Evosep One

User Guide

v. 17

Contents

1 Preface

1.1 About this manual

This manual has been written for laboratory technicians who use the Evosep One system for execution of analytical runs. It is assumed that the user of this manual has basic knowledge of how to use menu-driven software and that this person is familiar with standard laboratory and HPLC terminology and practices.

1.2 Safety and Special Notices

Make sure to follow the safety practices presented in this guide as well as those received from Evosep personnel.

Observe all written safety precautions during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument and might result in damage to the instrument, personal injury, or loss of life.

1.3 Contacting Us

Support: support@evosep.com

Sales: sales@evosep.com

1.4 Declaration of Conformity

We:

Company name	Evosep Aps
Postal address	Buchwaldsgade 35, 3rd floor
Postcode	5000
City	Odense C
Country	Denmark
Telephone	+45 2566 2322
E-mail	mba@evosep.com

Declare that this DoC is issued under our sole responsibility and belongs to the following product:

Apparatus model/Product	Evosep One	
Type	Laboratory equipment (HPLC)	
Manufacture site	Made in Denmark	
Manufacture year	From 2017	
Serial number	S000001 and later	

The object of the declaration described above is in conformity with the relevant Union harmonization legislation.

Applicable directives	EMC Directive 2014/30/EU Low Voltage Directive (LVD) (2014/35/EU) RoHS Directive 2011/65/EU WEEE Directive 2012/19/EU
The following harmonized standards and technical specifications have been applied	EN61010-1: Safety requirements for electrical equipment for measurement, control, and laboratory use EN61326-1: Electrical equipment for measurement, control and laboratory use. EMC requirements.

I, the undersigned hereby declare that the equipment specified above conforms to the above Directive(s) and Standard(s).

Michael Barrett Andersen,
Head of Product Management

July 4th, 2018,

2 Introduction

Evosep aims to improve quality of life and patient care by radically innovating protein based clinical diagnostics. We will make sample preparation and separation for mass spectrometry analysis 100 times more robust and 10 times faster to enable truly large cohort studies and provide the foundation for precision medicine.

The Evosep One is an optimized front-end to a mass spectrometer for large-cohort experiments. The Evosep One ensures:

More uptime with improved reliability and robustness owing to:

- Partial elution that leaves impurities from each sample on the disposable tips that act as pre-columns
- Low pressure elution & gradient formation that cause less wear and tear

Increased productivity with higher duty cycle utilization owing to:

- Fewer LC household steps
- Minimized dwell time since gradient formation happens at a high flow rate, close to the column

Increased performance with better data quality owing to:

- Reduced cross-contamination using disposable tips, and
- High flow-rates during autosampler washing steps

The Evosep One technology is centered around the Evotip and integrates sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with a small plug of C18 stationary phase at the bottom of the tip. The Evotips are used to desalt and clean samples prior to LC-MS analysis however, the traditional subsequent steps of eluting, drying down, re-suspending the samples from tips are

completely omitted and instead the tips are loaded directly into Evosep One for analysis. This new process leads to significantly less sample loss and variation as well as much simpler and faster work flows. The Evosep One sample tray accommodates up to 6 racks of 96 tips, i.e. 576 rinsed samples may be lined up for fast analysis. Upon starting an analysis, the autosampler places one tip at the time (with the pre-loaded sample) in-line with the solvent system, see Figure 1.

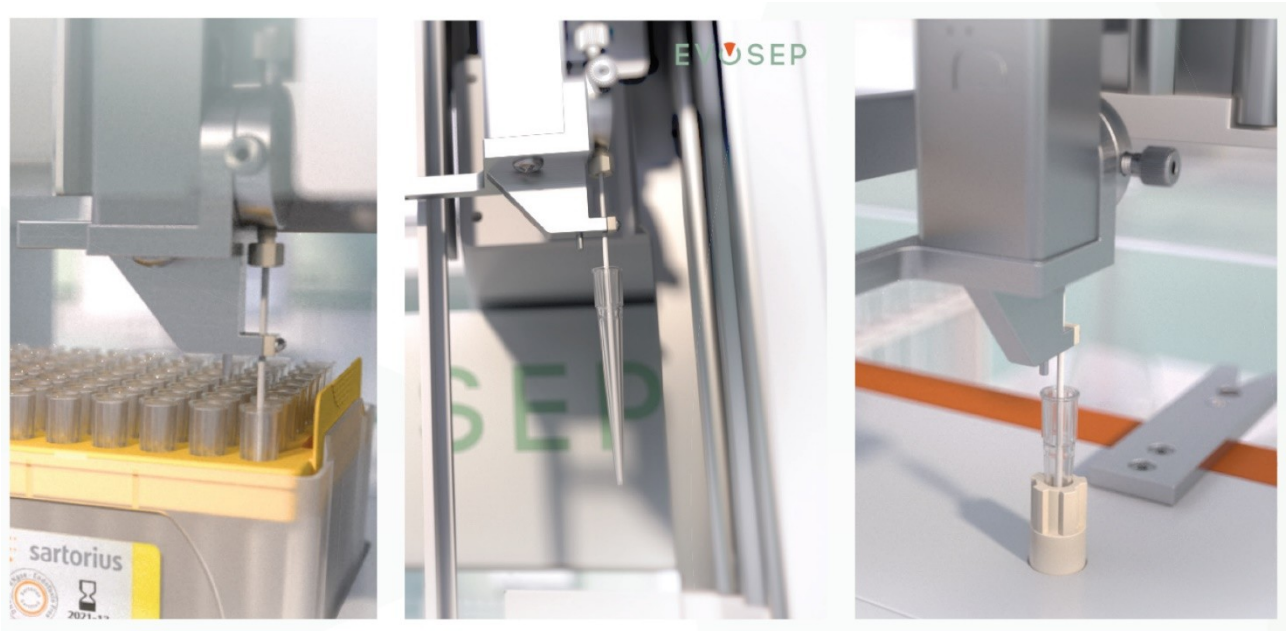


Figure 1. Upon starting an analysis, the autosampler places one tip at the time (with the pre-loaded sample) in-line with the solvent system

3 Installing the Evosep One hardware

To install or move the instrument from one laboratory benchtop to another please follow the instructions in this chapter.

Please note that these instructions do not replace a required instrument installation by an Evosep service engineer.

3.1 Lifting instructions

The instrument weighs approximately 37 kg, for safety reasons use two people to move the instrument.

Important Notes:

> *DO NOT lift the instrument with side panels mounted! As they are magnet mounted, they can come off during lifting.*
> *Use a cart for moving the instrument and only lift it to put it on a table*

Before lifting/moving the instrument, verify that the following actions has been performed:

1. That the two side panels have been removed
2. That the autosampler has been parked in lock position
3. That the instrument has been switched off
4. *Network, power and contact closure cables have been disconnected from the backside of the instrument*
5. *Transfer line has been disconnected from MS ion source*
6. *Waste tubing has been removed from the waste container (Not for instruments with waste container on door)*
7. *Evotip boxes have been removed from the Evosep One sample tray*
8. *The instrument can now be lifted by two persons, that lift underneath the instrument from each side.*

3.2 Table and trolley requirements

Evosep One dimensions including autosampler axis movement, safety bar, and terminal holder (working range):

Depth	880 mm	34.6 in
Width	690 mm	27.2 in
Height	910 mm	35.8 in
Weight	37 kg	81.6 lb

Evosep One footprint for installing on trolley:

Depth	440 mm	17.3 in
Width	420 mm	16.5 in

The table or the trolley must be stable and vibration free with wheels that can be locked and are able to support a minimum of two times the weight of the Evosep One.

The Evosep One should be placed as close to the MS ion source as possible. The distance between the right-hand front side of the Evosep One and the MS source should be less than 400mm.

3.3 Power requirements

Line Voltage:

The Evosep One requires two power outlets that operates within the range of:

100-240V ~ 2.5-3.0A, 50-60Hz

Grounding requirements:

The Evosep One, the MS and the data system hardware must have common grounding to avoid a ground loop that can cause noise and interference.

Please note the difference on the power supply plug for the autosampler and the pump box.

3.4 Temperature and humidity requirements

Evosep operating temperature: 15 – 30 °C (59 to 86 °F)

Temperature fluctuations < 1 °C/hour (2 °F)

Note; to meet the analytical specifications, temperature must be:

within range 22 °C ±3 °C (72 °F ±6 °F)

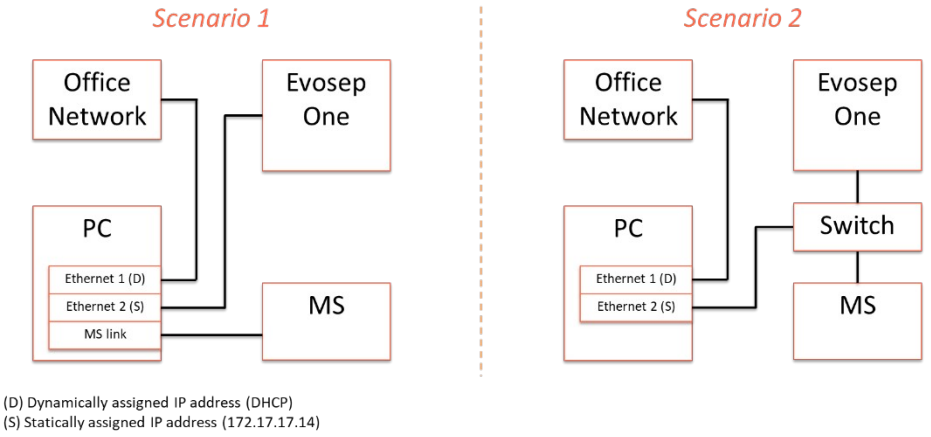
Operating Humidity: 20-80%, non-condensing

The maximum air conditioning load for the Evosep One is approximately 350 W

Avoid locations with high air humidity or changes in temperature, such as direct sunlight, drafts, directly below air conditioning or directly next to a mass spectrometer vent.

3.4.1 Connecting the ethernet communication cable and checking network adapter settings

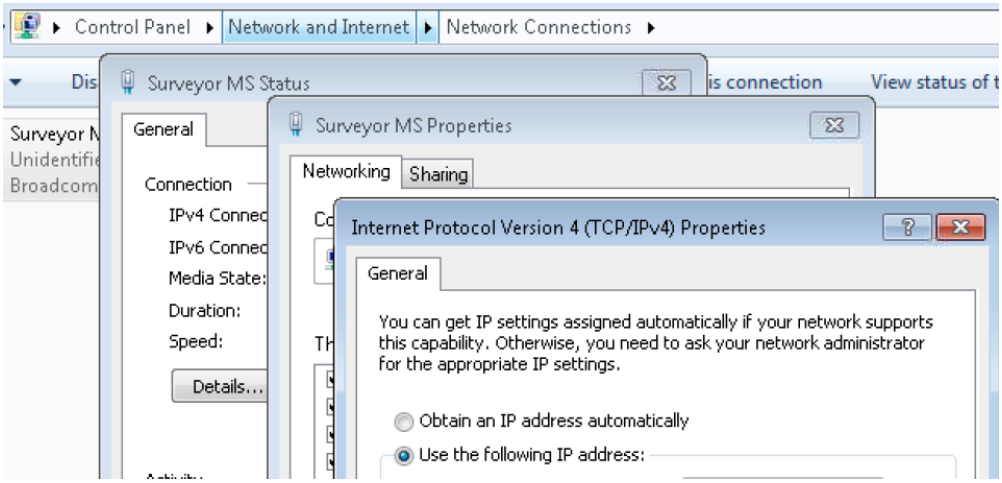
The Evosep One communicates with the MS data system through the Ethernet switch that is connected to the MS and MS data system or directly to a dedicated network card as outlined below.



(D) Dynamically assigned IP address (DHCP)
(S) Statically assigned IP address (172.17.17.14)

Important: The Evosep One must be connected via ethernet on a statically configured network.

Go to the PCs network connections for the specific network adapter card and make sure that it is configured with a static IP address.



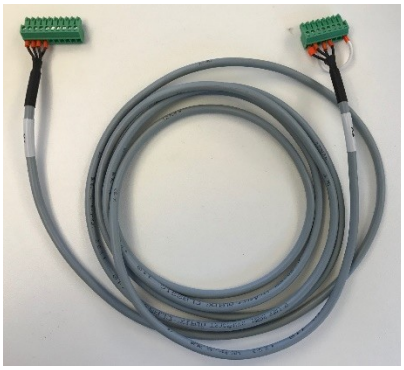
If in doubt how to set this up please contact your IT administrator.

