:

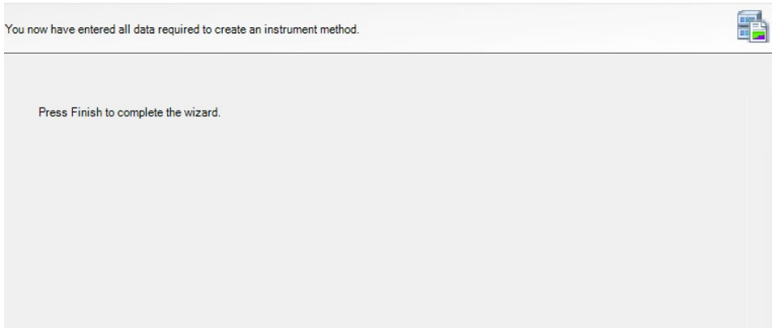


Figure 104: IMW Page 8 – Method Completion

Once the method wizard is complete, the tab “advanced autosampler settings” becomes visible.

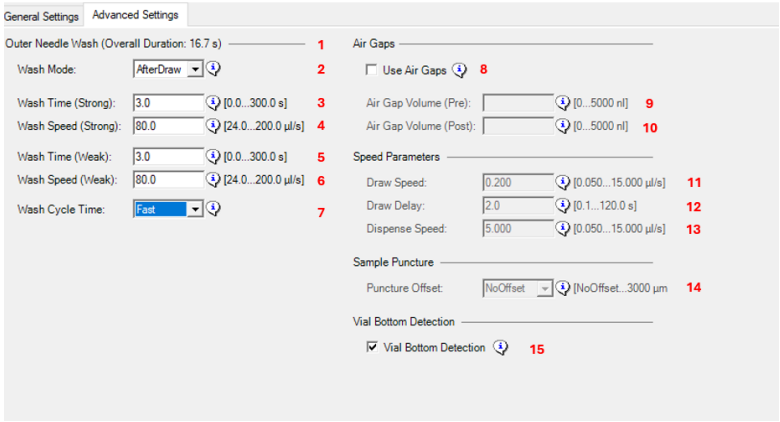


Figure 105: Instrument Method Editor Sampler: Advanced Settings. Note: This page will only be displayed in the instrument method editor and not in the instrument method wizard.

#	Description
1	Outer Needle Wash Overall Duration. – The duration for the needle wash must not exceed 30 seconds.
2	Outer Needle Wash mode performed in the autosampler wash port during the injection procedure.
3	Specifies the duration of the outer needle washing procedure in the autosampler wash port using the strong wash liquid (StrongSolventWp).
4	Specifies the flow speed of the strong wash liquid (StrongSolventWp) delivered by the wash pump to the wash port.

#	Description
5	Specifies the duration of the outer needle washing procedure in the autosampler wash port using the weak wash liquid (WeakSolventWp).
6	Specifies the flow speed of the weak wash liquid (WeakSolventWp) delivered by the wash pump to the wash port.
7	Defines the speed of the needle leaving the wash port after the outer needle wash procedure. The setting “fast” is the default for tandem workflow methods to decrease the sampler cycle time.
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 32: Key to Figure 105 IME Sampler Advanced Settings

8.2 Optimized Default Methods

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

Content	Flow Rate Range	Fluidic Config.	Workflow	TN reference
Deep Dive Proteomics	100nL/min - 1 µL/min	Nano/Cap	Direct Injection	8.2.1
High throughput / translational proteomics	≥1 - 100 µL/min	Cap/Micro	Direct Injection	8.2.2

Table 33: Summary of Tandem Workflow Methods Published in Tech Notes

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

8.2.1 Maximizing Sample Throughput and Sensitivity in Nano and Capillary LC-MS (TN-003335)

Exploring the potential of a novel, intelligent tandem LC-MS workflow with near 100% MS utilization for deep-dive and high-throughput proteomics

This TN showcases the performance and efficiency gains afforded by the Vanquish Tandem Direct injection workflow for high sensitivity, deep-dive proteomics workflows using 3 nano LC columns at flow rates from 100 nL – 1µL/min.

The data quality proves equivalent to that afforded by their Direct Injection workflow counterparts, whilst at the same time the MS utilization is increased to between 74 and 99% depending on column and flow rate.

Furthermore - the superior washing capability afforded by the tandem-workflow methodology was demonstrated for TMT-labeled samples, where carryover levels could be reduced to yield 0 peptide identifications in long (180 min gradient) methods.

8.2.2 A Dual-Column Single-Spray Configuration for Capillary and Micro-flow LC-MS applications (TN-003314)

Demonstrating a tandem LC-MS configuration for high-throughput proteomics and peptide quantification with near 100% MS utilization

This Technote showcases proof-of-principle experiments for Capillary (1 – 5µL/min and micro-flow (5 – 100µL/min) flowrate applications using a post column valve to switch the active column in-line with a single spray MS ion source. Applications are showcased using 4 different diameter column types from the novel 150µm x 150mm I.D. DNV150150PN capillary columns, through 300µm I.D. – 1mm I.D, spanning flowrates from 1.5 – 50µL/min affording MS utilization rates of 85 – 96%.

Column to column reproducibility was also assessed, comparing 8 x the novel 150 µm x 150 mm columns, yielding only a 4% variation in retention time over 30 injections and less than 6% CV of the median protein abundance when enabling match between runs. Methods are described which permit a throughput of up to 225 samples per day in direct injection mode with a minimum MS utilization of 90%. Column carry was also investigated using the 150µm x 150 mm column at throughputs of 60 – 180 samples per day using the trapezoidal washing pattern and yielding carryover levels from 0.03% - 0.008% for the 60 and 180 samples per day methods respectively.

9 Tandem Direct Injection Best Practices, Tips and Tricks and System Care

This chapter details best practices and tips and tricks on everything from installing columns and creating sequences to troubleshooting

9.1 Best practices- System Care and Use

9.1.1 Installing the columns into the Dual Spray Source

The following describes how to set-up the Sonation lab solutions DBO dual spray source with 2 x with Acclaim™ PepMap™ RSLC C18 75 µm x 15 cm column (2 µm dp) (P/N 164940) complete with emitters. Note: This guidance assumes that the Sonation double barrel oven, complete with mounting kit (P/N B51004433) has been fitted to the nanoSpray Flex Ion source and that that the Sonation Lab Solutions CO-Control application software has been installed on the Control PC. (see section 7.1.9 and the Sonation Lab Solutions User Manual for more details

NOTICE: The configuration described is required for the Vanquish Neo Tandem Workflow Instrument Qualification with the dual spray source in Nano/Cap fluidic configuration. For further details on Instrument Qualification, please refer to the current version of the Vanquish Neo System IQ Manual.

