Installation Guide – Thermo Scientific™ Vanquish™ Duo for Tandem LC or LC-MS

Observe the safety information and precautionary statements, unpacking and installation instructions presented in the *Operating Manual* for the respective Thermo Scientific™ Vanquish™ modules.

This guide provides condensed information for installing a Thermo Scientific Vanquish Horizon Duo or Flex Duo UHPLC system for Tandem LC or LC-MS.

Required Parts

Table 1: Modules required for installation of Vanquish Horizon Duo or Flex Duo UHPLC system for Tandem LC or LC-MS

Component	Vanquish Horizon Duo for Tandem LC or LC-MS			Vanquish Flex Duo for Tandem LC or LC-MS	
System: Base, Rack & Capillaries	• 1x System Base Vanquish Horizon/Flex (VF-S01-A-02) • Optional: 1x Passive Pre-Heater (6732.0174) • 1x Vanquish Duo for Tandem LC Kit (6036.2020) (includes further capillaries and valves) • For MS installation: MS Connection Kit for Vanquish (6720.0405) <u>Stack Stabilizer for stacks ≥ 5 modules</u> • Ion Bench with stack mounting kit (6036.1720) *or* • Bench Clamp Kit for benchtop installation (6036.1740) *or* • Stack Stabilizer Kit for benchtop installation (6036.1710)				
Pumps	• 2x Binary Pump H (VH-P10-A-02)			• 1x Dual Pump F (VF-P32-A-01) *or* • 2x Binary Pump F (VF-P10-A-01) *or* • 2x Quaternary Pump F (VF-P20-A) *or* • 1x Binary Pump F and 1x Quaternary Pump F	
Samplers	1x Split Sampler HT (VH-A10-A-02)			1x Split Sampler FT (VF-A10-A-02)	
Optional Sample Extension	1x Charger (VH-A90-A)				
Column Compartments	1x Column Compartment H (VH-C10-A-02)				
Any Combination of Two Detectors	Diode Array • HL (VH-D10-A) • FG (VF-D11-A-01)	Variable Wavelength • F (VF-D40-A)	Fluorescence • F (VF-D50-A) • F Dual-PMT (VF-D51-A)	Charged Aerosol • H (VH-D20-A) • F (VF-D20-A)	ISQ EC (ISQEC-LC)
Flow Cells	Selection of wide range of flow cells per each optical detector			n.a.	
Mass Spectrometers	Selection of wide range of mass spectrometers				

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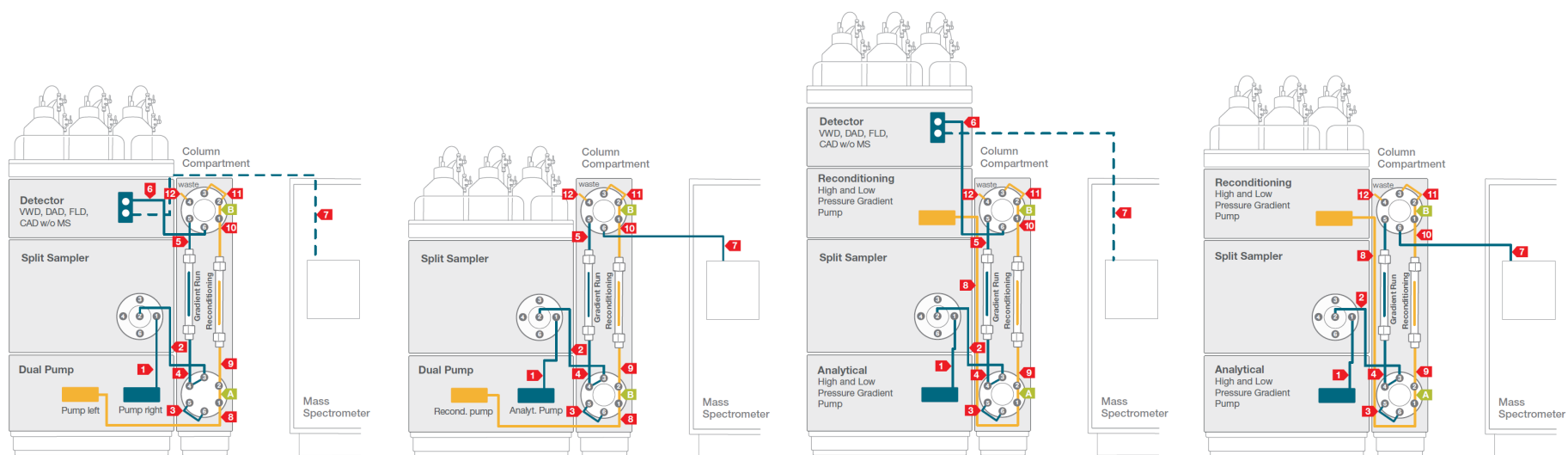*© Thermo Fisher Scientific, All rights reserved.*

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Figure 1: Vanquish Duo for Tandem LC or LC-MS flow schemes

Table 2: Required system tubing and items, compatible with any configuration from Table 1.