Please plug the ethernet cable into the LAN port on the pump box backside and plug the other end into the MS ethernet switch.

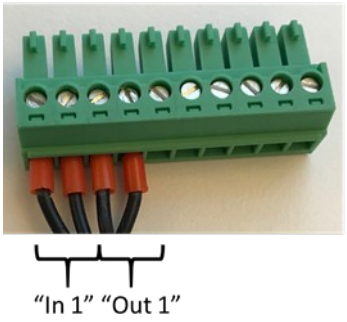
3.4.2 Connecting the contact closure cable

A contact closure cable between the Evosep One and the MS detector synchronizes the run timing. (For Bruker Compass HyStar systems run timing is performed via LAN)

A number of MS-specific contact closure cables exist, and can be ordered with the instrument.



The Evosep One terminal block is labeled X1 and the MS terminal block is labelled X2. The Evosep One terminal block is wired as depicted below:



Wire	Signal
Pin1	In1 (-)
Pin2	In1 (+)
Pin3	Out1
Pin4	Out1

Connect the X1 terminal block to the green contact closure connector on the lower left side on the back of the pump box.

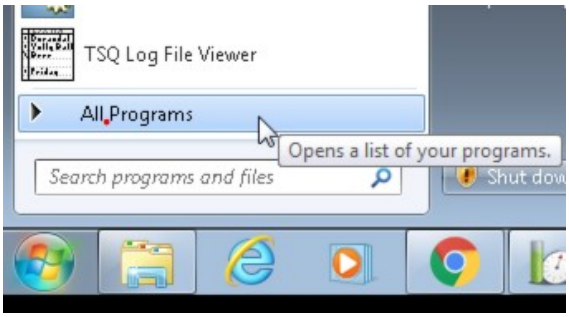
Please refer to the MS documentation on how to connect and establish contact closure for your specific Mass spectrometer.

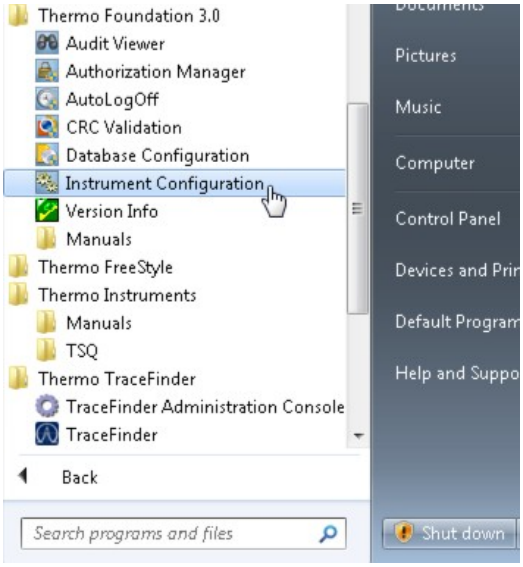
3.4.3 How to remove other LC devices from MS system configuration

If other LC/autosampler devices are configured in the MS instrument configuration contact closure will not work correctly when running the Evosep One.

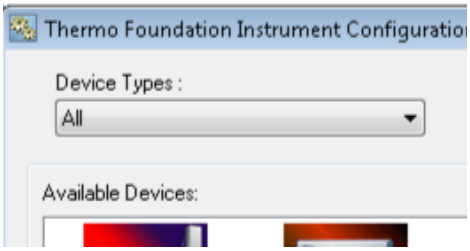
Please check for and remove other connected LC/Autosampler devices from the MS Instrument configuration. Example given for Xcalibur.

1) Close Xcalibur
2) From windows Start button, click “All Programs” and navigate to and open the “Instrument Configuration” program. Normally the program can be found in one of the Thermo specific folders.

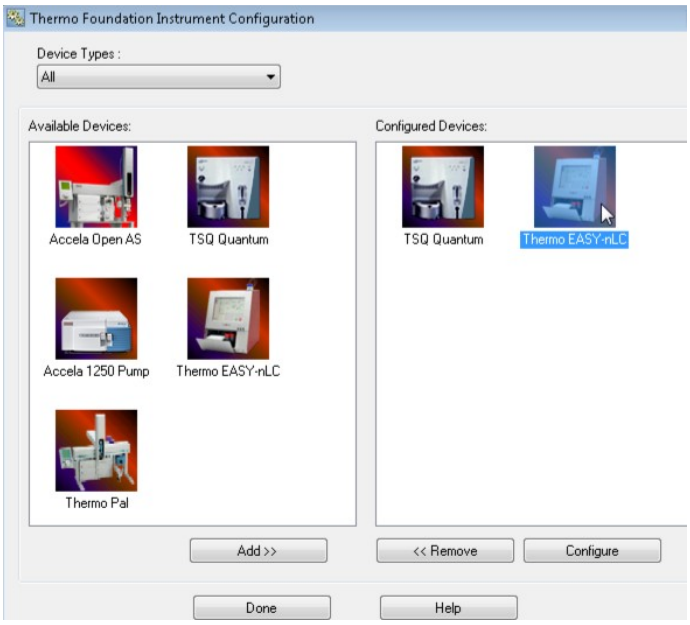




3) In “Instrument Configuration” Choose all device types



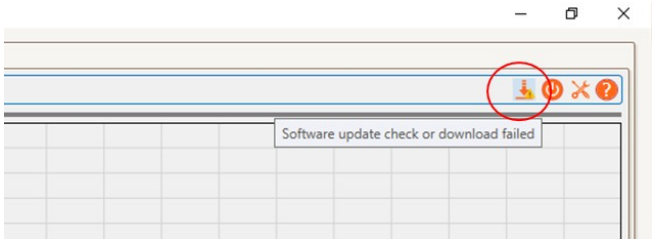
4) IF any LC systems are visible in the “Configured Devices” window then mark them and click remove, which will remove them from the configuration. (Do not remove the MS from the configuration). Then click “Done” and re-open Xcalibur.


Thermo Foundation Instrument Configuration
Device Types :
All
Available Devices:
Configured Devices:
Accela Open AS
TSQ Quantum
Accela 1250 Pump
Thermo EASY-nLC
Thermo Pal
TSQ Quantum
Thermo EASY-nLC
Add >>
<< Remove
Configure
Done
Help

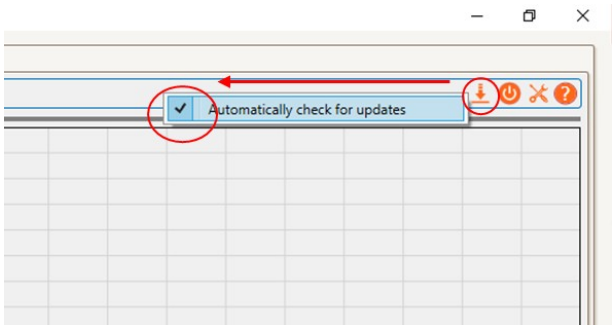
4 Installing control software

4.1 Automated software plugin update

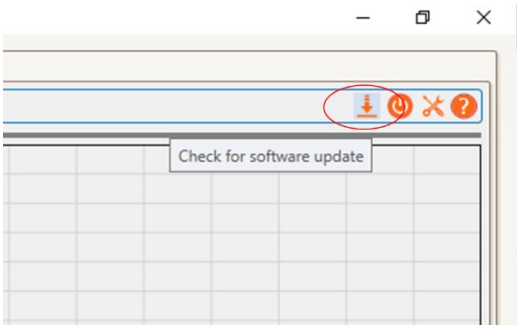
The plugin software will automatically detect if a new version is released and assist the user in the update process (available from plugin v1.4 for Chronos and v1.2 for HyStar). For this feature to work, the PC must have access to evosep.com. If this page is blocked, a warning will be displayed on the software update button on the graphs page. Contact your IT administrator to enable access.



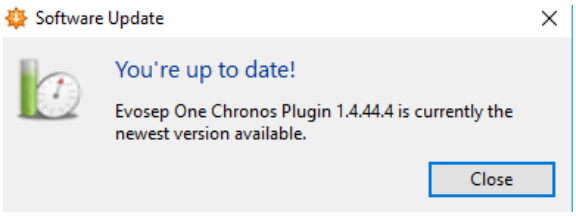
1. The software update process can be triggered in two ways:
    a. Automatically during restart of Chronos/HyStar. The automated feature can be turned on/off by right clicking “Check for software update” button and checking /unchecking the box.



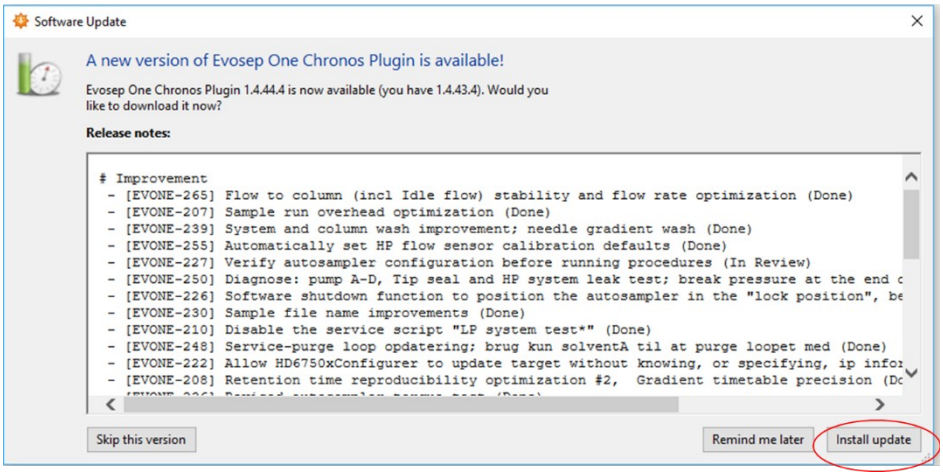
    b. Manually by pressing the “Check for software update” button on the graphs page:



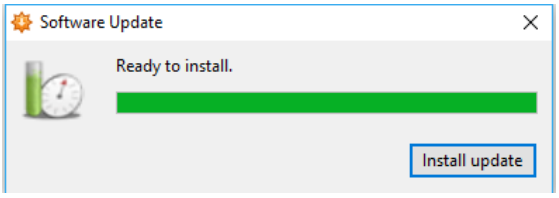
2. The software update window will open and show if the installed plugin is up to date



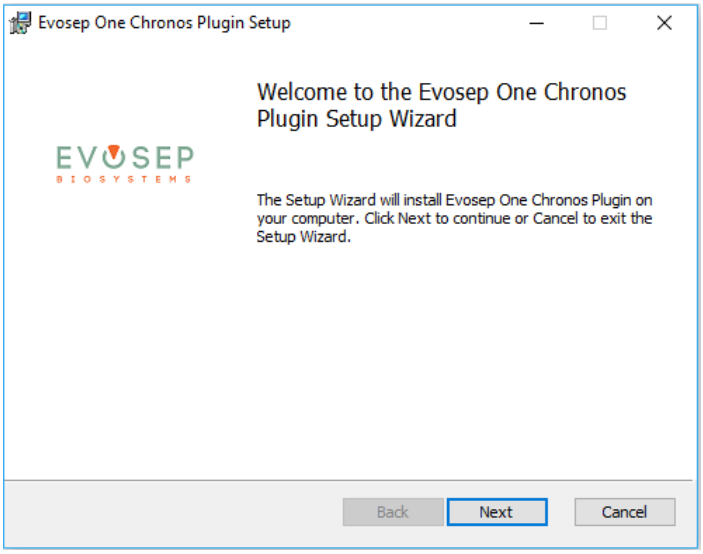
3. If not, the release note for the most recent version of the plugin will be displayed, read carefully and then Press “Install update” to proceed.



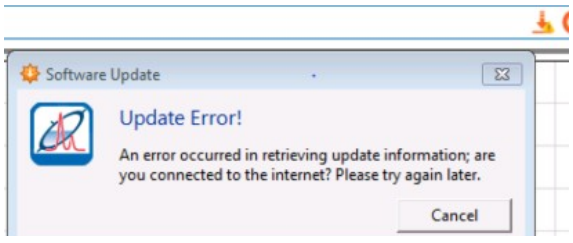
4. The new software plugin will be downloaded from evosep.com. Press Install updates to open the software installer program.



5. Press Next, to proceed with the installation procedure.



Please note: That the automated software update will only work if the PC is connected to the internet. If not, the newest SW plugin can be downloaded manually from the support zone at www.evosep.com.



