Installation Guide – Thermo Scientific™ Vanquish™ Duo for Inverse Gradient

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 Inverse Gradient.

Parts required

Table 1: Modules required for installation of Vanquish Horizon Duo or Flex Duo UHPLC system for Inverse Gradient

<table>
<tr><th>Component</th><th colspan="2">Vanquish Horizon Duo for Inverse Gradient</th><th colspan="2">Vanquish Flex Duo for Inverse Gradient</th></tr>
<tr><td>System: Base, Rack & Capillaries</td><td colspan="4">• 1x System Base Vanquish Horizon/Flex (VF-S01-A-02)
• 1x Vanquish Duo for Inverse Gradient Kit (6036.2010)
<u>Stack Stabilizer for stacks > 4 modules (optional)</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)</td></tr>
<tr><td>Pumps</td><td colspan="2">• 2x Binary Pump H (VH-P10-A-02) or
• 1x Binary Pump H and 1x Quaternary Pump F</td><td colspan="2">• 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</td></tr>
<tr><td>Samplers</td><td colspan="2">1x Split Sampler HT (VH-A10-A-02)</td><td colspan="2">1x Split Sampler FT (VF-A10-A-02)</td></tr>
<tr><td>Optional Sample Extension</td><td colspan="4">Charger (VH-A90-A)</td></tr>
<tr><td>Column Compartments</td><td colspan="4">1x Column Compartment H (VH-C10-A-02)</td></tr>
<tr><td rowspan="2">Detectors</td><td rowspan="2">Charged Aerosol
• H (VH-D20-A)
• F (VF-D20-A)</td><td colspan="3">Optional</td></tr>
<tr><td>Diode Array
• HL (VH-D10-A)
• FG (VF-D11-A-01)</td><td>Fluorescence
• F (VF-D50-A)
• F Dual-PMT (VF-D51-A)</td><td>Variable Wavelength
F (VF-D40-A)</td></tr>
<tr><td>Flow Cells</td><td>n.a.</td><td colspan="3">Selection of wide range of flow cells per each optical detector</td></tr>
</table>