9.1.1.1 Connecting the emitter to the column

Parts required:

- 2 x Pulled ESI-Emitters (P/N TIP36003010-5)
- 2 x Beige Sleeves to attach the Emitters (P/N SC603)
- 2 x Black Sleeves to attach the Columns
- 2 x MicroTight® Union Assemblies (P/N 13040420)
- 2 x Low Dispersion Y-pieces with insert (P/N 6250.1009)
- Viper Plugs (P/N 6040.2303)
- 2 x Acclaim™ PepMap™ 100 C18 1 mm x 15 cm column (2 µm dp)

TIP All the parts described in this section are either supplied with the Tandem Source Kit (P/N B51004433, see Table 21 for the detailed contents list.) or the Tandem Workflow Kit (P/N 6250.1030 - See Table 20 for detailed contents list)

1. The MicroTight® Union Assembly contains four parts. A body, two screw fittings and a white gauge plug. Attach the white gauge fitting

to one end of the body. Place the assembly on a clean surface (aluminum foil or lint free cloth).

2. Take a pulled ESI- Emitter from the package

3. Remove the protective sleeve by sliding it up the emitter away from the sharp emitter end (see Figure 106)

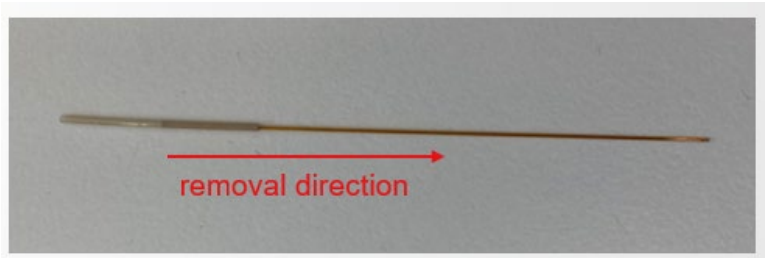


Figure 106: Electrospray Ionization Emitter complete with protective sleeve. The arrow indicates the direction in which the sleeve should be removed to avoid damage to the emitter tip

TIP When handling the emitter, never touch the bare fused silica (tip of the emitter) handle it from the coated part (Figure 107). To prevent the emitter from being contaminated by substances on the skin, it is important to always wear powder-free, sterile gloves when handling it.

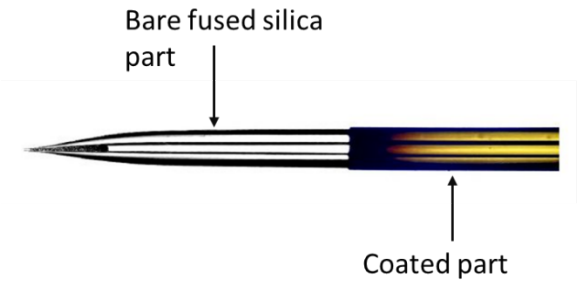


Figure 107: Parts of the Pulled ESI emitter (Figure taken from www.coanntech.com)

4. Slide the sleeve (P/N SC603) onto the back end of the pulled emitter taking care not to touch the sharp end.

5. Take the emitter complete with sleeve and carefully slide the back end into the red screw fitting (found in the MicroTight® union package). Both the sleeve and the emitter should be pushed in far enough so as to extend slightly out of the front end of the screw fitting (see Figure 108)

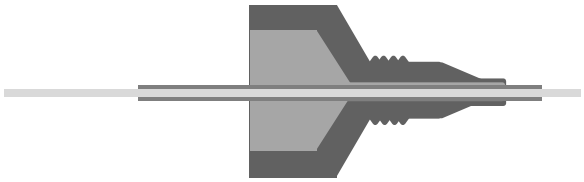


Figure 108: Schematic of the screw-tight fitting with the emitter and sleeve extended out of the front end

6. Hold the MicroTight® screw fitting vertically in one hand with the white gauge plug facing towards the floor and insert the screw fitting into the top of the union (Figure 109).

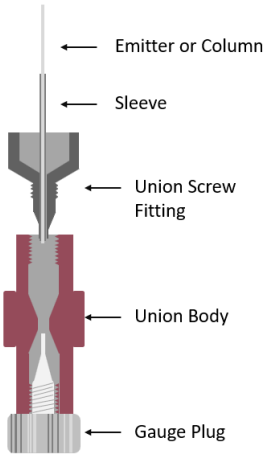


Figure 109: Schematic illustrating the orientation and positioning of the emitter into the union to ensure a dead-volume free connection

7. Carefully screw down the red fitting ensuring that the sleeve and emitter are both pushed into the fitting as far as they will go and tighten securely by hand.

TIP Ensure that the emitter and sleeve are securely fixed in the union by gently pulling on the glass emitter.

8. Remove the white gauge plug from the union. Place the black sleeve (SC903) inside the red fitting of the MicroTight Union assembly and insert the open fused silica end of the column into the sleeve. Ensure that the column fused silica and the sleeve extend out of the end of the screw fitting (as in Figure 108) before inserting the fitting into the open end of the red union. Screw down the fitting until the

column is firmly secured in the fitting. Pull very gently on the column to ensure it is tightly connected.

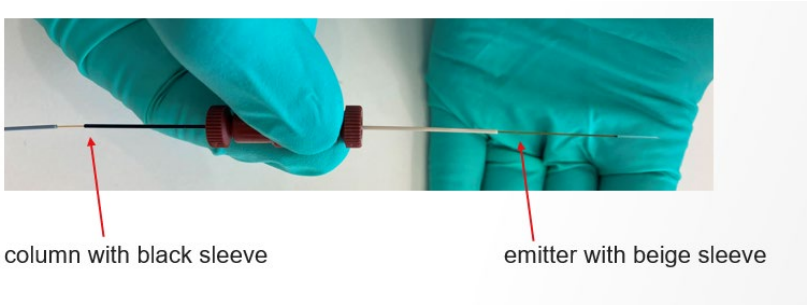


Figure 110: Complete Column and Emitter assembly

9. Repeat steps 1 - 8 for column 2.

10. Place the first column into the double barrel oven. The emitter clamps are spring loaded. Open them by pressing on the levers (Figure 111 a) and carefully place the emitter onto the bottom of the oven plate before releasing the emitter clamp (Figure 111 b).