4.2 Chronos for control of Thermo, Analyst (Sciex), Agilent and Waters MS

This section describes the software installations necessary for instrument control with Xcalibur.

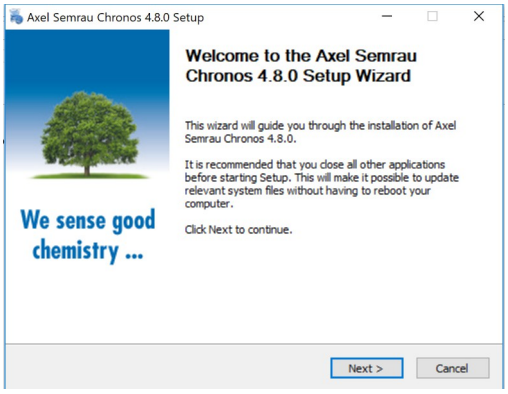
The Evosep One instrument is controlled through the "Chronos" sample acquisition software via an Evosep One plugin.

Chronos comes as a dongle-dependent version with the following limitations:

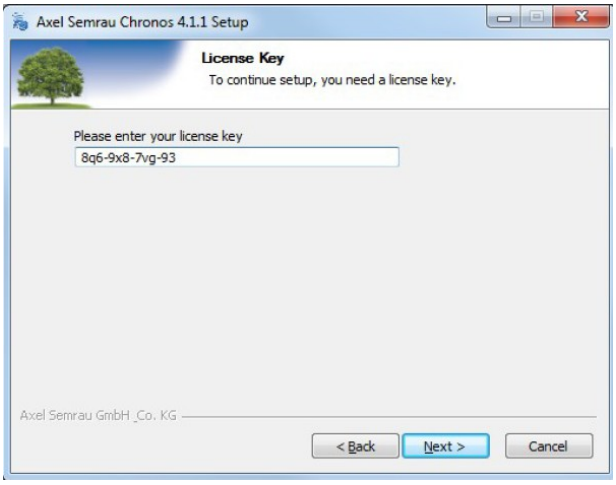
Dongle-dependent versions are full versions, without a time limit. They can be installed and used on any number of computers. To use Chronos, the USB dongle supplied with the software must be inserted and a drive letter must be assigned by Windows. No activation is necessary. If the dongle is not inserted or has not been assigned a drive letter by the operating system when the program is started, a corresponding error message will appear. The USB dongle must remain inserted when Chronos is running. If the dongle is removed during the runtime of Chronos an appropriate error message is shown.

Installing Chronos

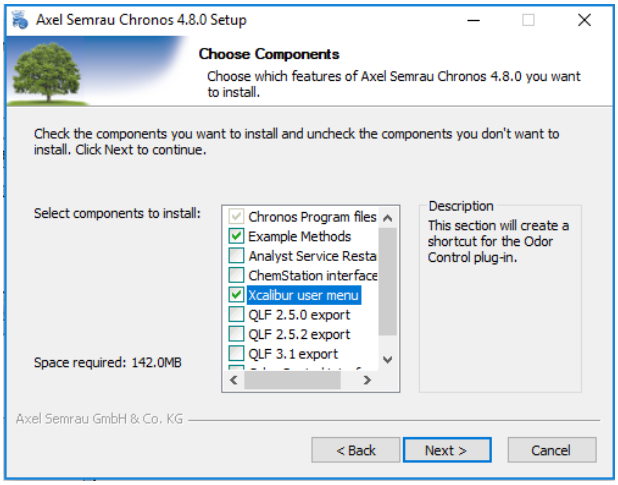
1. Insert the Chronos USB dongle into an available port on the MS data system
2. Run the Chronos setup application file found in the root of the dongle



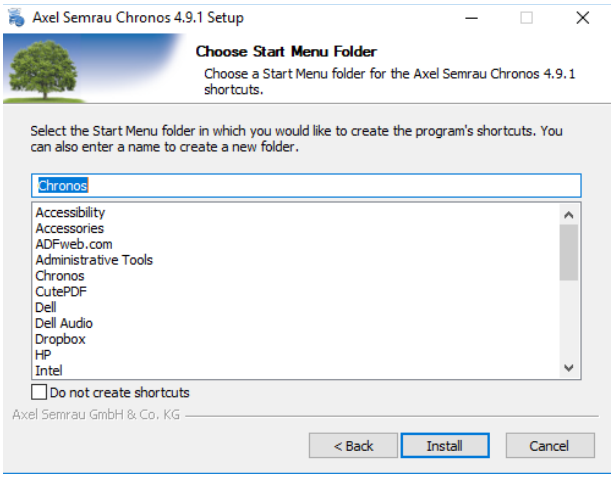
3. Click "I agree" to the software license agreement.
4. Type in the License key found in the USB dongle.



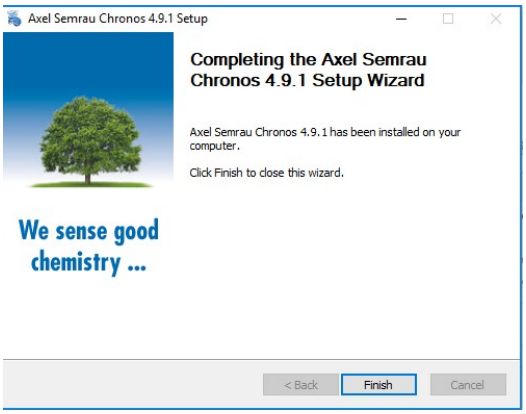
5. For installation on a Thermo MS data system please tick of the “Xcalibur user menu”. For Analyst tick of “analyst”



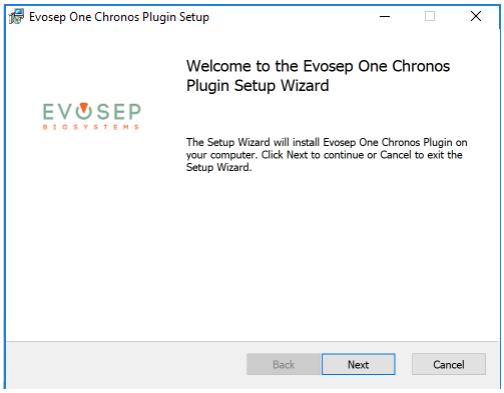
6. Click “Install” to start the installation.



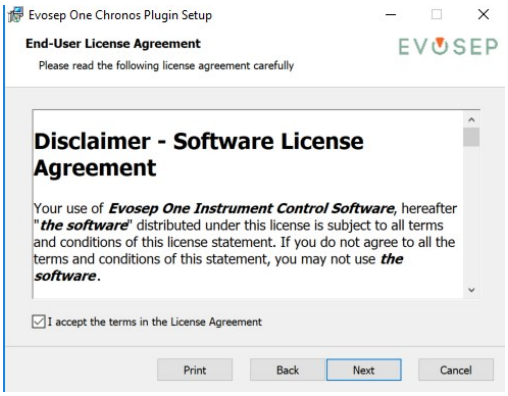
7. Click “Finish”.



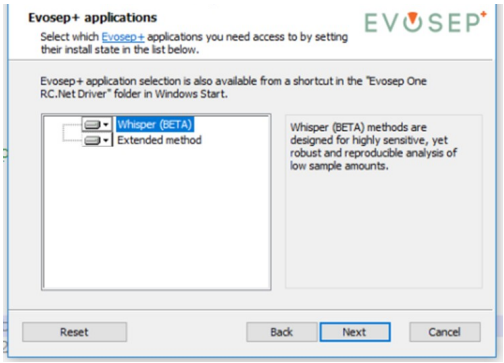
1. *Please make sure that Chronos is NOT running before starting the installer.*
2. With an ethernet cable connect the Evosep One instrument to the computer (optionally through a network switch).
3. **Make sure both the pump box and the autosampler of the instrument is switched on before starting the plugin installation.**
4. Insert the Evosep USB dongle into an available port on the MS data system.
5. Open the Evosep One software folder.
6. Click “Evosep One Chronos Plugin 2.x.x.x”, to run the installer.
7. Click “Next”.



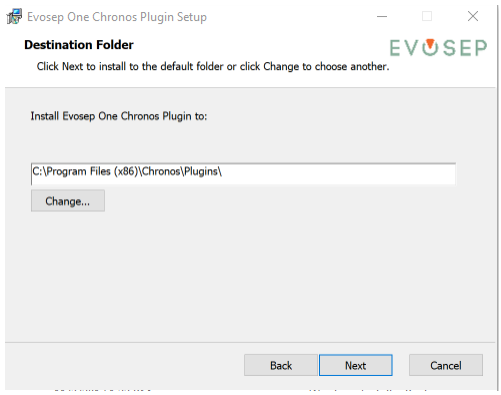
8. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.



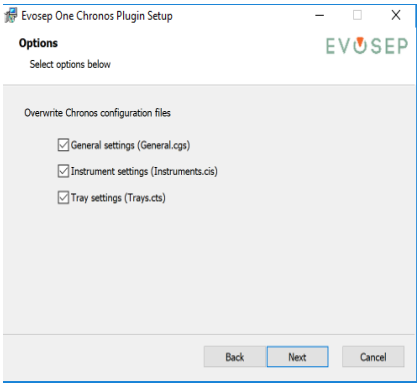
9. Please read the information in the “Prerequisites” window carefully, then click Next.



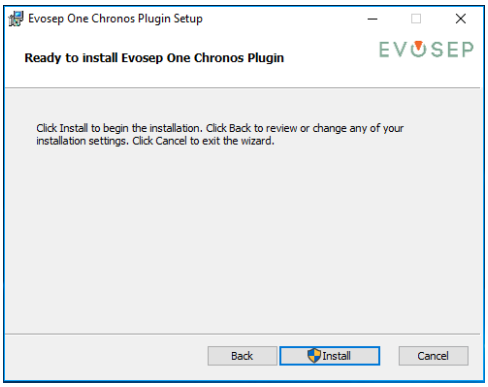
10. Choose which Evosep+ application you wish to install in addition to the standard methods
11. Click “Next” to install the plugin in the suggested folder.



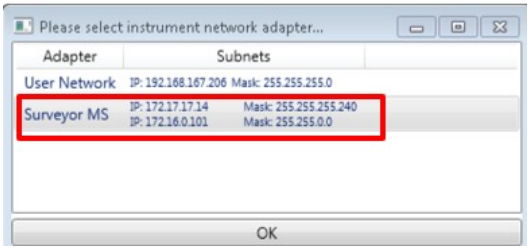
12. Verify that all three checkboxes are ticked to overwrite the Chronos configuration files with the Evosep One configuration files, then click “Next”.



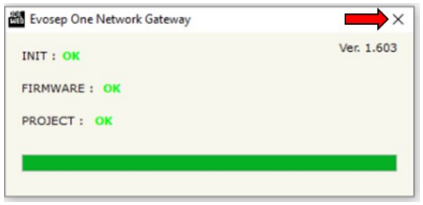
13. Click “Install” to begin the installation and click “Yes” to any popups during the installation.



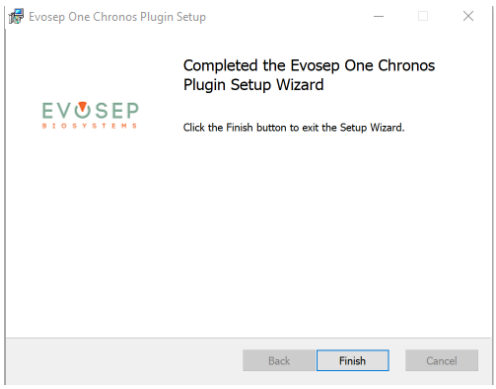
14. If asked to select instrument network adapter, always choose the MS network adapter and then click “OK”.



15. If the firmware update dialog is shown during the installation, please verify that status is OK for INIT, FIRMWARE and PROJECT.



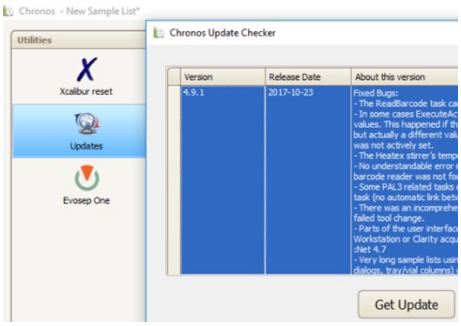
16. When the installation is complete, click “Finish”, to exit the installer.