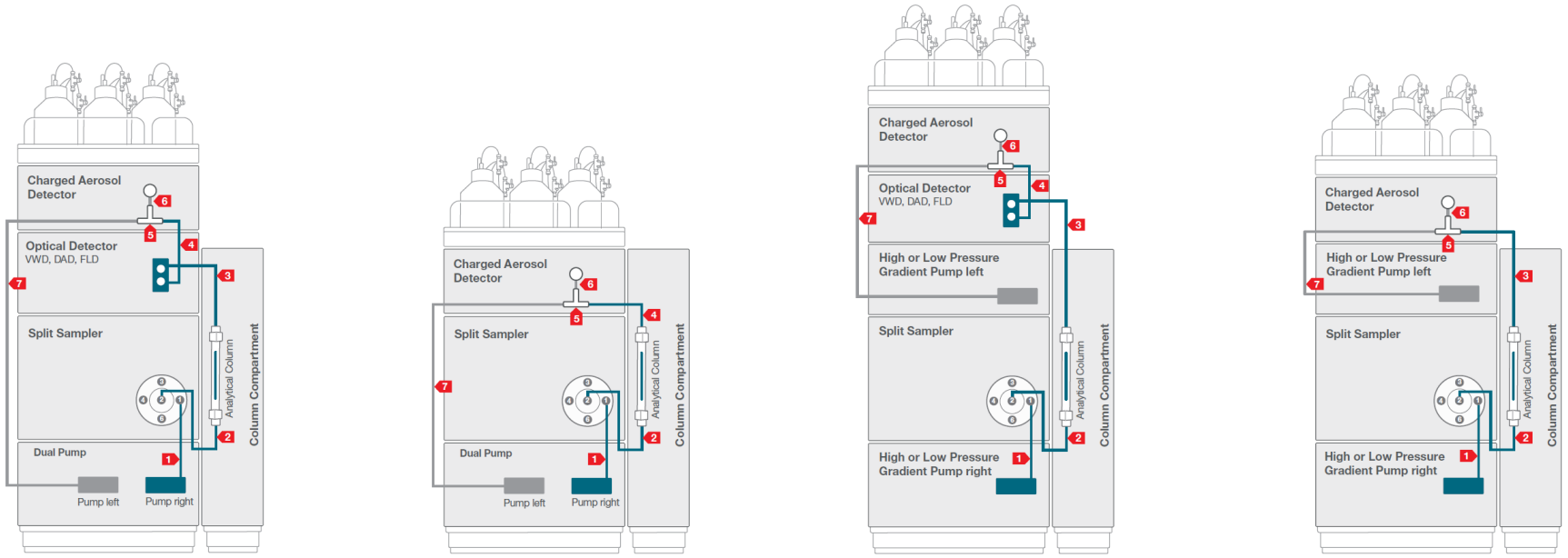

Figure 1: Flow Schemes Overview Vanquish Duo for Inverse Gradient

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

No.	Description	No.	Description
1	Viper Capillary, 0.10 x 350 mm, MP35N (6042.2340)[2]	6	Viper Capillary, 0.10 x 65 mm, MP35N (6042.2306)[1]
2	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]	7	Viper Capillary, 0.10 x 950 mm, MP35N (6042.2395)[1]
3	Only used if optical detector is present[4] For two separate pumps: Post Cooler 1µL, 0.1 x 590 mm, VH-C10 (6732.0520)[1]	8	*Flow Schemes Vanquish Duo for Inverse Gradient* (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]
4	Viper Capillary, 0.10 x 350 mm, MP35N (6042.2340)[5]		Optional: Set color marker labels (blue and red) for capillaries, solvent lines and wash lines[1]
5	Tee Piece ID 0.020“ (0.5 mm) for 1/16" capillary (6263.0035)[1]		

[1] Included in Vanquish Duo for Inverse Gradient Kit (6036.2010), [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 optical detector ship kit,[5] Included in Charged Aerosol Detector ship kit

Hardware and Software Installation

1. Locate and unpack all required system components as shown in Table 1 and Table 2.

2. Identify your configuration out of the four *Flow Scheme* pictures and stack the modules accordingly. Some Vanquish configurations may require the stacking of more than 4 modules (e.g. Vanquish Duo setups with two separate pumps). 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 instructions of the stack stabilizing follow the respective instructions delivered with the stack stabilizing options.

3. 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 to the PC individually.

4. 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* belonging to your configuration and part of the deliverd capillary 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.*

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

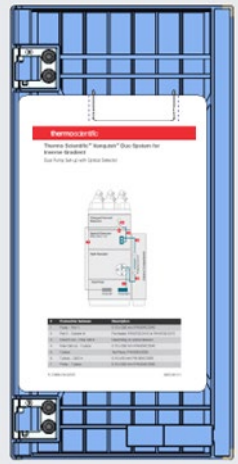

Figure 2:Flow scheme in sampler door

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

6. Install the columns.

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

7. Switch on all modules in the system.

8. Install the Thermo Scientific Chromeleon 7.2.8 (or higher) Chromatography Data System (CDS). Follow the instructions of section **Software Configuration** to configure and operate the Vanquish Duo for Dual LC system.

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 one **Instrument**

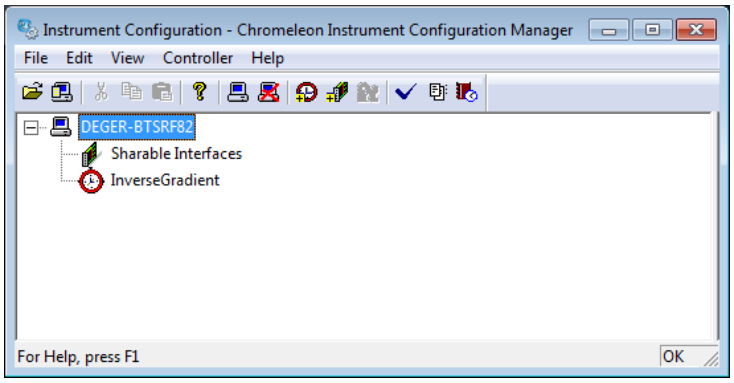

Figure 3: Instrument in Chromeleon

3. Add two pumps to the instrument

    a. If a Dual Pump is present choose **HPLC: Vanquish** followed by **Vanquish Dual Pump**. The left and right pump are automatically assign to the previously generated instrument on the **Devices** page. Ensure that the device names for the instrument devices are not identical (e.g., PumpLeft and PumpRight). It is recommended to add a descriptive extension to the respective device names and signal names. This will show up on the module panels and simplifies distinction.