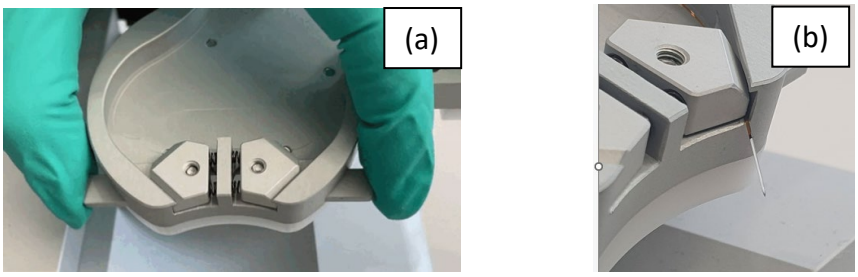


Figure 111: Open the emitter clamp on the DBO source (a). Insert the emitter by placing it on the bottom of the DBO oven before releasing the emitter clamp (b)

11. Repeat step 10 for column 2 (see figure 112)

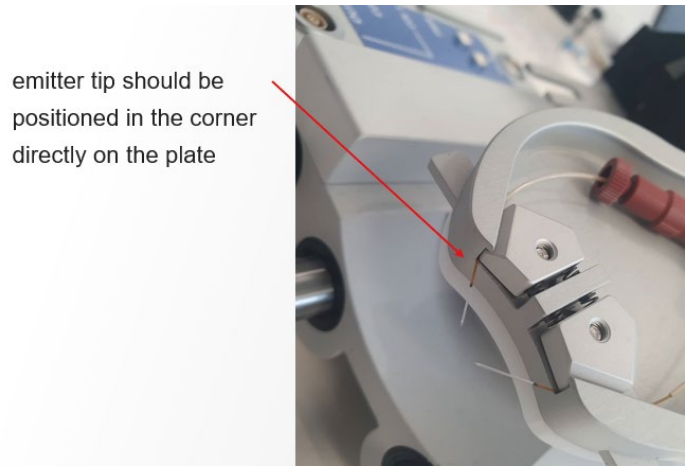


Figure 112: Double Barrel Oven complete with 2 x Emitters and Columns

12. Assemble the low dispersion Y-piece (P/N 6250.1009): Install the insert into the holder and use the white retaining / alignment plug to keep it in place.

TIP The Y piece inlet is a UHPLC compatible low-dispersion connector. It serves both as a liquid junction interface to transfer the voltage from the ion source to the solvent as well as the connection between the capillary inlet from the LC and the column.

13. Connect the Y-piece to the Viper Plug and the column inlet using the Viper Torque Tool. Disconnect the alignment plug and place the y-piece into the UHPLC liquid junction holder (Figure 113).

Figure 113: Low dispersion Y-piece with Viper Blind Plug Installed (and alignment retaining plug removed (a) Low dispersion Y-piece attached to the column inlet and mounted on DBO Liquid Junction Holder (b)

14. Connect the connections for the second column
15. Connect the outlet from the LC to the low dispersion Y-pieces to complete the fluidic setup for the columns.

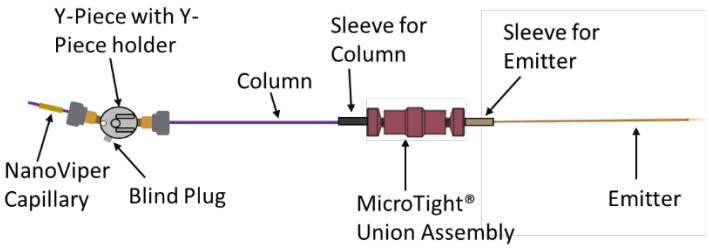


Figure 114: Schematic depicting the complete flow path for a single column configuration.

NOTICE Ensure that the left hand column is connected to valve port 1 on the TCC and the right hand columns is connected to valve port 3.

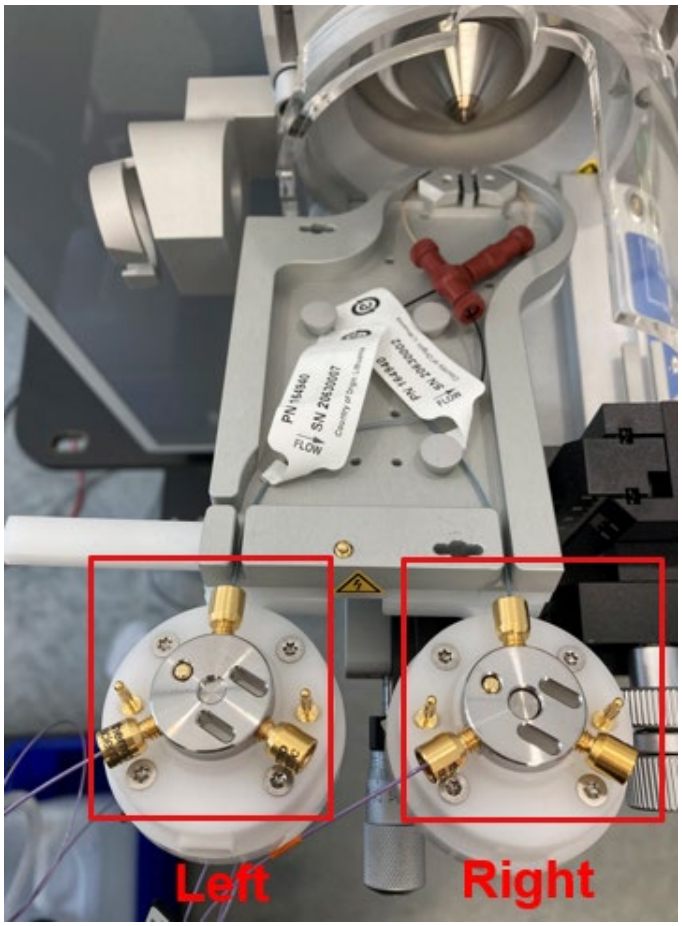


Figure 115: Left and Right Column Connectivity in the DBO

16. Place the top cover of the Y-holders onto the Y-pieces and attach the top part of the oven as instructed in the Sonation Lab Solutions User Manual.

TIP Be aware that the oven plate must be completely closed (without any gaps between the oven and the upper heating plate otherwise the heating element will not be recognized.