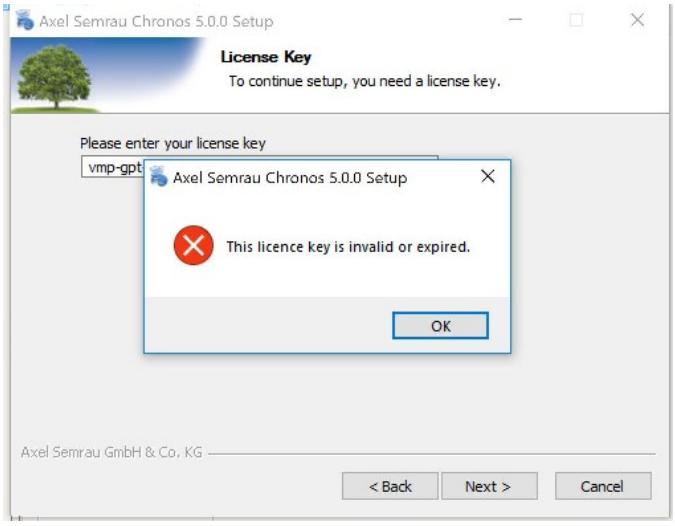
Please note the following, when updating Chronos:

- The newest version of Chronos can be installed by opening Chronos and clicking on Updates under Utilities.

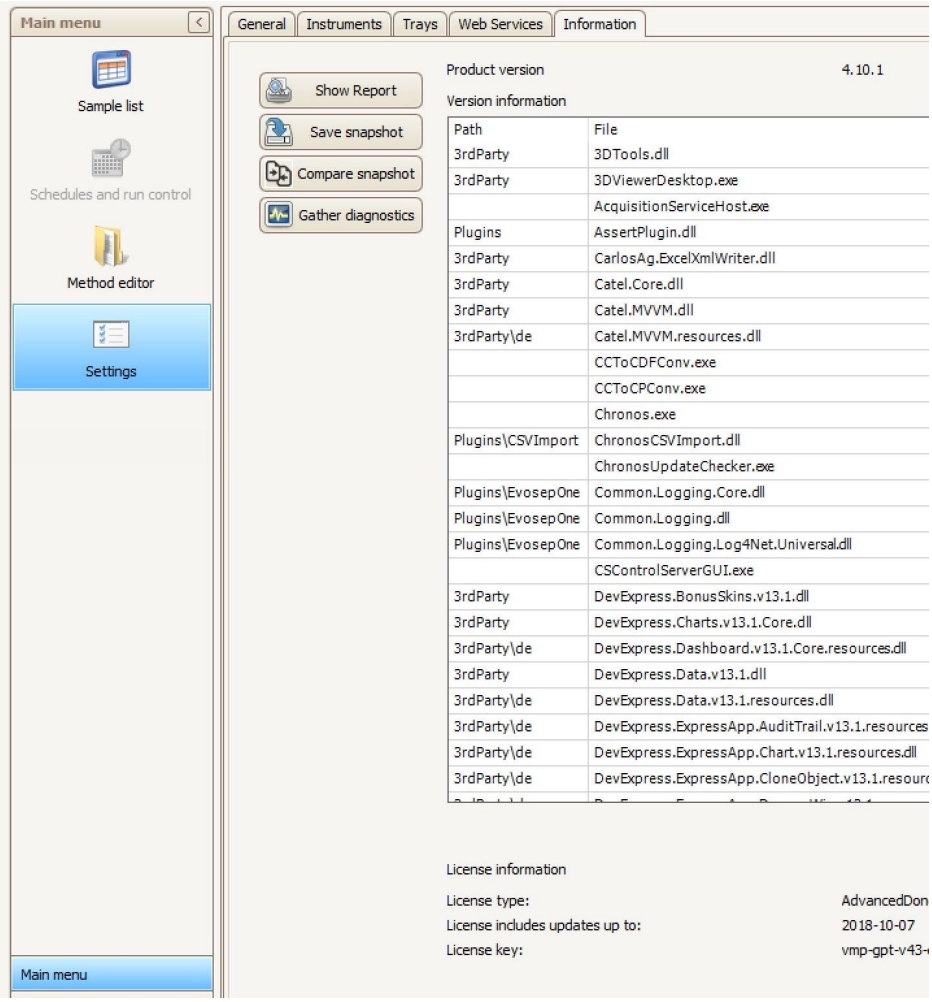
  Note: Before updating, please check for compatibility issues in the release notes for your version of the Evosep One plugin.



- Following error message during the install/upgrade of Chronos “The license key in invalid or expired” most likely means that the 1-year free updates is expired.



- The License free update period can be checked by clicking the Information tab in the Settings menu.



- **Note:** During the update of the Evosep One Plugin, settings can be overwritten, therefore always re-install the Evosep One Plugin after a Chronos update.

Please note the following, when updating the Evosep One plugin:

- If updating the Evosep One plugin to a newer version, please note that all Chronos method files will be overwritten.
- If updating with the same version of the Evosep One plugin the Chronos method files will not be overwritten.
- In case you need to reinstall the plugin, using the same version number, please uninstall the Evosep One plugin using the Windows program uninstaller feature.

4.2.1 IP configuration

By default, the Evosep One instrument will be set up automatically during plugin installation as a subnet with the following IP addresses

- Netmask: 255.255.255.240
- Host PC address: 172.17.17.14
- Modbus gateway address/pump: 172.17.17.1
- PAL address: 172.17.17.2

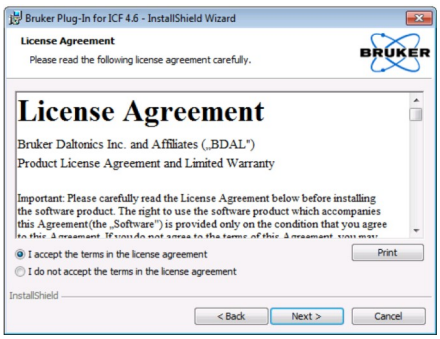
4.3 Evosep drivers for control of Bruker MS

4.3.1 Installing ICF for Bruker Compass HyStar

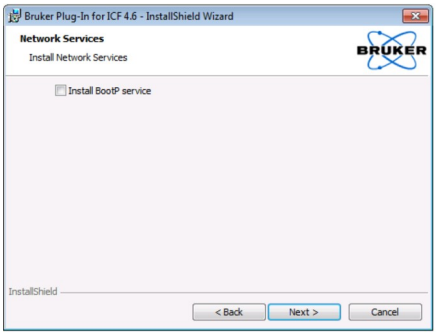
Install the Plugin on a system with appropriate ESI Compass / HyStar software already installed.

1. Insert media containing the ICF plugin for HyStar.
2. Navigate to the ICF plugin X.X for HyStar Y.Y folder for either Win 7 or Win 10
3. Run the “CD Start” application file and click “Install” to install the Plugin.

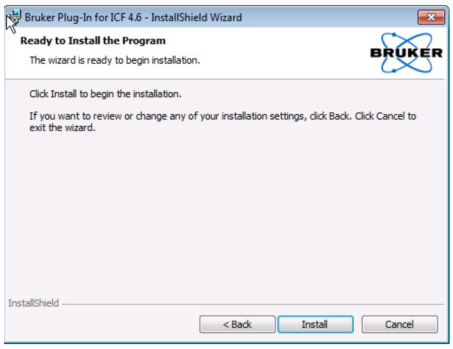
4. Follow the various pop-up windows with info regarding the installation.
5. Accept the terms in the License Agreement and click “Next”.

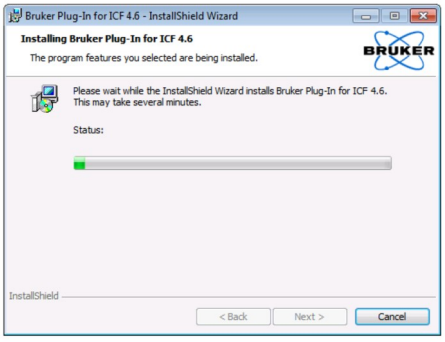


6. In the Network services window, do not check the “Install BootP service”.

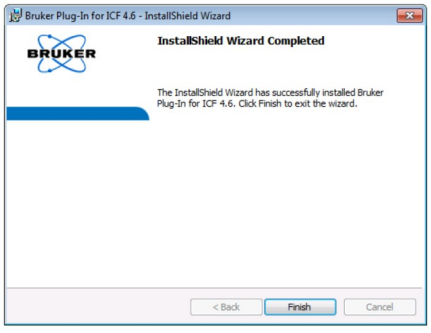


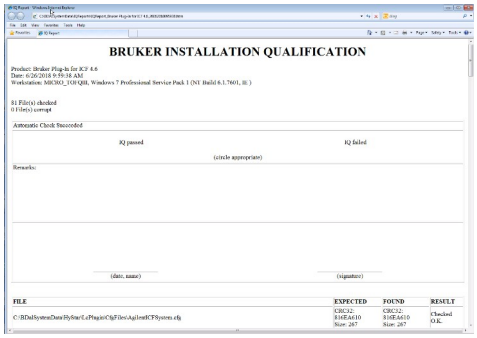
7. Now click “Install” to install the program.



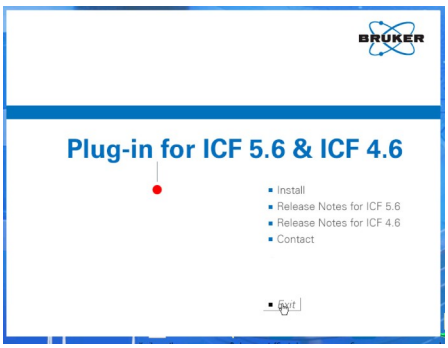


8. Click “Finish” and in the Bruker installation qualification pop up window check that all parts of the installation have been checked OK.





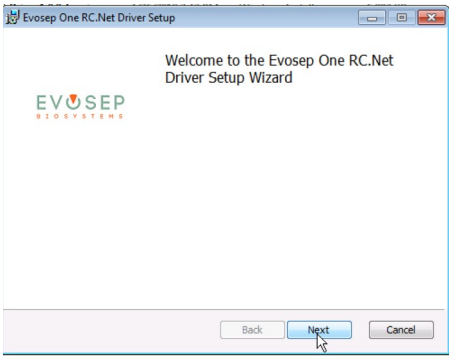
9. Finally click “Exit” to close the CD start menu.



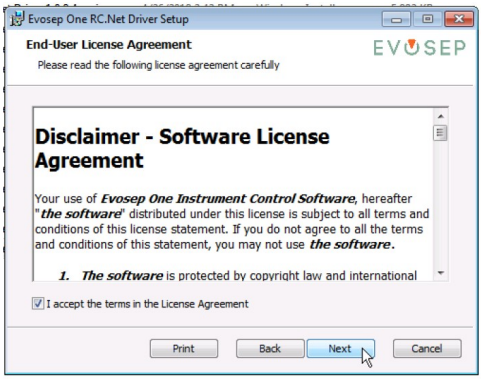
4.3.2 Installing the Evosep One RC.Net driver 2.x.x.x.msi

Make sure the ICF plugin for HyStar is already installed.

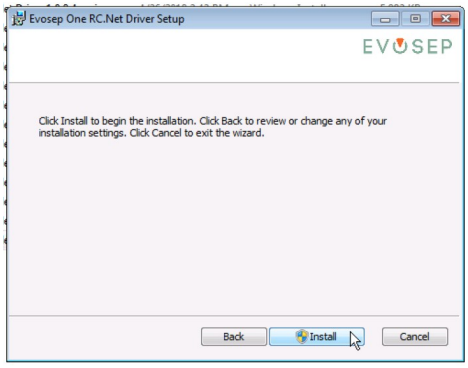
17. Connect the Evosep One instrument to the computer via ethernet cable (optionally through a network switch).
18. Insert media containing the Evosep One RC.Net driver.
19. Run the Evosep One RC.Net Driver 2.x.x.x Windows installer package file.
20. Click “Next”.



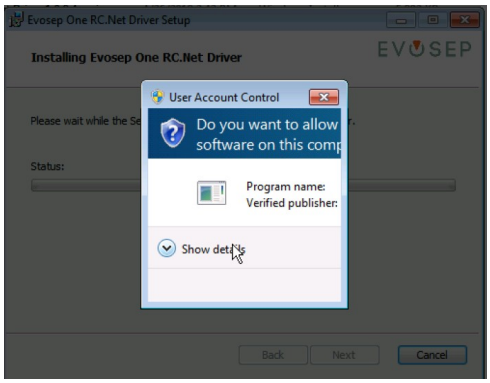
21. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.