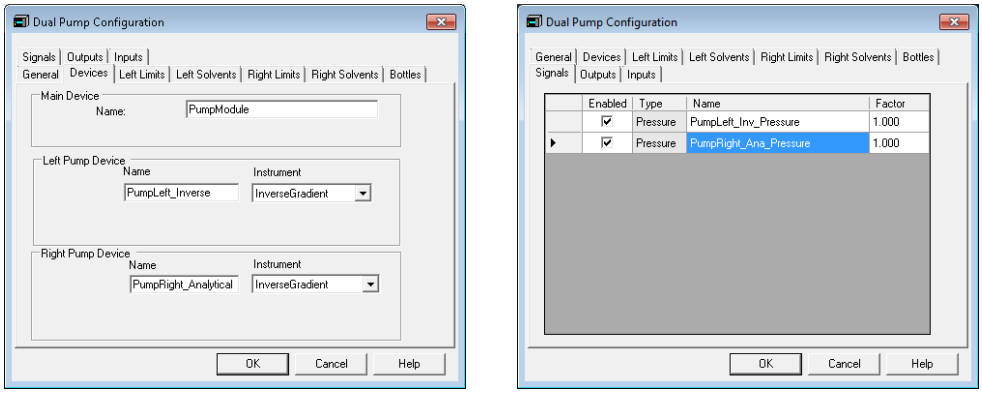

Figure 4: Dual Pump configuration

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. This will show up on the module panels and simplifies distinction.

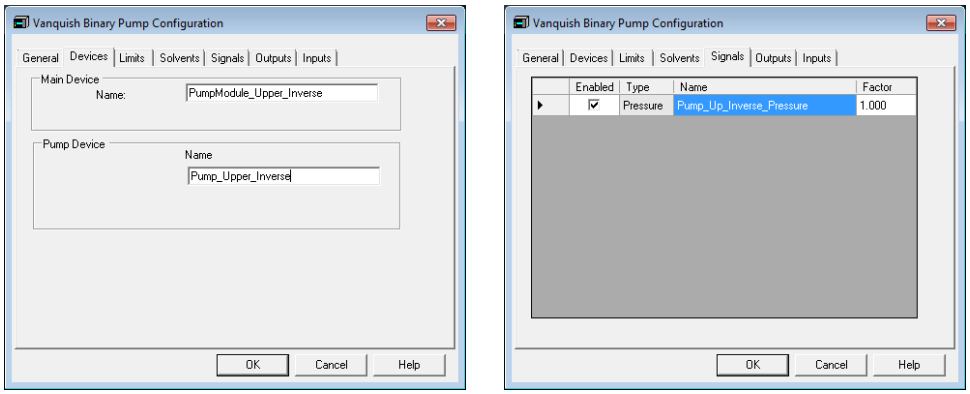

Figure 5: Two pumps configuration

4. Add the Sampler to the instrument. Choose **HPLC: Vanquish** followed by **Vanquish Autosampler**. Assign the appropriate pump links if applicable (e.g., *VPUMP*_**R**_*STRK* for the default Dual Pump configuration). Add a Vanquish Charger module if present in the configuration.