No.	Description	No.	Description
1	Viper Capillary, 0.10 x 450 mm, MP35N (6042.2350)[2]	9	Active Pre-Heater, 0.1 x 380 mm, VH-C10 (6732.0110)[1] optional: Passive Pre-Heater, 0.1 x 530 mm, MP35N (6732.0174)[3]
2	Viper Capillary, 0.10 x 450 mm, MP35N (6042.2350)[1]	10	Viper Capillary, 0.10 x 150 mm, MP35N (6042.2320)[1]
3	Viper Capillary, 0.10 x 150 mm, MP35N (6042.2320)[1]	11	Viper Capillary, 0.10 x 150 mm, MP35N (6042.2320)[1]
4	Active Pre-Heater, 0.1 x 380 mm, VH-C10 (6732.0110)[2] optional: Passive Pre-Heater, 0.1 x 530 mm, MP35N (6732.0174)[2]	12	Waste tubing, VH-D1 (6083.2425)[1]
5	Viper Capillary, 0.10 x 150 mm, MP35N (6042.2320)[1]	A	Valve 2-p 6-p 150MPa bio VH-C1 (6036.2520)[1]
6	Post Cooler 1µL, 0.1 x 590 mm, VH-C10 (6732.0520)[1]	B	Valve 2-p 6-p 150MPa bio VH-C1 (6036.2520)[1]
7	Capillary to mass spectrometer (for Tandem LC-MS setups only)[5] Optical detector waste fluidic (for Tandem LC setups only)[4]		*Flow Schemes Vanquish Duo for Tandem LC-MS* (4820.3615): four different flow schemes are included in the delivery showing the stack and tubing for four different configuration options. Flow Schemes can be clipped into right sampler door[1]
8	Viper Capillary, 0.10 x 950 mm, MP35N (6042.2395)[1]		Optional: Set color marker labels (blue and red) for capillaries, solvent lines and wash lines[1]

[1] Included in Vanquish Duo for Tandem LC Kit (6036.2020), [2] Included in System Base Vanquish Horizon/Flex (VF-S01-A-02), [3] not shipped with system, to be ordered separately, [4] Included in respective detector ship kit,[5] Included in MS Connection Kit for Vanquish (6720.0405)

Hardware and Software Installation

1. Locate and unpack all required system components as shown in Table 1 and Table 2 and follow the instructions in the *Vanquish System Operating Manual*.

2. Identify your configuration out of the four *Flow Schemes (Figure 1)* and stack the modules accordingly. Some Vanquish configurations may require the stacking of more than four modules (e.g. Vanquish Duo setups with two separate pumps and two detectors). This installation mandatorily requires an appropriate protection against tilting.

    > **NOTICE**: Thermo Fisher Scientific offers various technical solutions for tilting protection, e.g. Bench Clamp Kit (6036.1740) or Stack Stabilizer Kit (6036.1710) for benchtop installation or Ion Bench with Stack Mounting Kit (6036.1720). Make sure that an appropriate tilting protection is installed for safe operation*.

    > **TIP**: For installation instruction of the stack stabilizing follow the respective instructions delivered with the stack stabilizing options.

3. Install the two biocompatible Vanquish 2-position/6-port column switching valves following the instructions in the *Vanquish Column Compartment Operating Manual*.

4. Connect all power cords, USB and system interlink cables as described in the respective modules *Operating Manual*. Diode array detectors may need to be plugged into the PC individually.