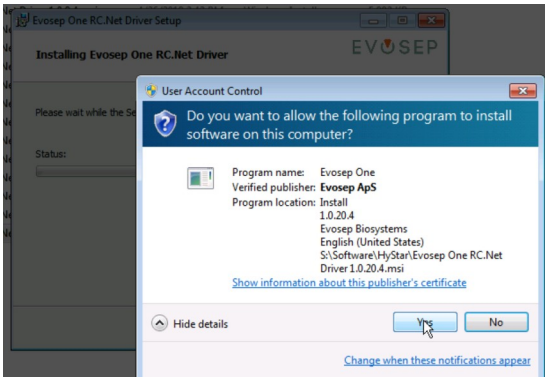


22. Please read the information in “Prerequisites” window carefully, then click “Next”.
23. Click “Install” to begin the installation and click “Yes” to any popups during the install.

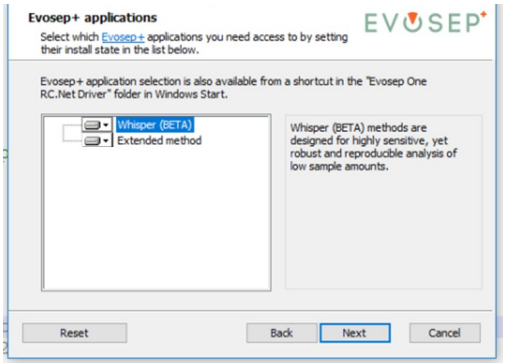


24. Click show details (if window not fully visible) then click “Yes” to allow the program to install the software.

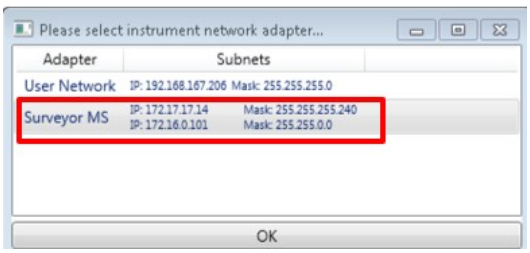




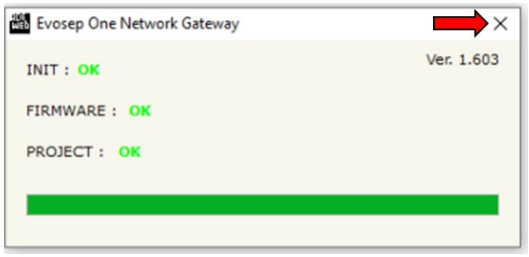
25. Choose which Evosep+ application you wish to install in addition to the standard methods.



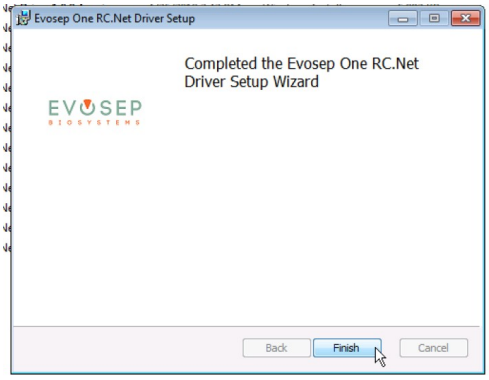
26. If asked to select instrument network adapter, always choose the MS/LC network adapter and then click “OK”.



27. If the firmware update dialog is shown during the installation, please verify that status is OK for INIT, FIRMWARE and PROJECT, then close the dialog by clicking the “X”.



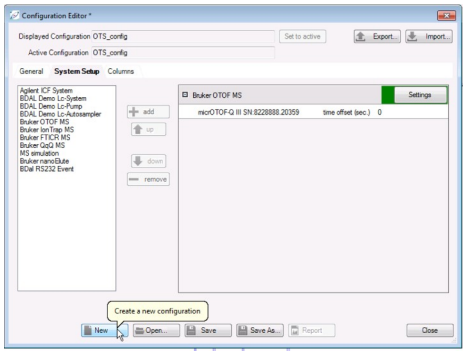
28. When the install is complete, click “Finish”, to exit the installer.



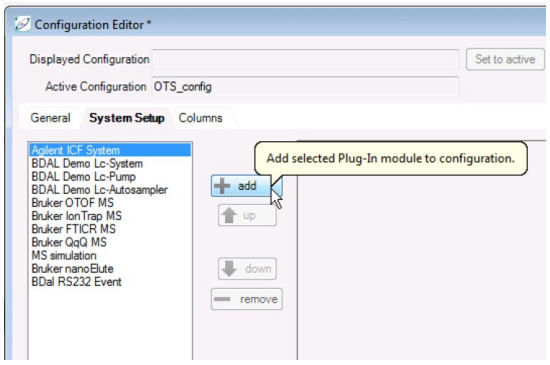
4.3.3 Create HyStar IFC configuration for Evosep One

1. Open Compass HyStar and click the gearwheel icon, to open the Configuration Editor.

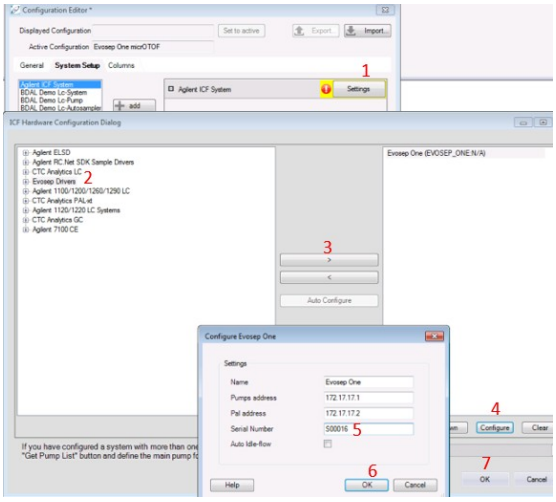
2. In the Configuration Editor window, click “New” to create a new configuration.



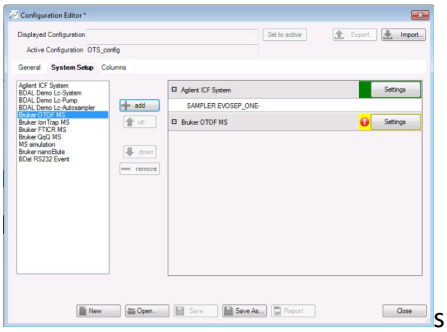
3. Mark “Agilent ICF System” and click “add”.



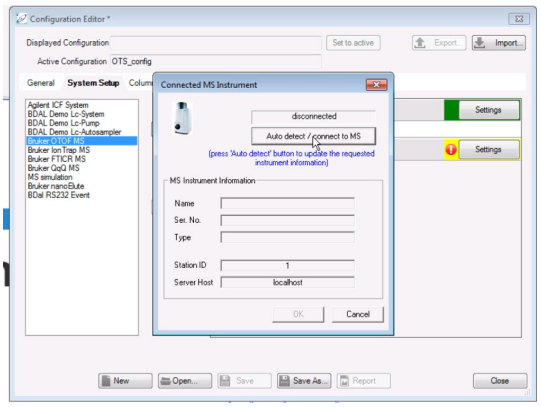
4. Click 1. “Settings”, 2. Mark “Evosep Drivers”, 3. Click the “>” button, 4. Click “Configure”, 5. Type-in the Evosep One Serial number and check “Auto Idle-flow” if needed, 6. Click “OK” and finally 7. click “OK” in the ICF hardware configuration Dialog.

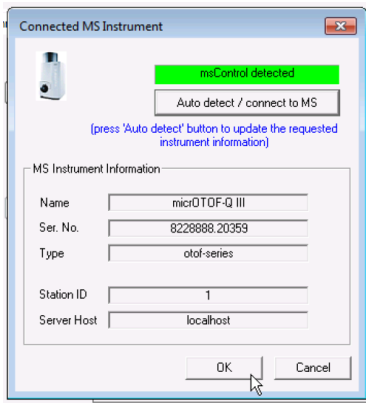


5. In the Configuration Editor mark the MS model being used then click “add” and then click “Settings” for the newly added MS.

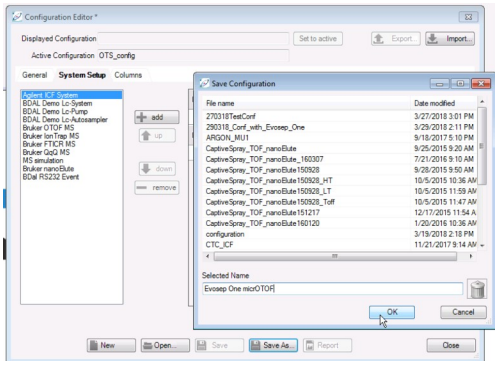


6. Click the auto detect button and verify that the MS is being detected and then click “OK”.

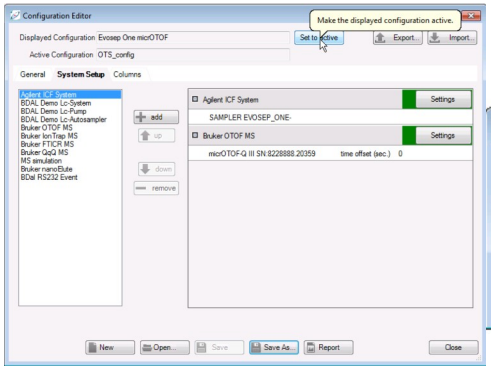


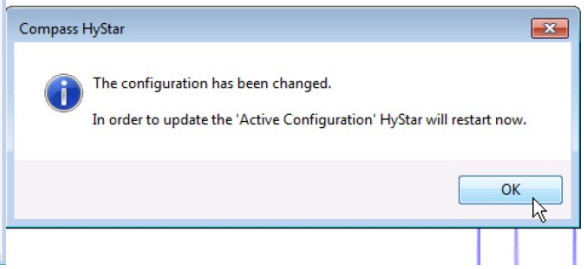


7. Click “Save As…” and give the configuration a name, e.g. “Evosep One MS model” then click “OK”.



8. Click “Set to active” to use the Evosep One configuration, click “Close” and click “OK” to restart HyStar.

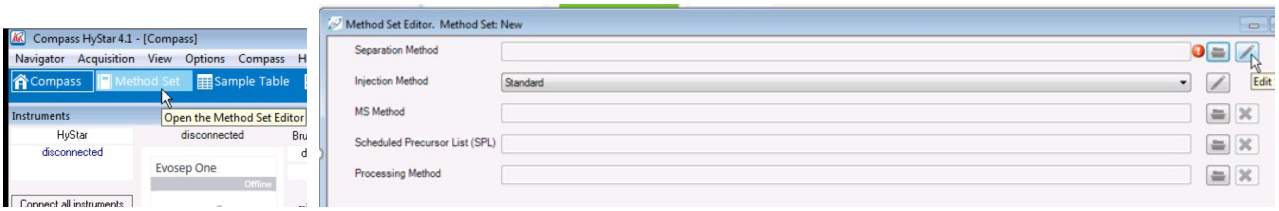




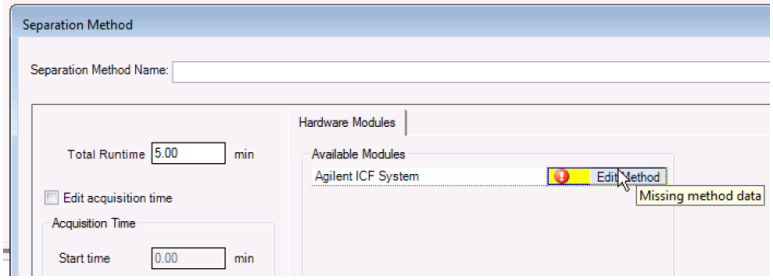
9. If upgrading from an earlier ICF plugin, carefully check all the HyStar hardware profiles (using ICF) and recreate if needed.

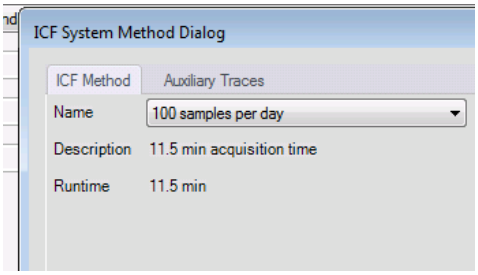
4.3.4 Create Evosep separation methods

1. Create Evosep One separation methods by clicking “Method Set”, then set Injection method to “Standard” and then click the small pencil to edit the separation method.