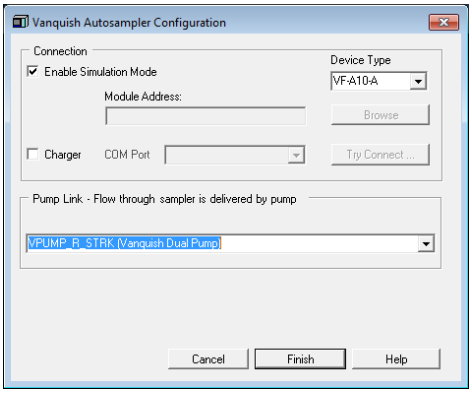

Figure 6: Sampler configuration with Dual Pump

5. Add the Column Compartment to the instruments. Choose **HPLC: Vanquish** followed by **Vanquish Column Compartment**.

6. Add the detector to the instrument. Choose **HPLC: Vanquish** followed by the respective detector type.

7. Save the configuration and launch **Chromeleon**.

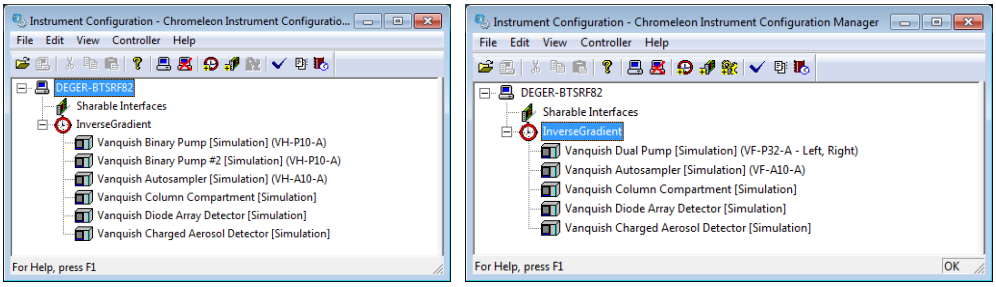

Figure 7: Final instruments configuration

8. Select one instrument and launch the **Fluidic Configuration** wizard.

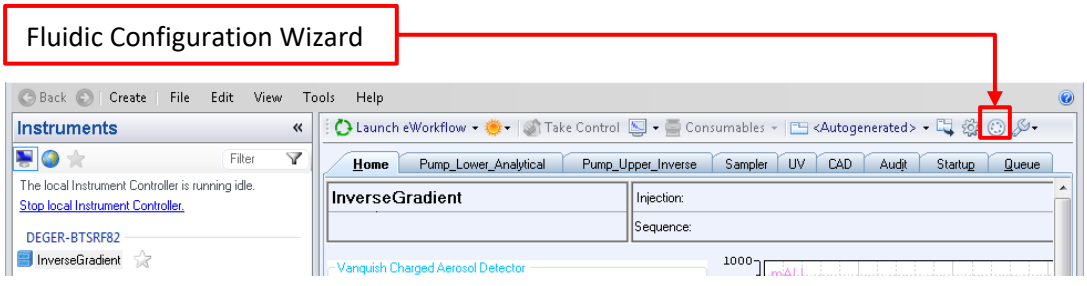

Figure 8: Fluidic configuration in Chromeleon

9. On the first wizard page select the installed capillary kit from the drop down list, here **Vanquish Inverse Gradient**.

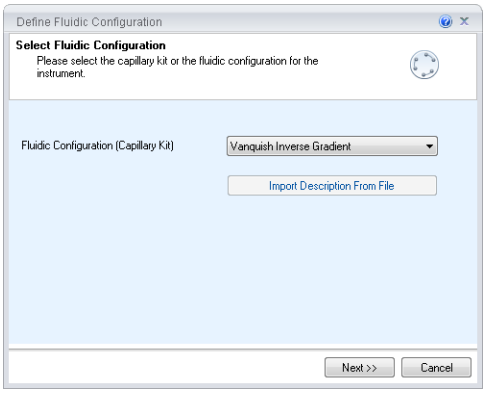

Figure 9: Workflow selection in Fluidic Configuration

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

10. Verify correct assignment of fluidic items, e.g. is the column in use connected to the left or right active pre-heater, and click **Next**. If columns are not configured in the **Vanquish Column Compartment Instrument Configuration** they will not show up in the list.