9.1.1.2 Adjusting the Emitter Tip Position

The Emitters should be positioned as close as possible to the MS source Ion Transfer Tube (ITT) without the flow being drawn into the vacuum zone (.. The positioning in X, Y, Z axis ((X axis: horizontal emitter adjustment; Y axis: vertical emitter adjustment; Z axis: forward and backward adjustment (toward or away from the MS ion transfer tube)) could be fine adjusted by XYZ-manipulator knobs. Typically, the emitter to spray cone outlet distance is 1-3 mm, and it is possible to finely adjust and verify this distance using the MS source camera monitors.

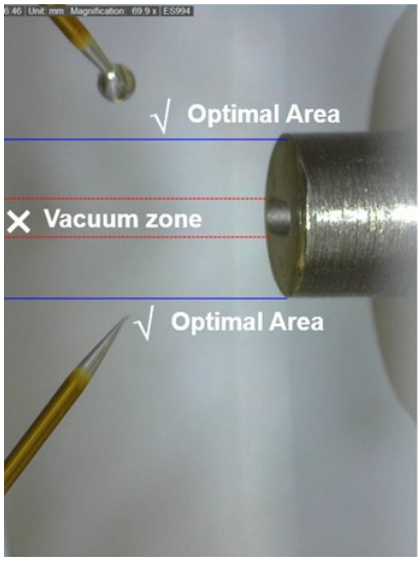


Figure 116: Correct emitter positions for the DBO source

CAUTION To ensure personal safety, check that the MS is set to stand-by mode or that the high voltage is turned off before opening the DBO and installing or removing the columns or emitters.

TIP Ensure that the positioning in X, Y, Z axis for both emitter tips is equal by verifying the alignment using the left and upper cameras in the NanoSpray Flex Ion Source mounting kit.

9.1.2 Troubleshooting the Sonation COControl Software Error

If the top of the Double-Barrel Column Oven is not sat tightly on the body of the oven then the heating element in the top part of the oven will not be recognized. The dialogue box for the Double-Barrel Column Oven control software displays an error along with an implausible column temperature (See Figure 117). This is most commonly caused by a fluidic capillary or the column being trapped between the heating plate and the body of the oven. Coil the offending item carefully back into the oven and replace the top plate.

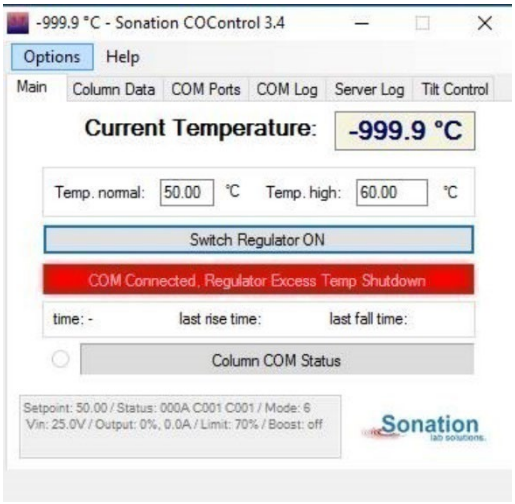


Figure 117: Dialogue box for Sonation COControl 3.4 Software when the regulator is off

9.1.3 Monitoring Spray Stability Prior to Sequence Start

Before starting the run, check the spray stability by selecting each column in Column Compartment N direct control module box (for details see section 7.2.3).

TIP Use the “Tune” Control Software App to monitor the Ion Spray Stability and optimize the MS instrument Tune settings. Use the “Spray Check” direct control option on the VUI Column Compartment Module box to switch the voltage between the columns

9.1.4 Sequence submission, deletion and resubmission

9.1.4.1 Selecting the Column for running the first sample in the sequence

Prior to running the first sample in a Tandem sequence, the column intended for the first sample separation must be conditioned (washed and equilibrated) and loaded with sample. During this time, the gradient programmed in the method is run on the second column. The process can be considered as a “hidden blank” at the start of the sequence.

TIP The hidden blank run is always equal in length to the duration of the method. During the hidden blank, the VSC displays the message “Running- Prepare for Run”. No data are recorded and the Mass Spectrometer remains waiting for the contact closure start signal.

LC system data (logfiles downloaded from the Vanquish Neo VSC) pressure profiles for the first injection in the sequence include the data from the hidden blank run (outlined in yellow in Figure 118 below).

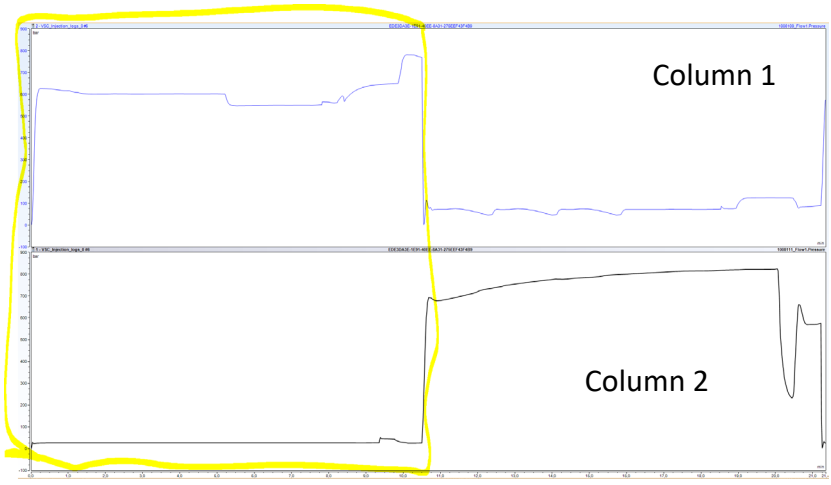


Figure 118: Pressure profiles for the first run in a sequence revealing the “hidden blank” run – highlighted in yellow. In this case, data acquisition starts using column 2.

For the sequence corresponding to the first injection shown in Figure 118 above, the first data acquisition file will be run using column 2 although the separation pump was set to run on column at the beginning of the sequence. If no column has been specified, the column which is currently subjected to flow from the separation pump will be exposed to the hidden blank at the beginning of the next sequence.

NOTICE: If Column 1 (Left) should be used to generate the first data set in the sequence, set Separation Pump Flow to -> Column 2 (Right) and vice versa. Instructions on how to select a specific column prior to sequence start are given in section 7.2.3. Note: If the workflow is configured for single spray analysis and the valve position configuration does not reflect a tandem compliant state, the system will default to column 1 as the starting column.