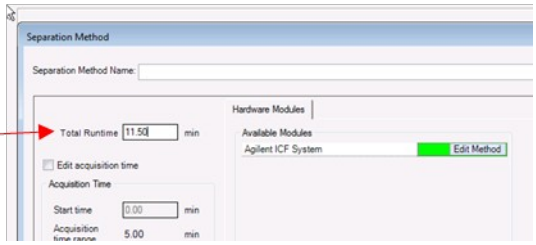
2. Click “Edit Method” and choose one of the predefined Evosep methods, e.g. “100 samples per day” and click “OK”. Please note the Runtime for the chosen method name in the ICF System Method dialog.



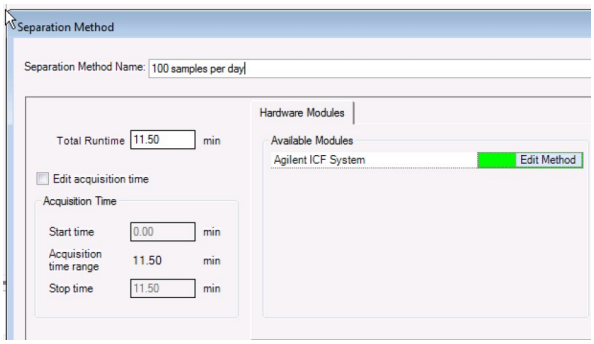


3. Now, if not done automatically, set the Total Runtime for the chosen method, using below table and or Runtime from above dialog, below example given for the 100 samples per day method.

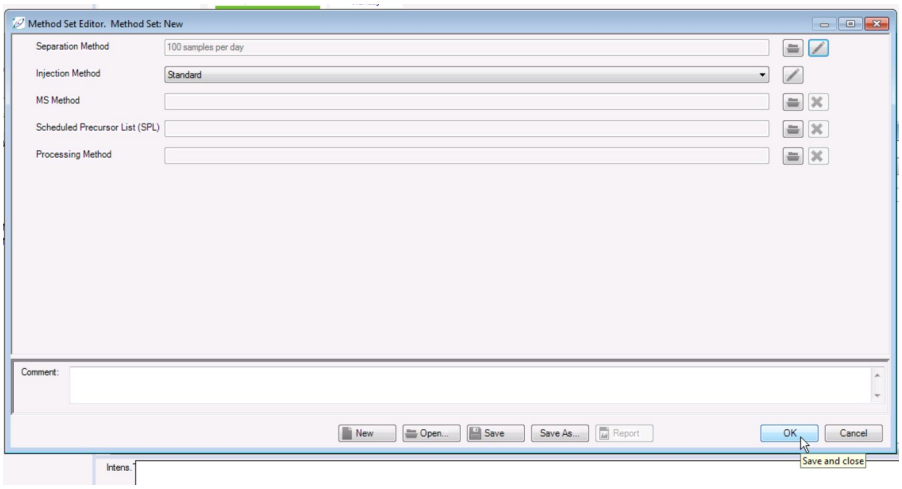
Throughput	Gradient Length
Samples/day	Minutes
300	3.2
200	5.6
100	11.5
60	21
30	44



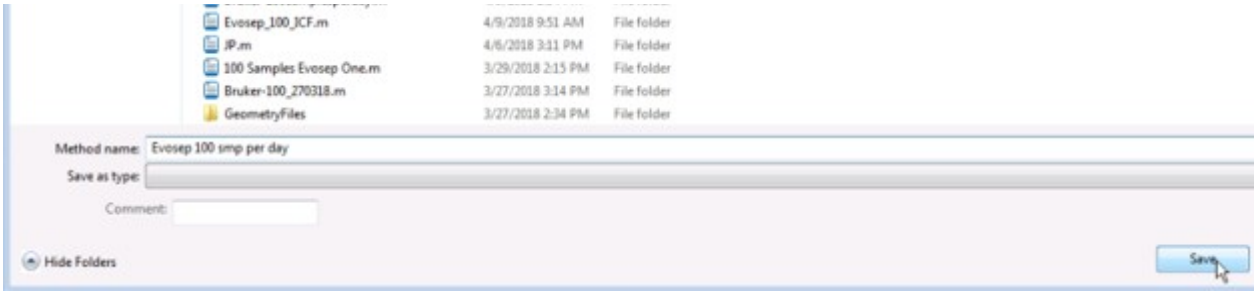
4. Give the Separation Method the same name as chosen in the ICF System Method Dialog, e.g. “100 samples per day” and click “OK”.



5. In the Method Set Editor, click “OK” to save and close.



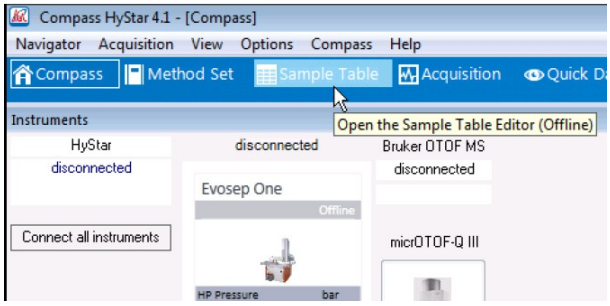
6. Save the method with the Separation method name e.g. “Evosep 100 samples per day” for the 100 samples per day method, “Evosep 60 samples per day” for the 60 samples per day method etc.

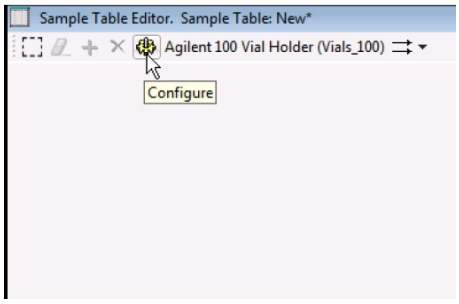


7. Go back to the start of the “Create Evosep separation methods” section and create separation methods for the remaining methods.
    a. 30 samples per day
    b. 60 samples per day
    c. 100 samples per day
    d. 200 samples per day
    e. 300 samples per day

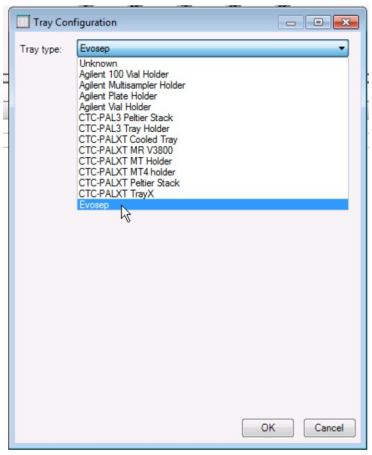
4.3.5 Create Evosep One tray type and Sample Table

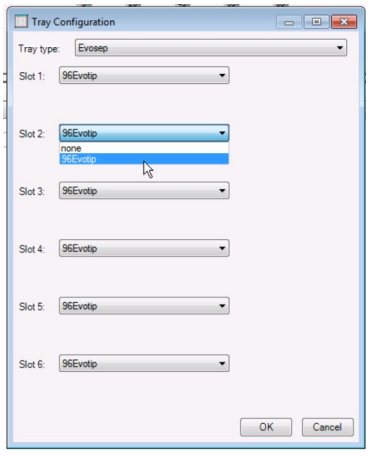
1. When HyStar has restarted, create the Evosep One tray type by clicking “Sample Table” and then the small gearwheel icon in the Sample Table Editor.





2. Choose “Evosep” as tray type and then choose “96Evotip” for Slot 1 to 6 and click “OK”.





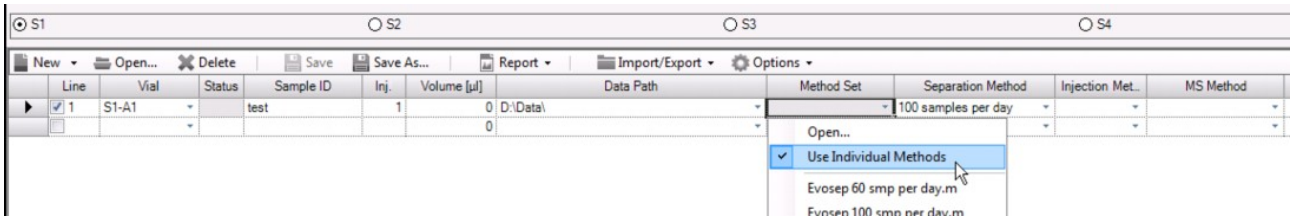
3. In the sample table line 1 set following:

    a. Vial: S1-A1

    b. Sample ID: test

    c. Method Set: Click the small arrow and check mark “Use individual Methods”

    d. Separation Method: choose Evosep 100 samples per day



4. Now click Save As… and set name as “Evosep One Sample table” and then click ok to save the sample table.

5. Click Close to close the Sample Table Editor window.

4.3.6 Flow to column – 20% Sol B enables MS calibration

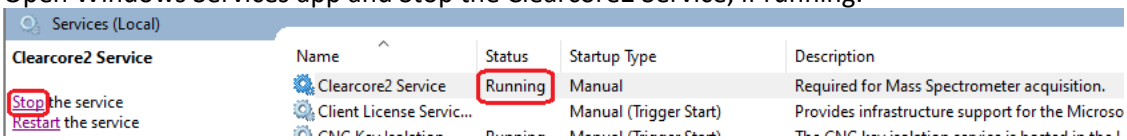
1. The service program, Flow to column – 20% sol B, is intended to aid MS calibration. It will run 20% solvent B to the transfer line at 250 nl/min for 10 min. The program requires a Evotip in position S1-A1.

4.4 Evosep One driver for SCIEX OS

4.4.1 Installation

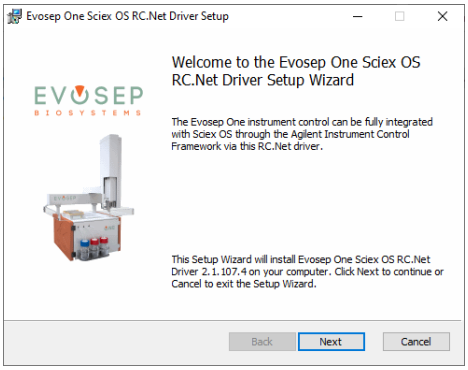
Prerequisites:

A. Make sure SCIEX OS 2.0, or newer, is installed.
B. Close SCIEX OS, if running.
C. Open Windows Services app and Stop the Clearcore2 Service, if running.

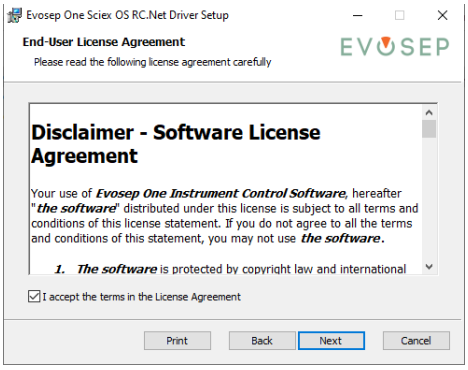


Installation procedure:

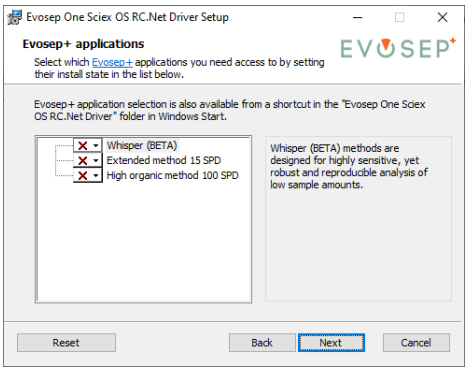
1. Connect the Evosep One instrument to the computer via ethernet cable, and make sure that the instrument is switched on.
2. Run the Evosep One SCIEX OS RC.Net Driver 2.x.x.x Windows installer.
3. Click “Next”.



4. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.



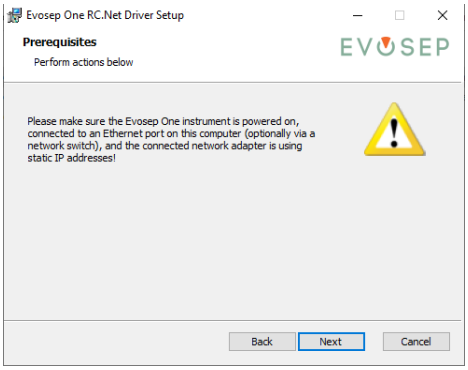
5. In the Evosep+ applications window, click “Next”.