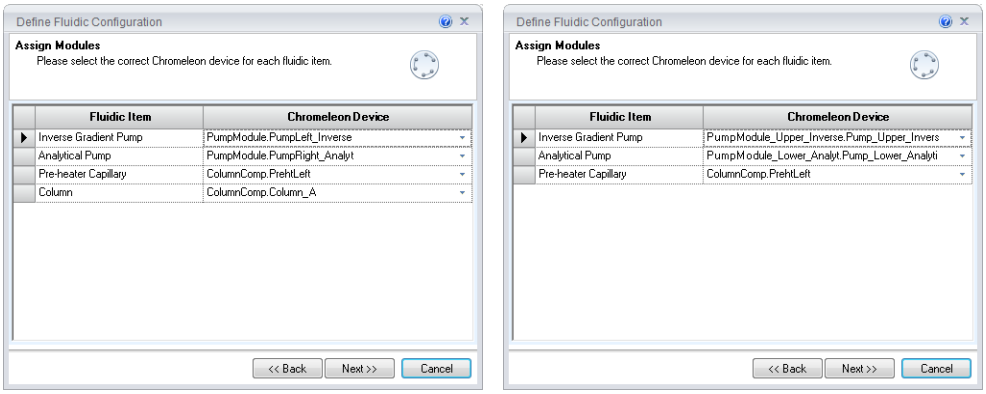

Figure 10: Assignment of fluidic items

11. Define the column volume.

    a. If the columns are configured in the **Vanquish Column Compartment Instrument Configuration** and the written column tag is used the column parameters are filled and calculated automatically. Click **Finish**.

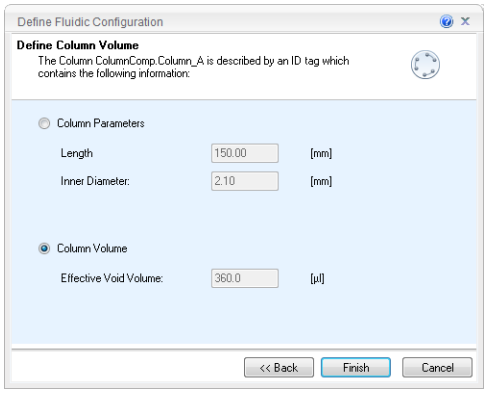

Figure 11: Automated fill of column information

b. If the columns are not configured in the **Vanquish Column Compartment Instrument Configuration** or no column tag is used the column parameters need to entered and the void volume will be calculated. If the column void volume was experimentially determined select column volume and enter the value accordingly Click **Finish**.

$$V = \pi * \frac{{d_c}^2}{4} * L * \varepsilon_T$$

V: Column Void Volume [µL]
d_C: Column Diameter [mm]
L: Column Length [mm]
ε_T: Total porosity (0.63)