5. Connect all system tubing and solvents

    a. For solvent lines follow the *Vanquish Pumps Operating Manual*.

    b. For rear seal tubing follow the *Vanquish Pumps and Sampler Operating Manual*.

    c. For needle wash tubing follow the *Vanquish Sampler Operating Manual*.

    d. For system capillaries install each capillary according to the numbering on the *Flow Scheme* for your configuration and part of the Vanquish Duo for Tandem LC Kit. Instructions on how to install the passive pre-heater are outlined in the *Installation Note* of the passive pre-heater. For installing the capillaries correctly, please read the *Viper Installation and Operating Guide.*
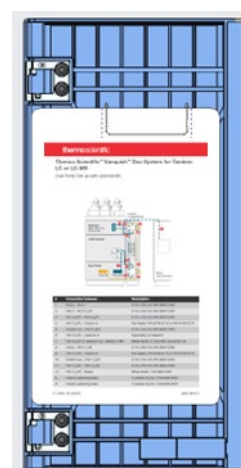

Figure 2: Flow scheme in sampler door

> **TIP**: The *Flow Scheme* of the installed system configuration can be clipped into the right sampler door (Figure 1).

    e.   For the waste fluidic follow the *Quick Installation Guide* delivered with the waste fluidic.

    f.   For Vanquish Duo Tandem LC-MS setups follow the instructions in the *MS Connection Kit for Vanquish Quick Installation Guide* to connect the instrument to the corresponding mass spectrometer.

6. Optionally install tubing markers that are delivered with the system ship kit on the flow paths for optical distinction.

7. Install the two analytical columns.

> **TIP**: If operational qualification of the hardware is required please follow the *Operating Instructions for Operational Qualification/Performance Qualification for HPLC Instruments*.

8. Switch on all modules in the system.

9. Install Thermo Scientific Chromeleon 7.2.8 (or higher) Chromatography Data System (CDS). Alternatively, install SII for Xcalibur 1.4 (or higher) with the corresponding Thermo Xcalibur version. Detailed compatibility information can be found in the *SII for Xcalibur Release Notes*.
   Follow the instructions in the section **Software Configuration** to configure and operate the Vanquish Duo for Tandem LC or LC-MS system.

> **NOTICE**: Thermo Scientific Chromeleon 6.80 and DCMS[Link] for Xcalibur will not support Vanquish Duo workflows.

Software Configuration

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

2. Open the **Chromeleon Instrument Configuration Manager** and create 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 create a new Instrument (Figure 2).
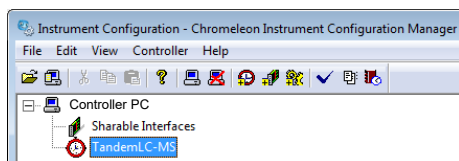
Figure 3: New Instrument

3. Add the pump modules

   a. If a Vanquish Dual Pump is present, choose **HPLC: Vanquish** followed by **Vanquish Dual Pump**. Rename the pump devices and signal names according their functionality. E.g. **ReconditioningPump** for the left pump device and **AnalyticalPump** for the right pump device (Figure 3).
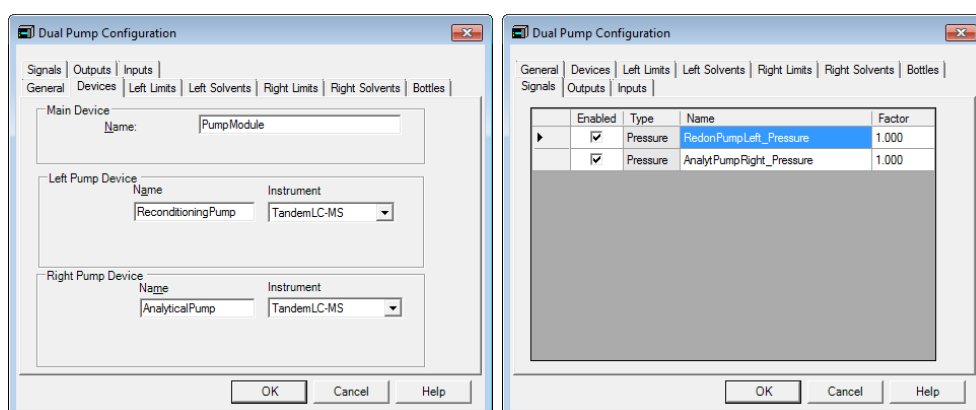

Figure 4: Pump configuration

> ***TIP** The names used here (AnalyticalPump; ReconditioningPump) are examples. Feel free to choose your own.