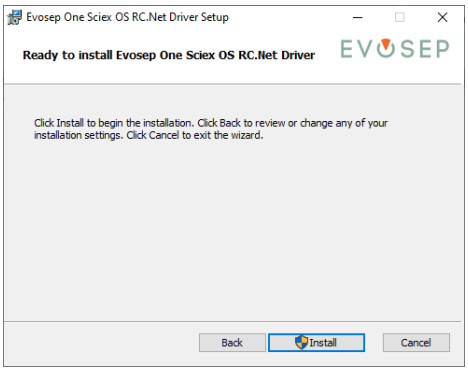
The +applications are not installed during a standard installation, because Evosep prefers that the customer actively chooses the +applications they need.

During the user training, the +applications, and how to select them from the Evosep folder from the windows start menu, should be discussed.

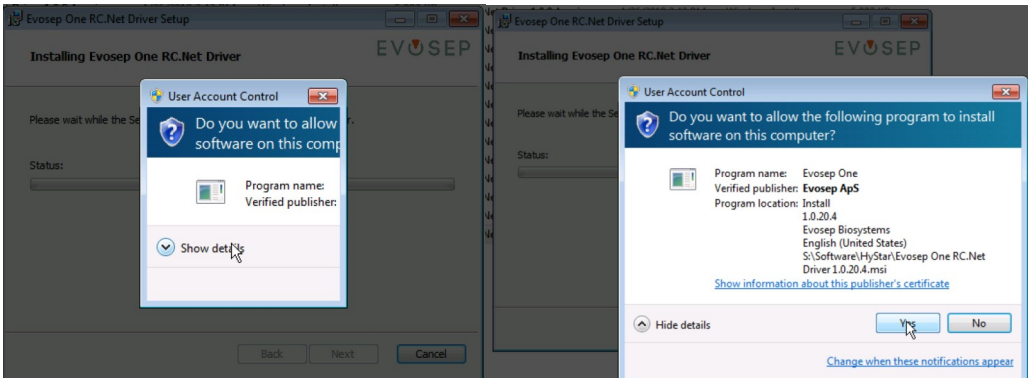
6. Please read the information in the “Prerequisites” window carefully, then click “Next”.



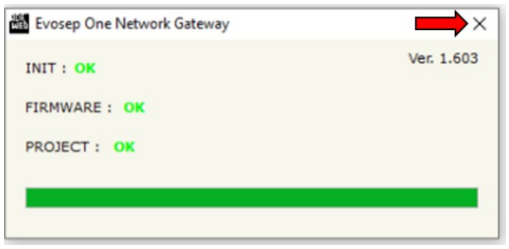
7. Click “Install” to begin the installation and click “Yes” to any pop-ups during the install.



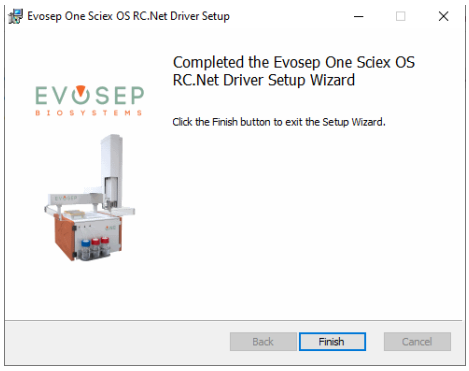
8. Click show details (if window not fully visible) then click “Yes” to allow the program to install the software.



9. If the firmware update dialog is shown during the installation, please verify that status is ok for INIT, Firmware and Project, then close the dialog by clicking the “X”.

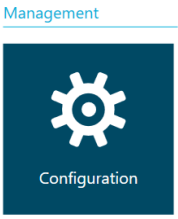


10. When the install is completed click “Finish”, to exit the installer.



4.4.2 Create SCIEX OS hardware configuration for Evosep One

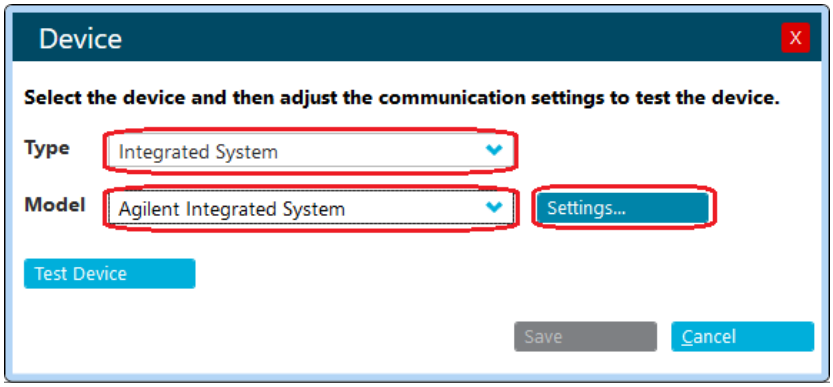
1. Start SCIEX OS (this will automatically start the Clearcore2 Service also).
2. Select “Configuration”



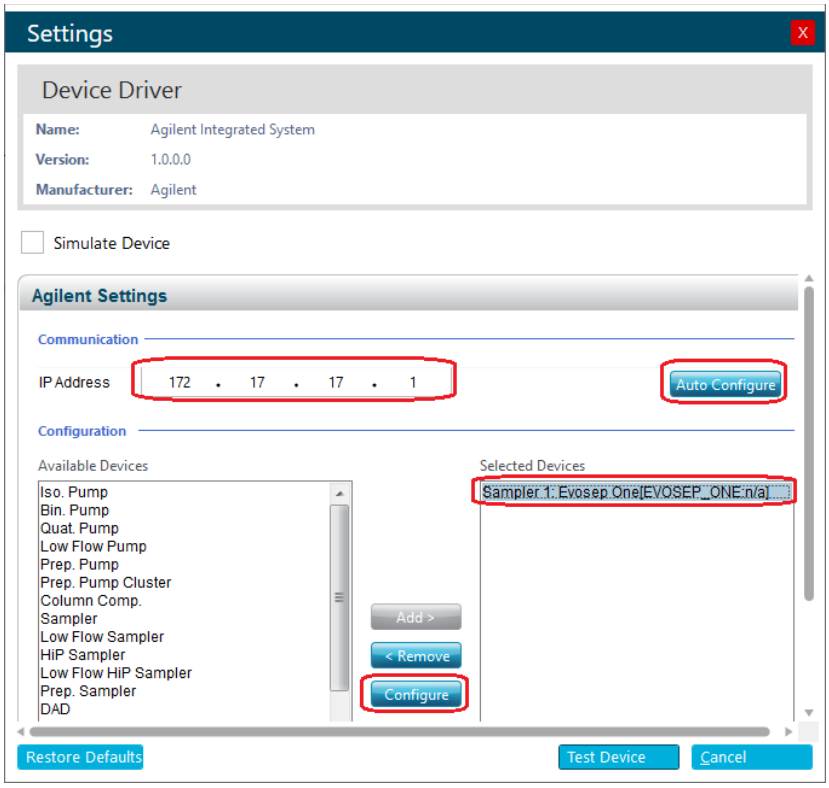
3. If the current configuration is active, click the “Deactivate” button on the toolbar.

4. Click the “Add” button on the toolbar.

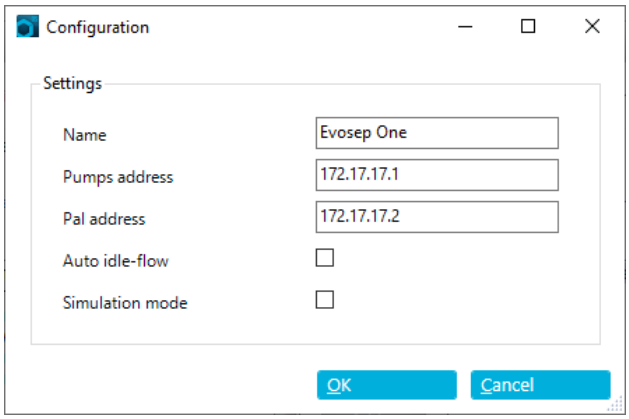
5. On the “Device” dialog, select “Integrated System” and “Agilent Integrated System” and click “Settings…”.



6. On the “Settings” dialog, enter 172.17.17.1 as IP Address and click “Auto Configure”.

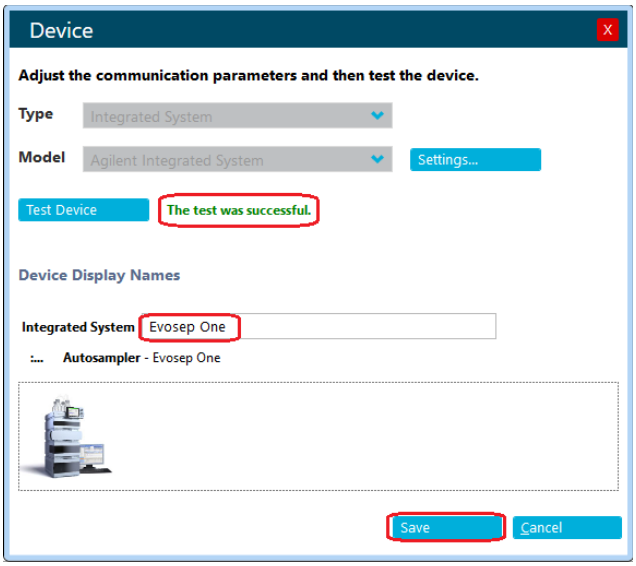


7. After a little while, the Evosep One instrument shows up in the “Selected Devices” list. Select it and click “Configure”.
8. On the Evosep One Configuration dialog, you can set some basic instrument settings:



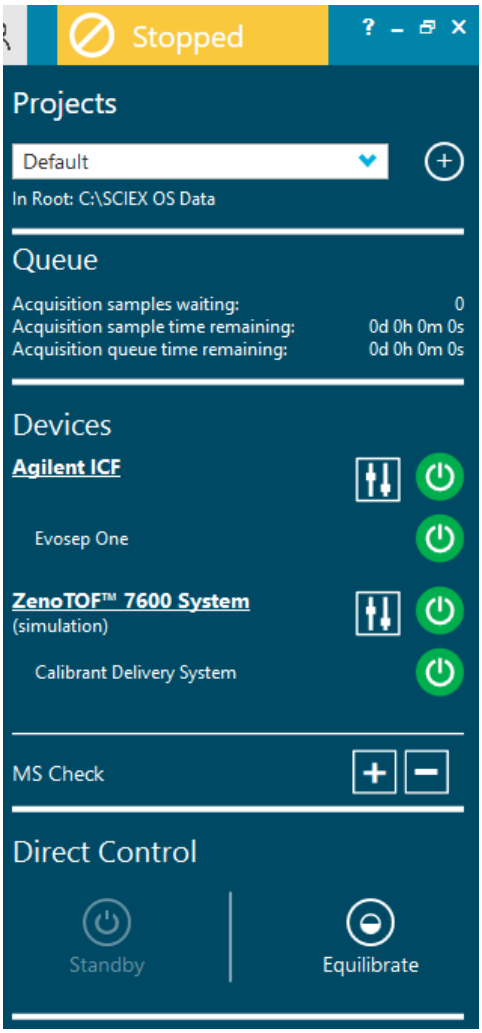
   a. Name: Used for display, leave at default.
   b. Pumps address: Communication setting, leave at default.
   c. Pal address: Communication setting, leave at default.
   d. Auto idle-flow: Start idle-flow after a few minutes of inactivity.
   e. Simulation mode: **Use for testing without a Evosep One device present.** This will offer some very basic methods for testing, including emitting generated pump trace data.
9. When satisfied with the configuration, click “OK” to save and close the dialog.
10. Back on the “Settings” dialog, click “Test Device”

11. On the resulting “Device” dialog, verify that it was successful, then change the “Integrated System” display name to “Evosep One”.



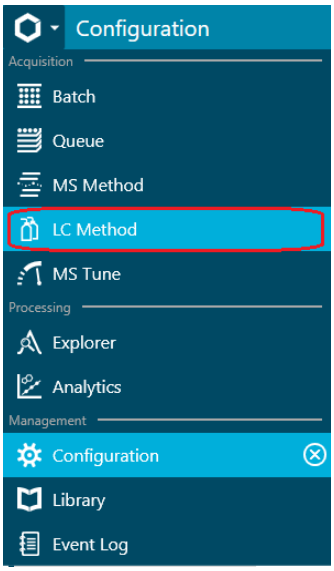
12. Click “Save”.
13. Click “Activate Devices”.