9.1.4.2 Sequence Submission

As mentioned above, the sequence start always necessitates the execution of a “hidden blank”. It is advisable to submit as many runs as possible in a single sequence to obtain the maximum benefit from the increase in detector utilization afforded by the tandem workflow.

Other considerations for sequence submission include:

- The LC method component must be identical for all runs in a sequence
- The MS method may vary within a sequence
- The injection volume may vary within a sequence however, it must remain within the limits set in the LC Instrument Method (Basic Tandem Settings Page)

TIP: During the last injection of a sequence, no sample pickup is performed. The separation column that was used for the final gradient in the sequence is not subject to the wash cycle that was programmed into the tandem workflow method. However, this column will be washed during the hidden blank injection at beginning of the subsequent sequence as long as the TCC valve positions are not manipulated by the user between the sequences.

9.1.4.3 Sequence deletion and resubmission

The nature of the tandem workflow operation is such that whilst an analysis is being performed on the first column, the second column is being washed, equilibrated and loaded with sample for the subsequent run. Because of this, injection “n+1” must be taken into consideration if an injection abort for injection “n” is carried out.

NOTICE: Carefully decide how many runs to submit in a sequence as each new sequence submission will require the execution of a hidden blank run. If it is necessary to delete a run in the middle of sequence table, it is best to delete the rest of the sequence from that point and re-submit to avoid sample assignment errors

9.1.5 System Care und Use

9.1.5.1 Cleaning of ESI-Emitters

Emitters should be cleaned with proper Optima™ LC-MS solvents (see Section 4.1.2) e.g., use Acetonitrile when clusters are seen and there is dramatic loss in signal intensity (See Figure 119) by dipping the emitter into a beaker with solvent for a couple of seconds.

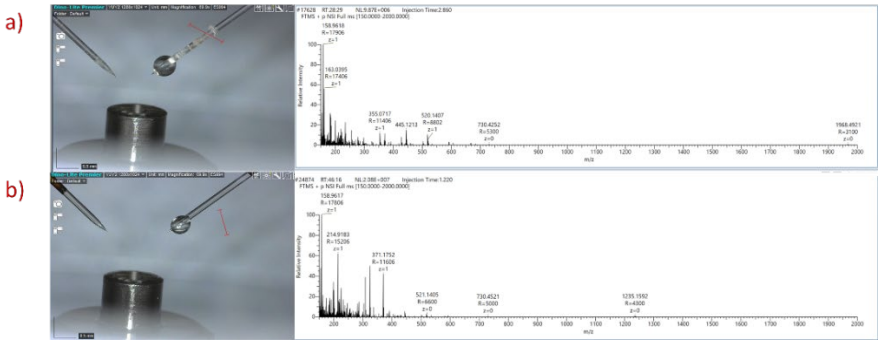


Figure 119: Representative comparison of emitter a) with clusters without any cleaning for a long time of operation b) cleaned emitter resulting 2 times better intensity and 2 times less injection time.

9.1.5.2 Considerations when switching to the Tandem Direct Injection Workflow from Direct Injection Workflow

This will most commonly occur when an already installed Vanquish Neo System is being upgraded to the Tandem Direct Injection Workflow.

During the installation of the upper (separation) pump, care should be taken to purge the pump sufficiently to flush the residual Isopropanol from the pump and flow meter.

If the upper pump is being re-activated after a period of long term shutdown. Care must also be taken to purge the attached solvent lines from any residual Isopropanol before putting the system into operation.

9.2 Tips and Tricks

9.2.1 Adding and Removing Vials/Wellplates to and from the Sampler

The precise scheduling of different tasks in the tandem direct injection is precisely controlled by the workflow. Any deviation from the allotted time windows will cause the workflow to abort.

For the autosampler, this impacts when the user can add and remove samples from the system because opening the sampler door inhibits the execution of scheduled tasks if a conflict occurs. For this reason, the user is given **precise instructions on when they can or cannot access the autosampler tray.**

This information is provided in the **Sampler Module Box on the VUI** whilst the tandem workflow sequence is running (Figure 120.)

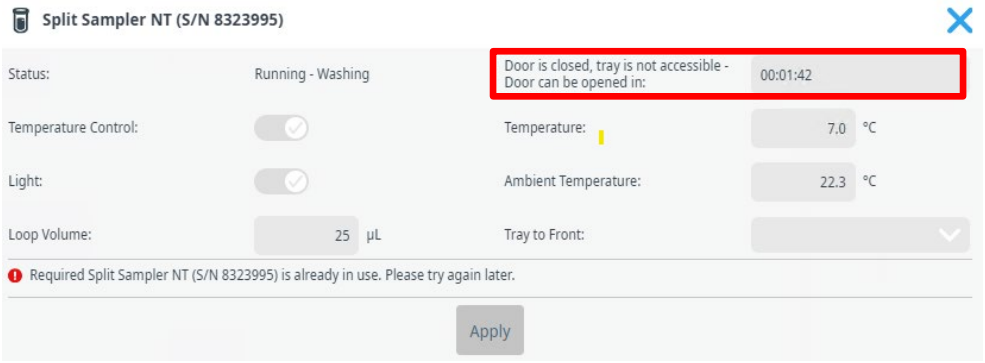


Figure 120: Sampler Module Box dialogue display indicating: sampler door status (open or closed); whether the tray can be accessed or not; duration until the next change in status (how long until it will be possible to open the door to add or remove samples)

A similar message is displayed once the door can be opened, Figure 122

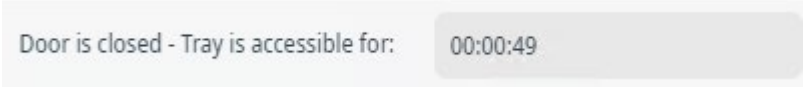


Figure 121: Dialogue Box indicating that the Sampler Door is closed, that the Tray can be accessed and also for what duration

TIP: The sampler carousel must be rotated manually to access the tray compartments whilst the sample sequence is running.

9.2.2 Method Transfer from Direct Injection to Tandem Direct Injection

9.2.2.1 Understanding the "Delay Time" in the Tandem Direct Injection Workflow

In low-flow LC-MS applications, the time it takes for the eluting gradient to travel from the outlet of the pump to the detector can be significant. This can lead to substantial time offset between the gradient window and the detection window.

The delay time setting in the Basic Tandem Settings of Direct Injection Workflow (see Figure 98 in section 8.1)is used to adjust the time delay between the gradient delivery from the pump and the data acquisition at the detector so that only the analyte elution chromatogram window is recorded for each sample run.