   b. If two separate pumps are present, choose **HPLC: Vanquish** followed by the respective pump type. It is recommended to add a descriptive extension to the respective device names and signal names (Figure 4). This will show up on the module panels and simplifies distinction.
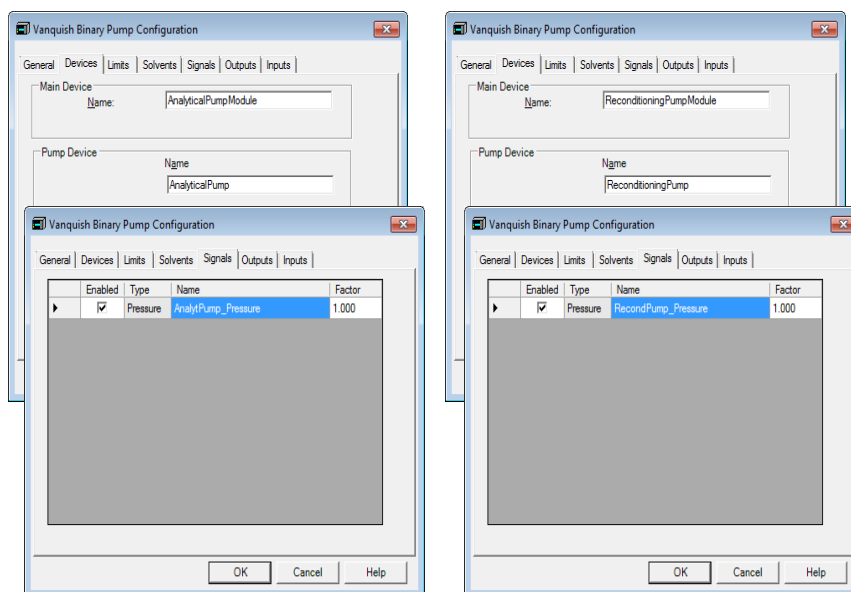
Figure 5: Pump signal channel

c. Add the Sampler to the instrument. Choose **HPLC: Vanquish** followed by **Vanquish Autosampler**. Under **Device Type**, choose the Vx-A10-A version that reflects the P/N of the Sampler present. Assign the sampler to the previously generated pump device; assign the appropriate pump link if applicable (e.g., *VPUMP_**R**_STRK*) (Figure 5). Add a Vanquish Charger module if present in the configuration and follow the instructions in the *Vanquish Charger Installation Guide*.
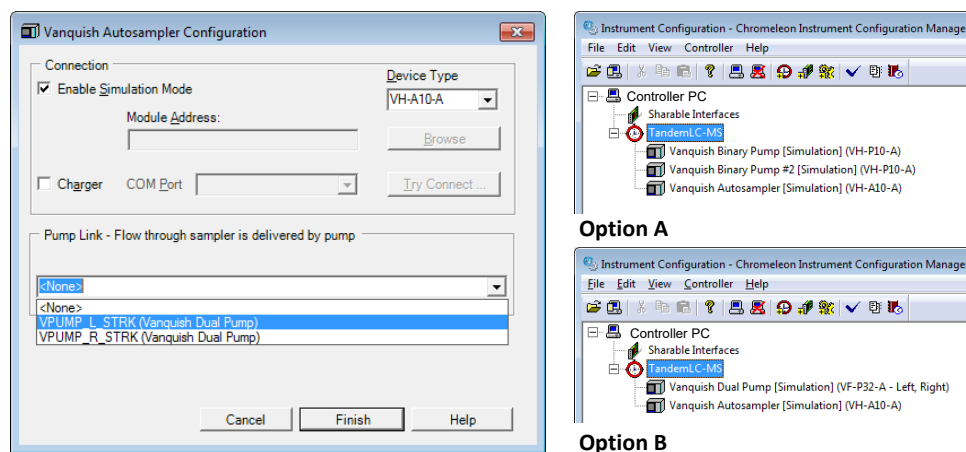

Figure 6: Sampler configuration, Option A based on two separate pumps and Option B with one Vanquish Dual Pump.

4. Add the Vanquish Column Compartment to the instrument. Choose **HPLC: Vanquish** followed by **Vanquish Column Compartment**. Under **Valves**, select the 2 Position 6 Ports Thermo Scientific 150MPa bio valve for the upper and lower valve port (Figure 6).
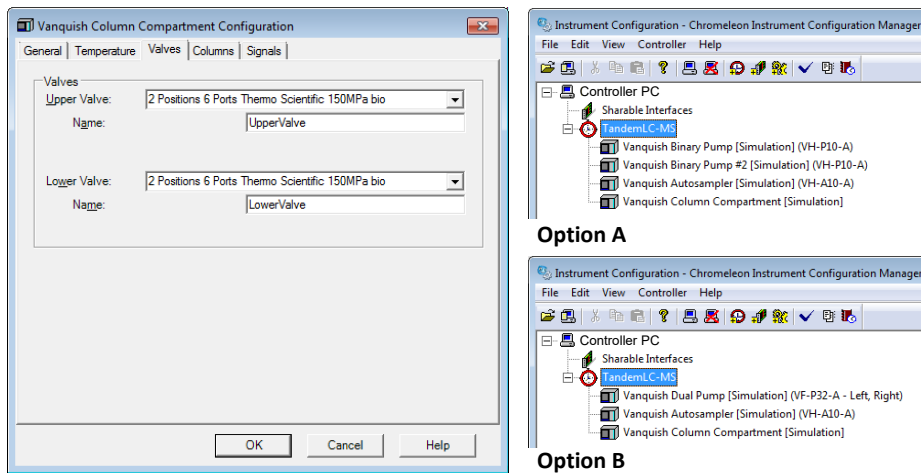
Figure 7: Configuration of column compartment, Option A based on two separate pumps and Option B with one Vanquish Dual Pump.