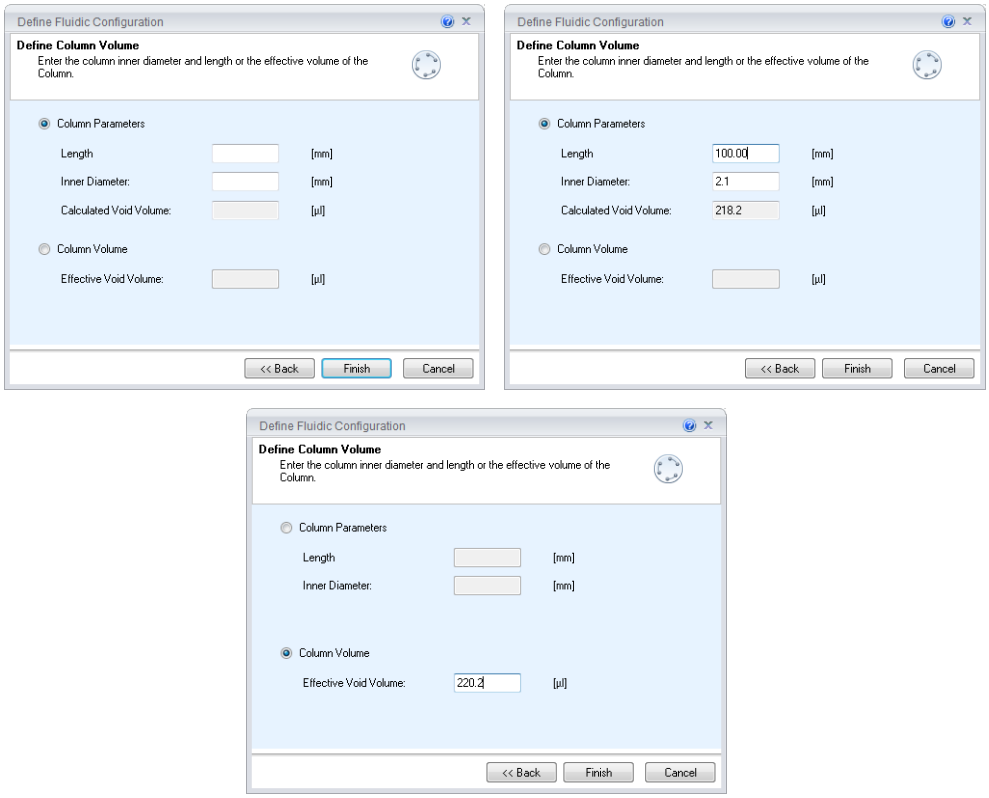

Figure 12: Manual column definition

12. Check the Audit Trail if the Fluidic Configuration was saved accordingly.

		Date	Time	Retention Time	Device	Message
7		3/16/2018	1:10:02 PM +01:00			The capillary kit / application 'Vanquish Inverse Gradient' has been selected.

Figure 13: Audit trail message from fluidic configuration

13. To create an instrument method click **Instrument Method…** in the **Create** drop down menu.

14. Select **Inverse Gradient** on the **Workflow Selection** page. Click **Next**.

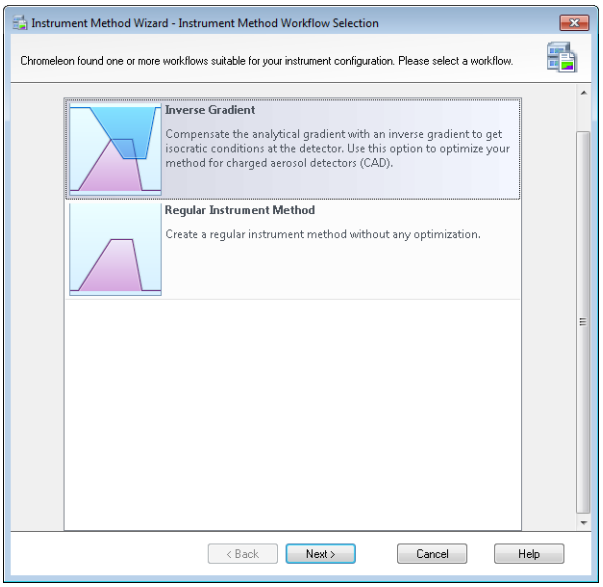

Figure 14: Workflow Selection Page

15. On the **Inverse Gradient Options** page select the desired **Calculation mode**. The Inverse gradient offset is automatically filled form the fluidic configuration taking the following volumes into account: System capillaries from installed kit, pump mixers, sampler fluidics, flow cell volumes. If the offset was determined by the user the value can be entered here overwriting the calculation from the fluidic configuration.

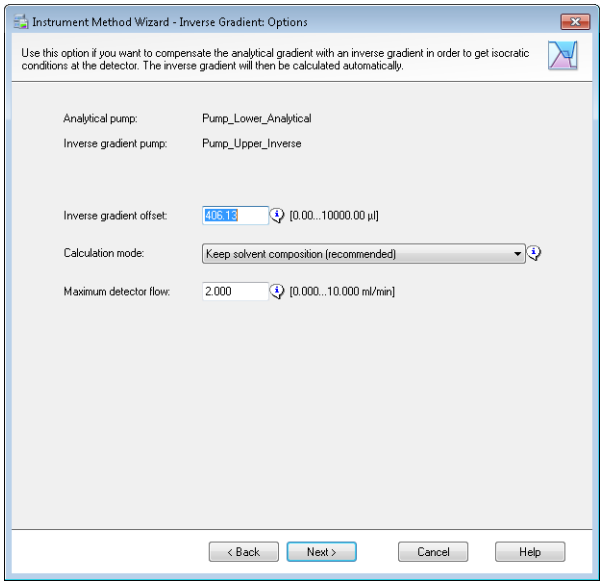

Figure 15: Inverse Gradient Options Page

16. Enter the analytical gradient in the gradient table on the **Inverse Gradient: Flow Gradients** page. The inverse gradient is automatically generated based on the previously selected **Calculation Mode** and is therefore here read only. To see the generated gradient table of the inverse gradient either the gradient image or the table tab can be clicked.