1. If no delay is programmed in the tandem method– the detector starts to acquire data as soon as the gradient is triggered –
   - An empty chromatogram in the first part of the elution window (as experienced in 'non-tandem' low-flow applications)
   - Risk of missing analyte data if the data acquisition window is not significantly longer than the gradient elution window. (The data acquisition stops before elution of the entire sample to the detector is complete).
2. If too much delay is programmed in the method – The detector starts to acquire data after a portion of the sample has already eluted. Instead, the detector will continue to acquire data during the wash phase of the workflow cycle = no sample data.
3. Optimized delay time – Data acquisition starts just before the first analyte reaches the detector and stops just after the last analyte has been eluted into the detector, before switching to record sample data for the next sample.

9.2.2.2 Example Method Transfer from Direct to Tandem Direct Injection

This example details how to successfully transfer a 10 minute gradient method running at 50 µL/min from a Direct Injection to a Tandem Direct Injection workflow. A schematic representing the flow composition gradient profile is shown below. (**TIP** The delay time is the time until the analyte reaches the detector. In this example, this occurs at around 2.7 minutes. **It is advisable to leave a buffer of approximately 0.5 minutes before the first peak elutes** as the chromatogram will tend to shift to the left as the column ages.

1. Program a new method. In the Basic Tandme Settings for the Workflow, changes the **Duration to 10 minutes** and the **delay to 2.3 minutes** (see Figure 128)

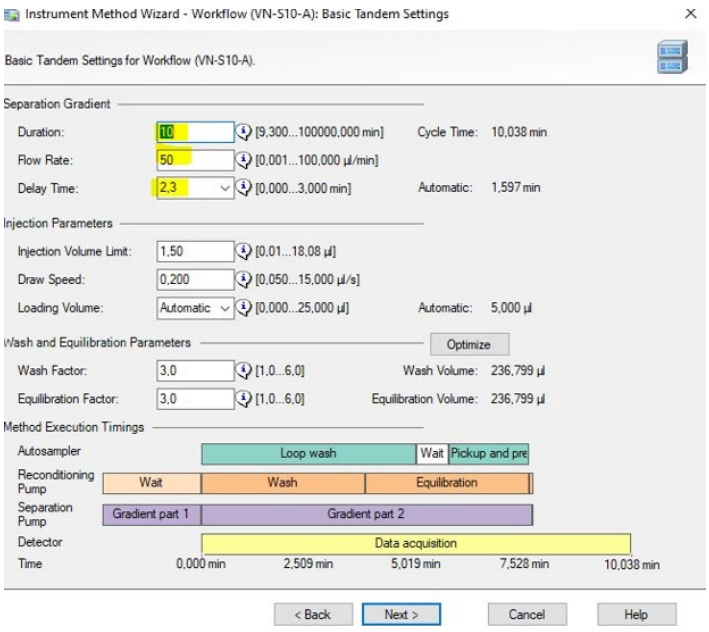


Figure 125).

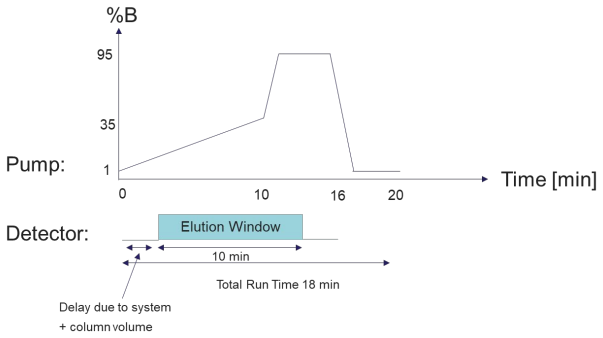


Figure 122: Schematic depicting gradient pattern for the test run

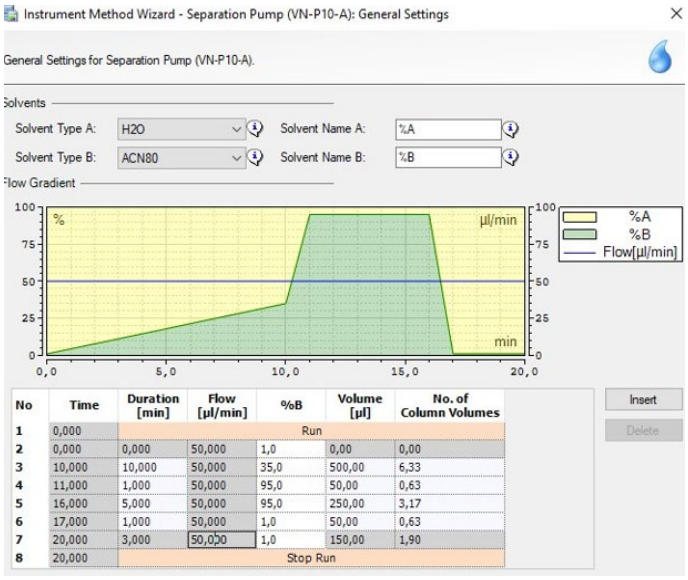
Proceed with the following steps:

2. Start the instrument wizard and specify a extended run time (e.g. 100 minutes).

3. In the Basic Tandem Settings (page 3 on the IMW) set the duration to 20 minutes (for fast gradient methods at high flow rates it is recommended to run a method which is twice as long as the intended gradient). For long columns/ low flow-rates, the extra time allowed can be less, but it should be sufficient to ensure the entire elution profile is detectable.

4. Set the "**Delay Time" in the Basic Tandem Settings to "0"**

5. Program the intended gradient. Make sure to include a wash and equilibration step in the method. (These will not be present in the gradient table for the final method.