5.   Optional: Add an optical detector to the instrument. Choose **HPLC: Vanquish** followed by the respective detector type.
For Chromeleon LC-MS installations add also 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* and the *Thermo Xcalibur Qual Browser User Guide.*
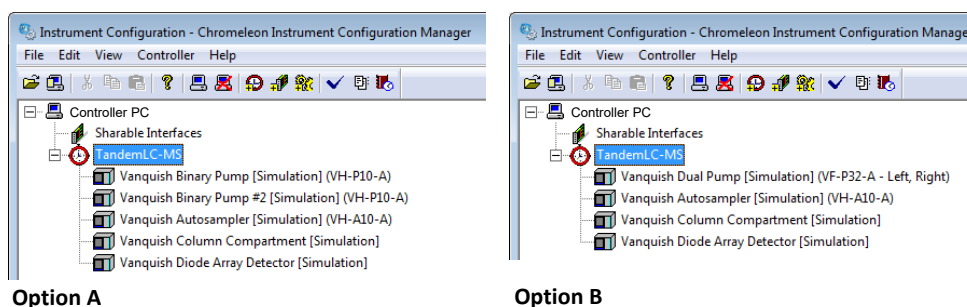
Figure 8: Save configuration, Option A based on two separate pumps and Option B with one Vanquish Dual Pump.

7.   Define the **Fluidic Configuration** for the Tandem LC or LC-MS workflow with the corresponding wizard, which can be started in

a. **Chromeleon** in the Chromeleon Console - Instruments Tab. Select the correct instrument and launch the Fluidic Configuration Wizard with the corresponding icon in the toolbar on top (similar to Figure 8).

b. **Thermo Xcalibur** in the **Direct Control** panel of SII for Xcalibur with the same icon in the toolbar (Figure 8).
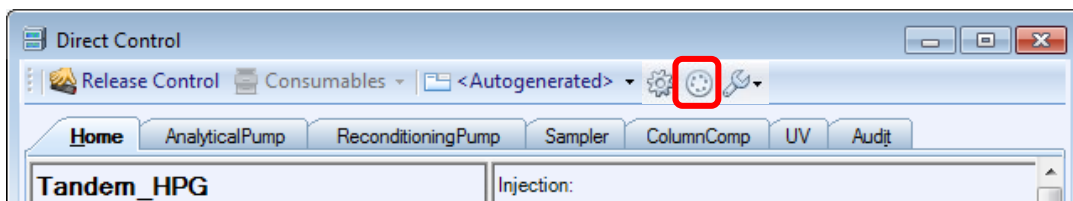

Figure 9: Start Fluidic Configuration Wizard (icon labeled in red)

8. The **Fluidic Configuration Wizard** will guide you through the configuration.

a. Select the corresponding **Capillary Kit**, in this case the Vanquish Tandem LC, on page 1 (Figure 9).

> **NOTICE**: The selection "Single LC" will allow to use the HPLC system in a classic way without using the Tandem LC functionality. The correct valve position of upper and lower valve has to be checked manually to ensure the use of the appropriate column in Single LC mode.

> **TIP**: The drop-down list entries are automatically tailored to the previously created hardware configuration.