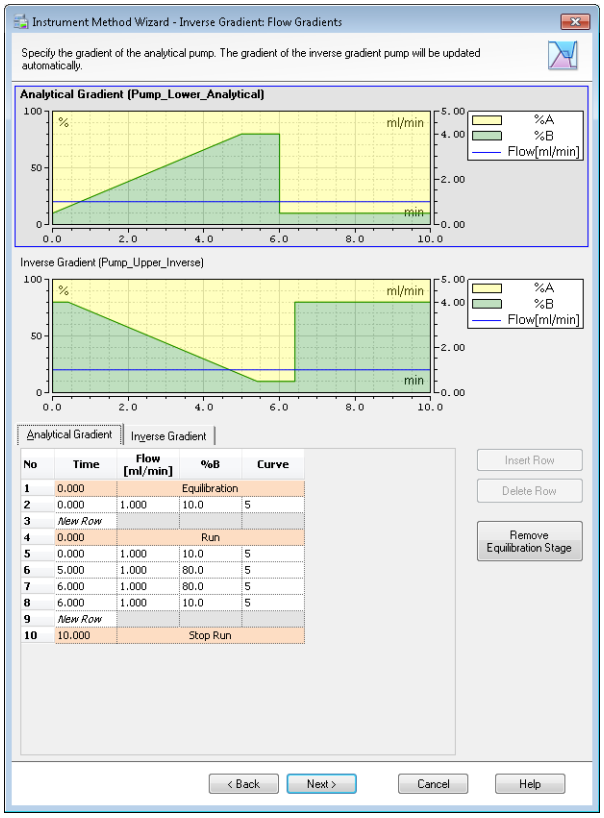

Figure 16:Inverse Gradient Flow Gradients Page

17. If the gradient or the calculation mode needs to be adapted this can be done at any time in the method editor under **Inverse Gradient (Flow Gradients)** and **Inverse Gradient (Options)**. The user interface is similar to the method wizard.

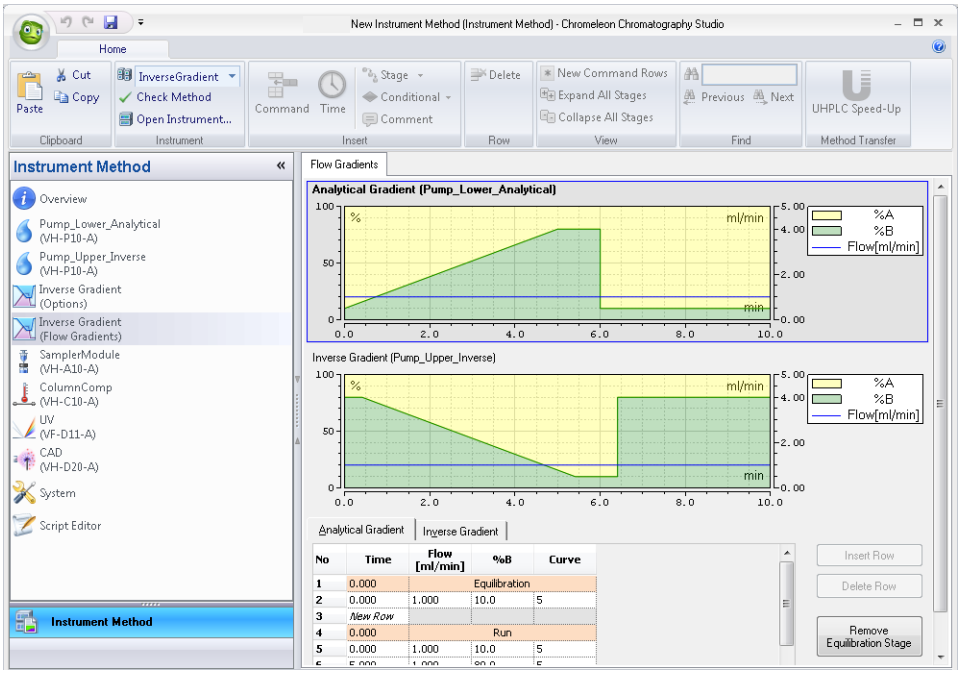

Figure 17: Method Editor of Inverse Gradient Method

18. Create a sequence by clicking **Sequence…** in the **Create** drop down menu and add the Inverse Gradient instrument method to start analysis.