No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0,000	Run				
2	0,000	0,000	50,000	1,0	0,00	0,00
3	10,000	10,000	50,000	35,0	500,00	6,33
4	11,000	1,000	50,000	95,0	50,00	0,63
5	16,000	5,000	50,000	95,0	250,00	3,17
6	17,000	1,000	50,000	1,0	50,00	0,63
7	20,000	3,000	50,000	1,0	150,00	1,90
8	20,000	Stop Run				

Figure 123: Example Gradient for 10 minute run including washing and equilibration

6. Run a short sequence. The data for this example appear as follows:

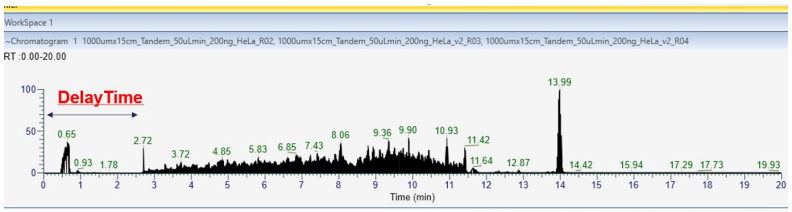


Figure 124: Elution profile prior to delay time adjustment

TIP The delay time is the time until the analyte reaches the detector. In this example, this occurs at around 2.7 minutes. **It is advisable to leave a buffer of approximately 0.5 minutes before the first peak elutes** as the chromatogram will tend to shift to the left as the column ages.

7. Program a new method. In the Basic Tandme Settings for the Workflow, changes the **Duration to 10 minutes** and the **delay to 2.3 minutes** (see Figure 128)

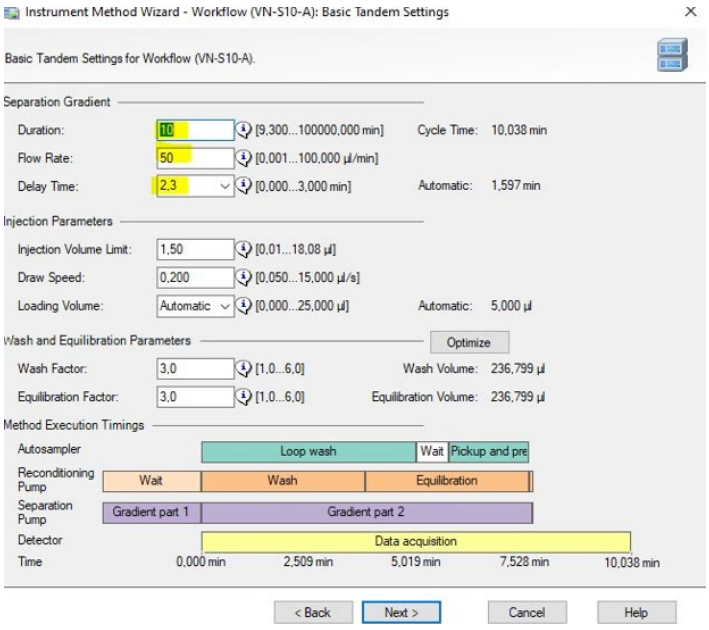


Figure 125: Updated Tandem Basic Settings for the Optimized Method

8. Program the gradient table.

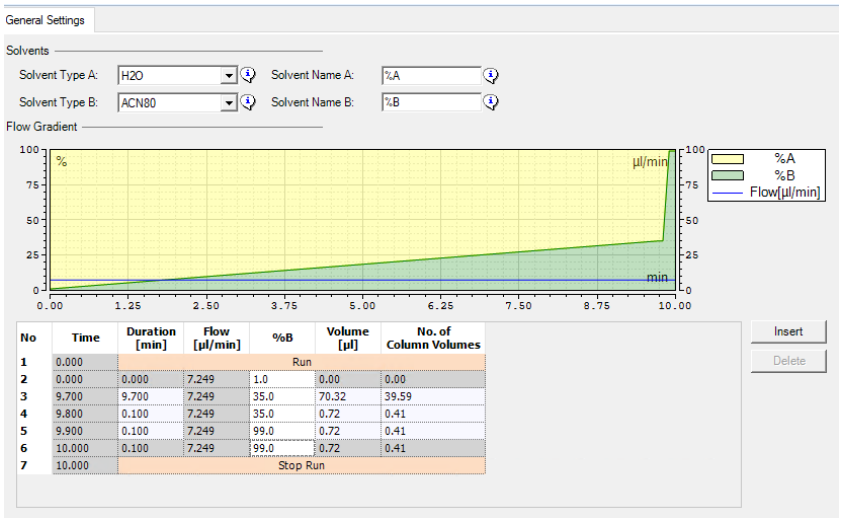


Figure 126: 10 minute Gradient Table

The Chromatogram with the final result after delay time adjustment with 10 min gradient:

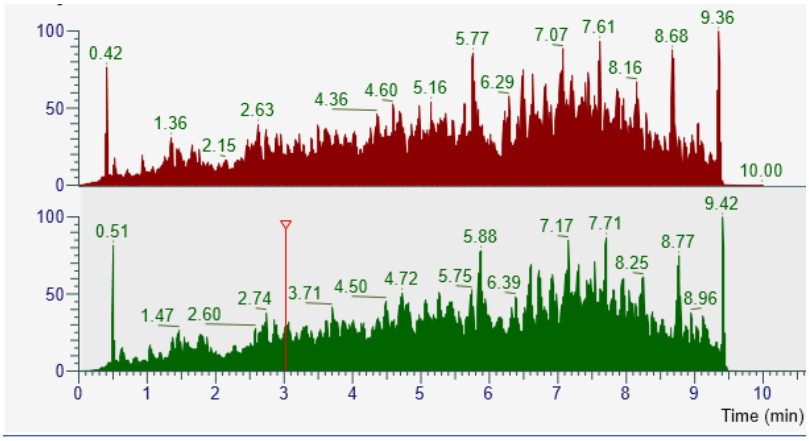


Figure 127: Example chromatograms comparing column 1 and 2 in tandem workflow after delay time adjustment

TIP The Gradient may need to be adjusted to maximize the elution profile.

9.2.3 Switching from the Tandem Direct Injection to Direct Injection or Trap-and-Elute Workflows

9.2.3.1 Switching to the Direct Injection Workflow whilst shutting down the Upper (Separation) Pump

Whist changing from the Tandem Direct Injection Workflow to the Direct Injection Workflow, the user is presented with a toggle option to shut down the separation (upper) pump. This option should only be selected if the upper pump will be out of use for an extended period (i.e. several weeks, due to repair of if the pump will be put in long-term storage). NOTE – Isopropanol is required to flush the pump during the long term shutdown procedure, after which the solvent lines on the pump are emptied. Once the procedure is complete, all open connections (e.g. pump inlets) should be plugged

9.2.3.2 Switching to the Trap-and-Elute Workflow

If you wish to change your workflow from Tandem Direct Injection to Trap and Elute,

4. Change the workflow to Direct Injection by running “Change Fluidics / Workflow: Tandem” script (Script A00)”

5. Change to the “Trap and Elute” workflow by running script A06 “Change Fluidics / Workflow.

TIP The A06 “Change Fluidics / Workflow” script is not visible whilst the Tandem Direct Injection Workflow is configured. It re-appears after the Direct Injection workflow has been configured.