b. Check the correct pump assignment for the **Analytical-** and the **Reconditioning Pump** on page 2 (Figure 9).
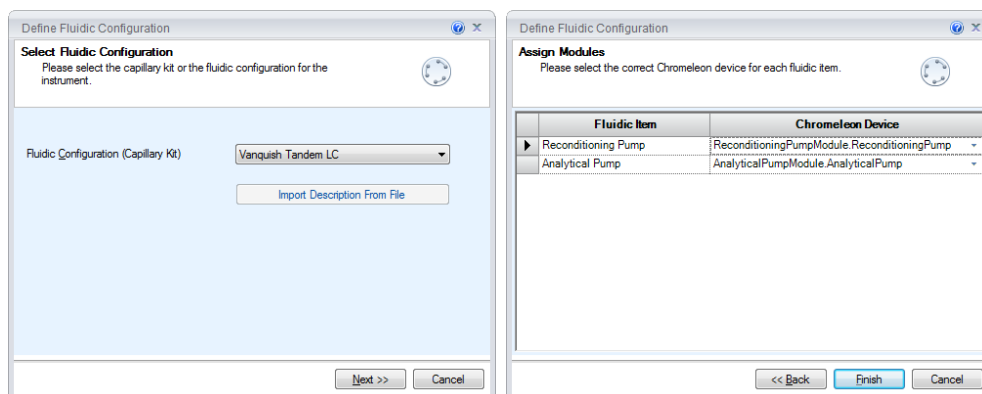
Figure 10: Fluidic Configuration Wizard

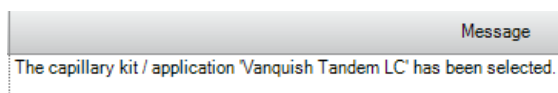c. Finish the wizard and check the Audit Trail if the Fluidic Configuration was saved appropriately (Figure 10).

Figure 11: Audit trail message for a successful Fluidic Configuration procedure

NOTICE: If the type of Pre-Heater is changed, the Fluidic Configuration has to be repeated to apply the changes of the fluidics. The same applies, if you switch from columns with column ID tags to columns without tag and vice versa.

9. Create an instrument method for the instrument by clicking **Instrument Method…** in the **Create** drop down menu. Select the instrument you want to write a method for and follow the prompts of the wizard. For Thermo Xcalibur installations follow the *Thermo Xcalibur Data Acquisition and Processing User Guide*.

NOTICE: The following examples are showing screenshots from Thermo Xcalibur and SII for Xcalibur 1.4. The wizard procedure in Chromeleon 7.2.8 is equivalent.

a. For Vanquish Duo Tandem LC setups, a Regular Instrument Method or a Tandem Instrument Method can be selected (Figure 11). Follow the prompts of the wizard. The first page of the wizard is the same for a Regular Instrument Method and will not be covered here in more detail.
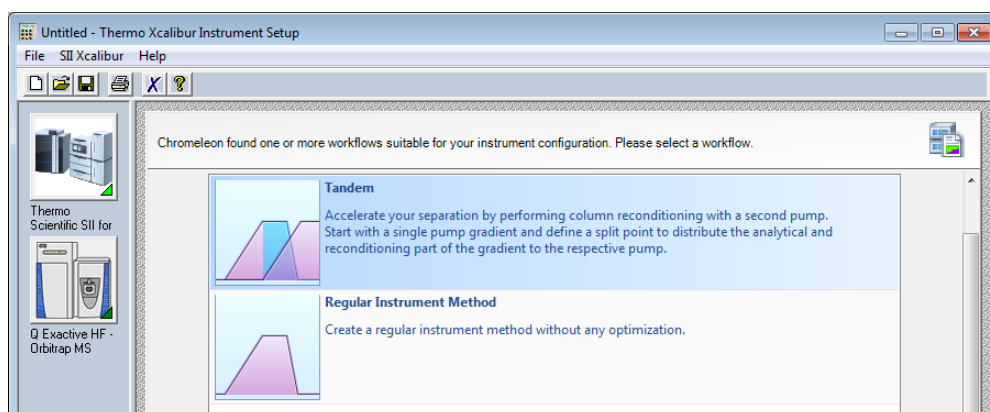

Figure 12: Instrument Method Wizard

NOTICE: The selection "Regular Instrument Method" will allow to use the HPLC system in a classic way without using the Tandem LC functionality. The correct valve position of upper and lower valve has to be checked manually to ensure the use of the appropriate column in Single LC mode.

b. Define the currently used **single pump method**, the wizard will create a Tandem Method automatically (Figure 12). By setting the **split point**, typically after the analytical gradient, the separation of the analytical part and the reconditioning part will be specified.
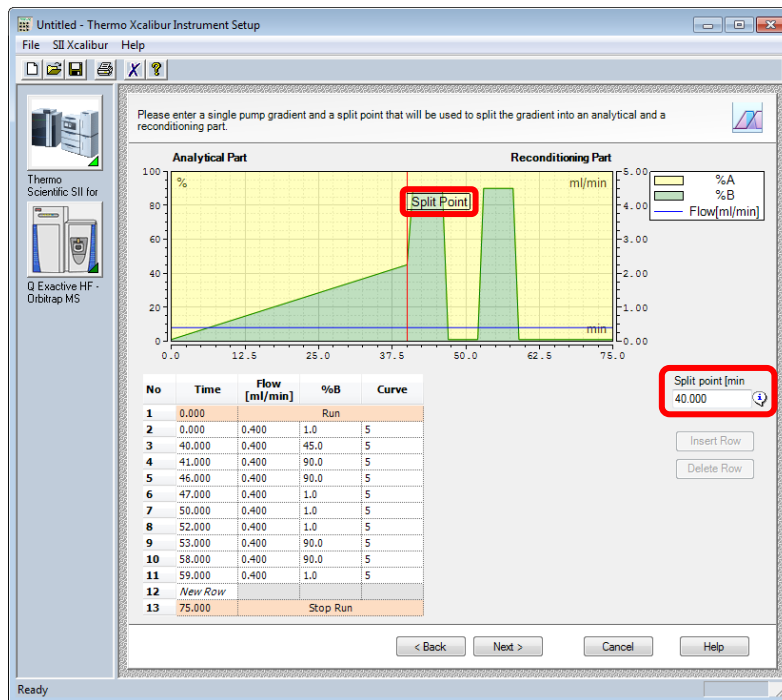

Figure 13: Single pump method

c. The following step is converting the previously defined single pump method into a Tandem LC method (Figure 13). The gradients for the Reconditioning- and Analytical Pump can be modified if required. The wizard is automatically adding a void volume purge section to the analytical gradient, which is required to purge the system fluidics for the next injection. The required time is automatically calculated and depends on flow rate, used modules and fluidics. The automatic calculation is based on the capillaries provided in the Vanquish Duo for Tandem LC Kit (P/N 6036.2020).
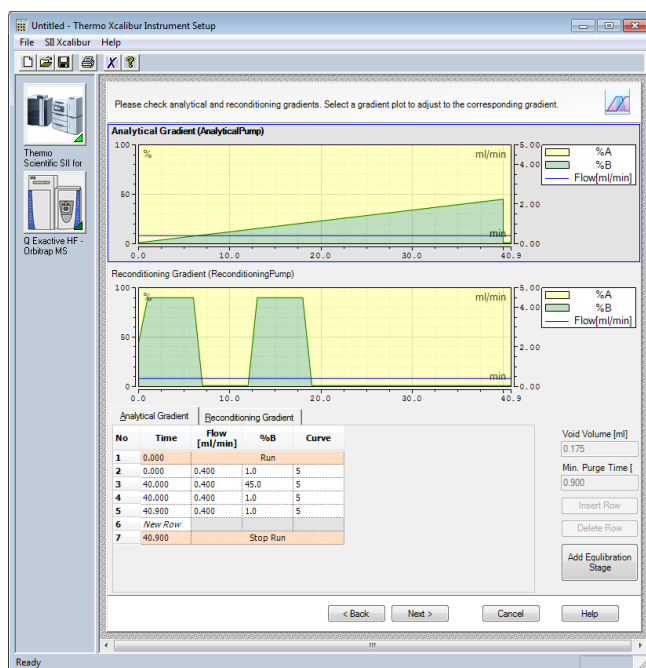
Figure 14: Tandem LC method

> **NOTICE**: The use of different capillaries than recommended and provided in the Tandem LC kit may affect the void volume purge performance, which can lead to retention time variation of target analytes.

> **NOTICE**: An important parameter for the void volume purge calculation is the used **pump mixer set**. If the default mixer set is changed, the correct mixers must be defined in the pump module properties accordingly. Please follow the instructions provided in all mixer kits in the *Capillary Mixer and Static Mixer Installation Settings for Vanquish Pumps manual* (P/N 4820.4420).

d. Finish the Instrument Method Wizard. The Instrument Method Editor allows final edits of the Tandem LC method and has an integrated Tandem Integrity check to detect possible errors after method edits (Figure 14).
    For Tandem LC-MS setups, define the MS instrument method and save the instrument method accordingly.
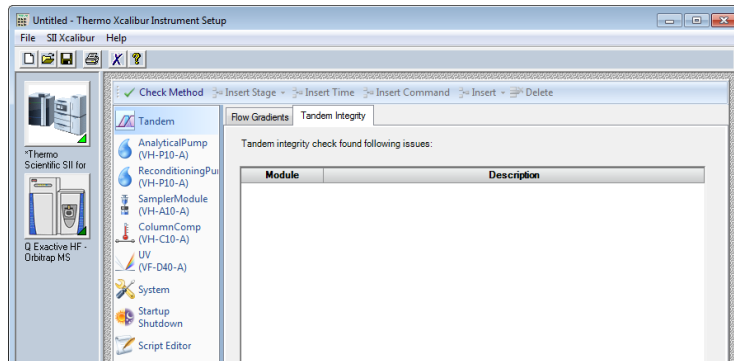
Figure 15: Instrument Method Editor