9.2.4 Determination of which was analyzed using which Column in the FreeStyle App

The information regarding which column was used to acquire which sample is stored in stored with the .raw data file and can be accessed via the FreeStyle App.

6. Open the .raw file in FreeStyle
7. Enable the “A/D Card” Channel (Sampler pressure trace appears)
8. Click on the Report Icon Tab in the Workspace Options Ribbon
9. Select Status log
10. The Active column during the sample analysis is displayed in the “Value” column in the table. Designations are “1 (Left)” or “2 (Right)”.

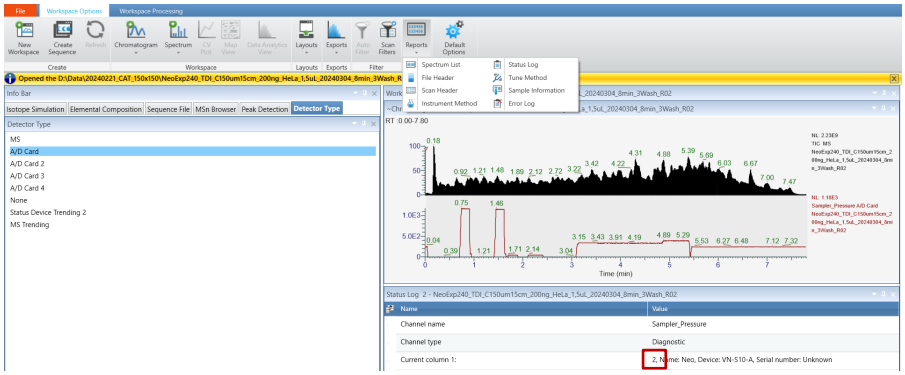


Figure 128: Sample chromatogram displayed in FreeStyle with the “active column” used to acquire the data file displayed.

TIP The active column used for the sample data acquisition is displayed under the name “Current column 1:” The integer value in the title is misleading. It is the value property (‘2’ in the example in Figure 128) which defines the column.

10 Appendix

Miscellaneous Information relevant to system operation, care, use and troubleshooting

10.1 Example Reference data from the Vanquish Neo System Installation Qualification

10.1.1 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 several 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.

10.1.2 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 129) and trap-and-elute (Figure 130) respectively.

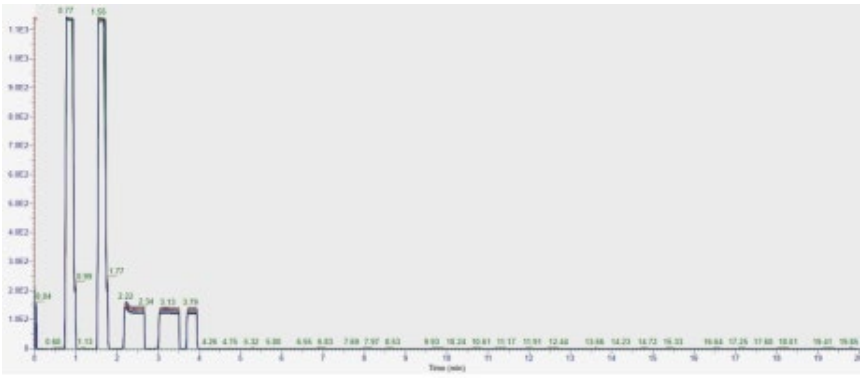


Figure 129: Sampler Pressure Profile for Direct Injection

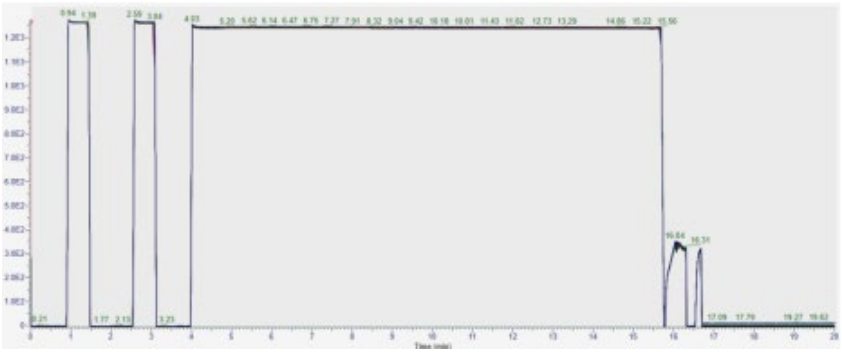


Figure 130: Sampler Pressure Profile for Trap-and-Elute

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

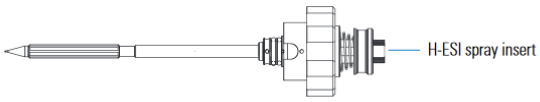
10.2 The Ion Max and Ion Max NG Ion sources for heated electrospray ionization

10.2.1 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 131).

Heated Electrospray Ionization (HESI-II) Probe for the IonMax 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 131: Ionization probes for the Ion Max and Ion Max NG sources.

10.2.2 Low-flow metal needle kit for microflow applications

A low-flow (50 µm I.D.) metal needle Figure 132 is required for low-flow experiments (5 – 100µL/min) 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 132.

Source version	Capillary to metal needle	Part number to order
NG	Fused silica	OPTON-30697, KIT NG, HESI LOW FLOW Metal Needle Insert 50umID 35G 50umID NanoViper (50µmX150mm, 1 piece)
	PEEK	OPTON-30138, KIT NG, HESI LOW FLOW Metal Needle Insert 50umID 35G 65umID PEEK Viper (65µmX150mm, 5 pieces)
Non-NG	Fused silica	OPTON-30136, KIT (see below) + 6041.5124 (NanoViper Capillary IDXL 50µm X150mm)
	PEEK	OPTON-30136, KIT HESI LOW FLOW Metal Needle Insert 50umID 35G 65umID PEEK Viper (65µmX150mm, 5 pieces)

Figure 132:Low-Flow Metal Needle Kit compatibility matrix for Ionization probes for the Ion Max and Ion Max NG sources

Figure 133: Low flow needle insert "L" (P/N OPTON-30697 Kit). Adjust the needle position closest to the ion transfer tube horizontally (to 1) and vertically (to L).

10.3 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 the most commonly 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.

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