10. Create a sequence by clicking **Sequence…** in the **Create** drop down menu. Follow the Sequence Wizard and save the new sequence.
    For Thermo Xcalibur installations follow the *Thermo Xcalibur Data Acquisition and Processing User Guide.*

11. Optional: For Tandem LC or LC-MS applications it is recommended to add a custom column to the sequence view in the Chromeleon Console, which shows the current column for the individual injection.

> **NOTICE**: The procedure to show the current column in Thermo Xcalibur is different, please follow the instructions in the *Thermo Xcalibur Qual Browser User Guide*.

    a. Right-click on the column header and select **Custom Columns – Insert Result Formula** or alternatively press the **Custom Columns** button in the tool bar and select **Insert Result Formula**.
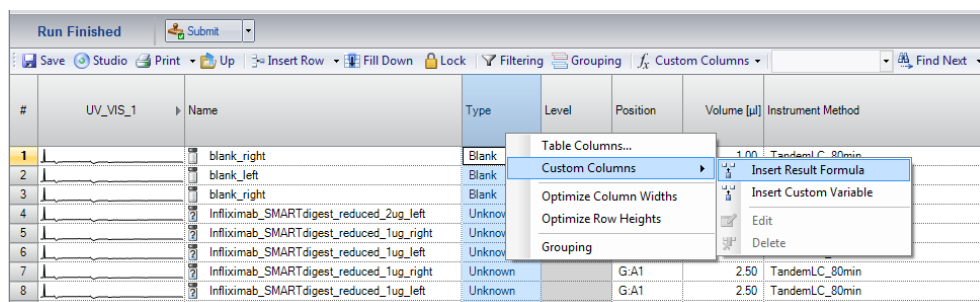
Figure 16: Insert Custom Column

    b. Follow the wizard and choose "**Create a new result formula"**, then select the category "**Preconditions**" with the variable "**Current Column**" and insert the new column (Figure 16).
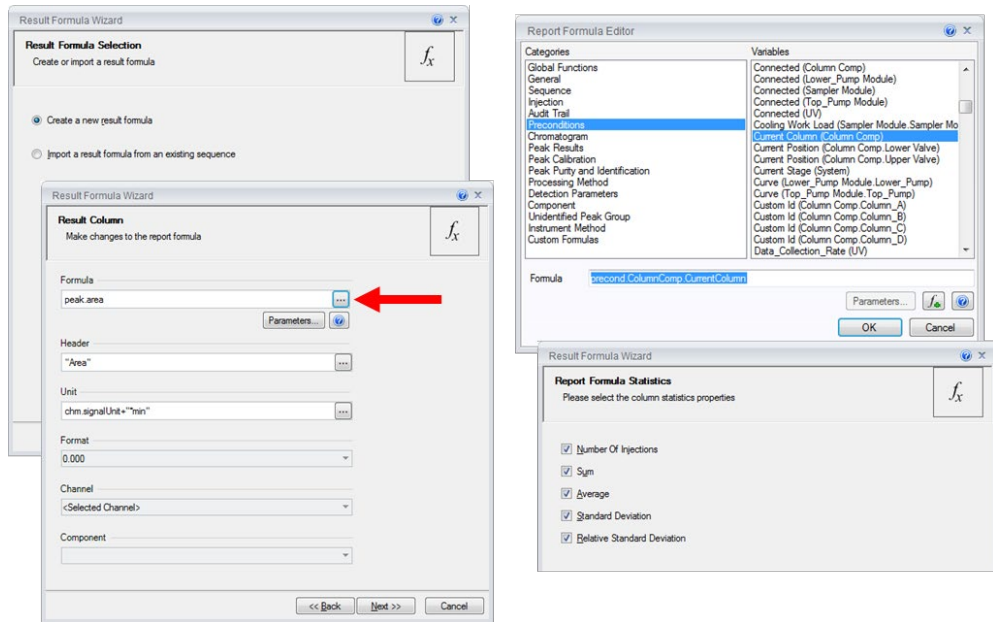
Figure 17: Result Formula Wizard

c. For each injection, the current column is now displayed in the sequence view of the Chromeleon console (Figure 17).

Figure 18: Custom Columns for column A and B (in red rectangle)