14. Click “Stopped” at the top-right in SCIEX OS to see the state of your configured devices, e.g.:



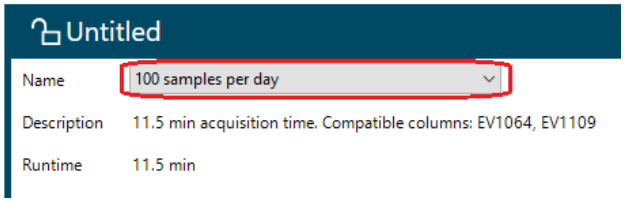
4.4.3 Create SCIEX OS LC methods for Evosep One

1. In the top-left dropdown menu, click “LC Method”

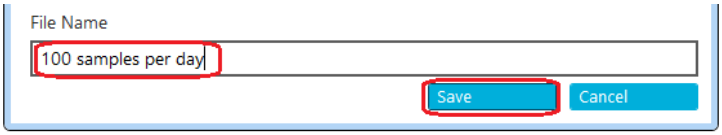


2. Click “New”

3. In the editor, select the desired method and click “Save”.



4. Name the LC file the same as the Evosep One method name and click “Save”.



5. Repeating step 3 and 4, create a LC method for each of the Evosep One methods you want to use in your project. Note the method acquisition runtime, which you will need when creating the corresponding MS method (MS dependent, not described here).
   The Evosep One standard methods have these gradient lengths:

Throughput	Gradient Length
Samples/day	Minutes
300	3.2
200	5.6
100	11.5
60	21
30	44

*Please note that the duration of the “System and column wash” method is column dependent (approximately 10 min) but that there is no need for collecting MS data during the wash, hence the MS acquisition time should be set to 1 min.

4.5 Evosep One driver for Thermo Chromeleon / Xcalibur

4.5.1 Chromeleon vs SII for Xcalibur

SII for Xcalibur is basically a Chromeleon installation repackaged. You should choose to install either one or the other, but not both at the same time as that will cause issues with license management. The current version, SII for Xcalibur 1.7, builds on Chromeleon 7.3.1.

The Evosep One Chromeleon driver can be used in two different scenarios:

1. Directly in the Chromeleon software package.
   or
2. In Xcalibur, through “Thermo SII for Xcalibur”.

SII is short for Standard Instrument Integration and SII for Xcalibur enables the use of Chromeleon drivers in Xcalibur.

4.5.2 Compatible software:

For operating system and environment in general, follow the requirements for the version of Chromeleon or SII for Xcalibur, which the driver will be running under. Currently, Windows 10 64-bit US English is supported.

For Thermo SII for Xcalibur 1.7, Thermo Foundation 3.0 SP2 or later is required.

No specific Xcalibur version is mentioned by the SII installer, but release notes states that validation was done with Xcalibur 4.5 and 4.5 SP1.

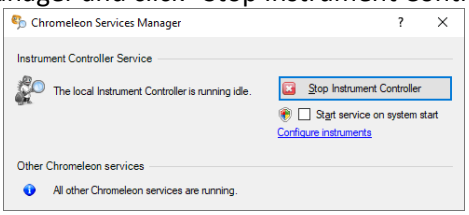
4.5.3 Prerequisites:

D. Make sure that either:
   a. Thermo Chromeleon 7.3.1 or newer is installed.
      or

b. Thermo SII for Xcalibur 1.7 or newer is installed.

E. Close Chromeleon and/or Xcalibur, if running.

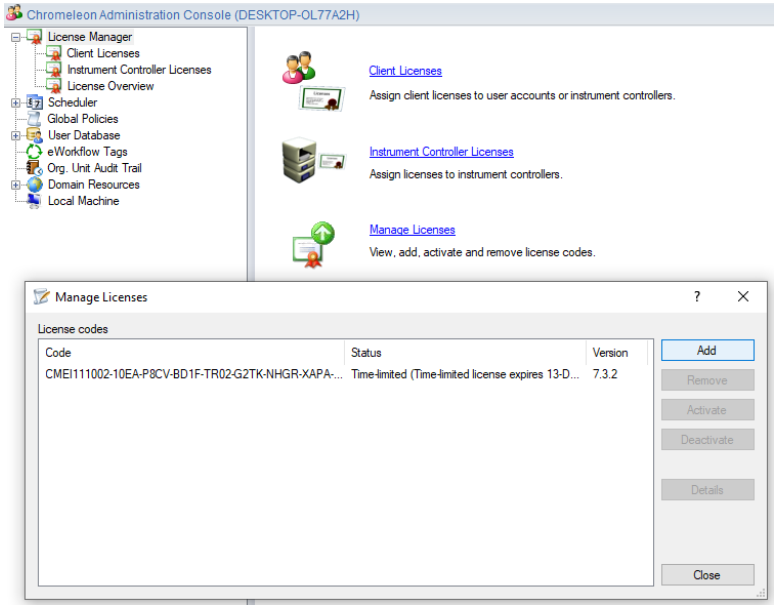
F. Open Chromeleon Services Manager and click “Stop Instrument Controller”, if running.



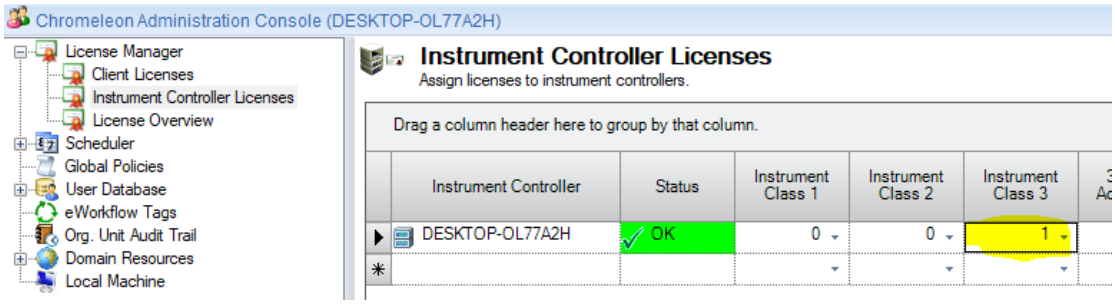
4.5.4 Chromeleon/SII for Xcalibur license

License can be added through the Chromeleon Administration Console.

Click “Manage Licenses” and “Add” the license key:

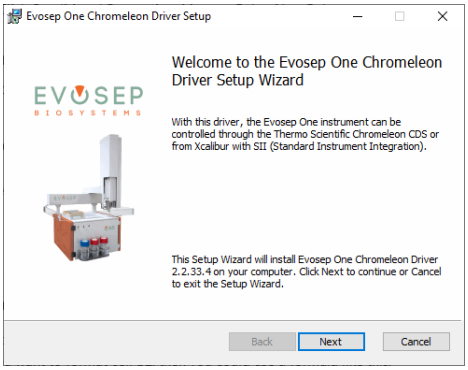


Under “Instrument Controller Licenses”, make sure that at least one Class 3 instrument is allowed:

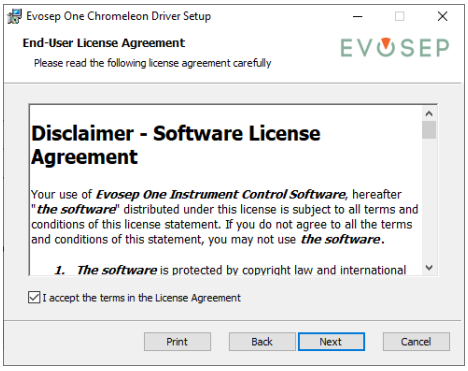


4.5.5 Installation procedure:

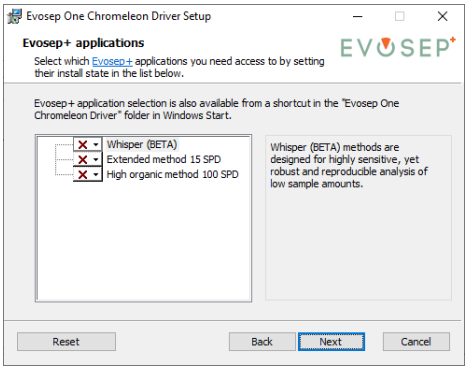
29. Connect the Evosep One instrument to the computer via ethernet cable, and make sure that the instrument is switched on.
30. Run the Evosep One Chromeleon Driver 2.x.x.x Windows installer.
31. Click “Next”.



32. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.



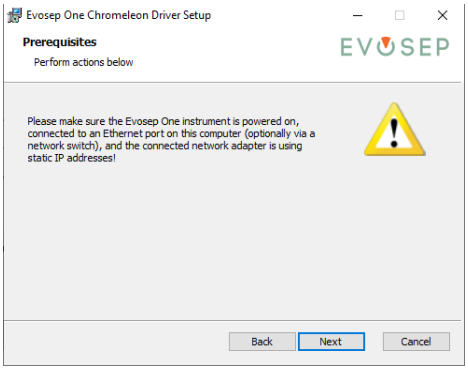
33. In the Evosep+ applications window, click “Next”.



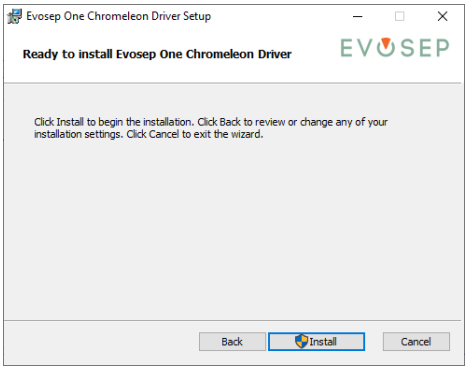
The Evosep+ applications are not installed during a standard installation, because Evosep prefers that the customer actively chooses the Evosep+ applications they need.

During the user training, the Evosep+ applications, and how to select them from the Evosep folder from the windows start menu, should be discussed.

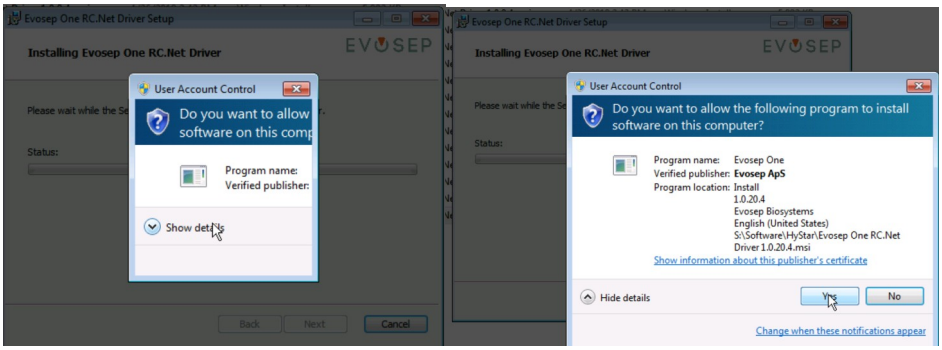
34. Please read the information in the “Prerequisites” window carefully, then click “Next”.



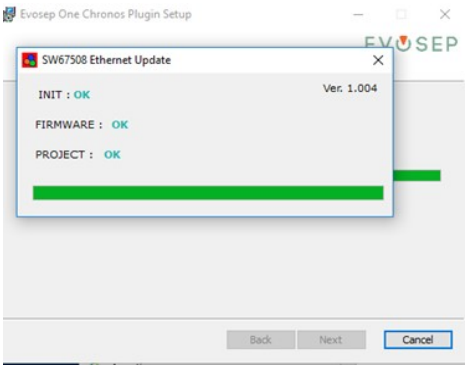
35. Click “Install” to begin the installation and click “Yes” to any pop-ups during the install.



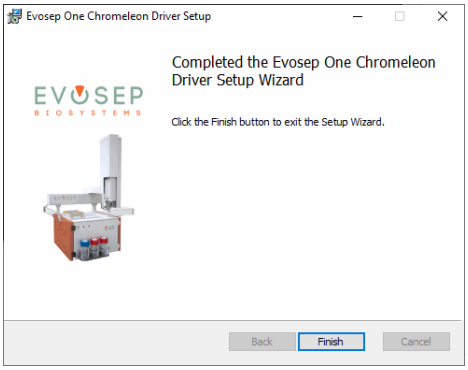
36. Click “Show details” (if window not fully visible) then click “Yes” to allow the program to install the software.



37. If the Evosep One is connected, firmware will be checked/updated.



38. When the installation is complete, click “Finish” to exit the installer.

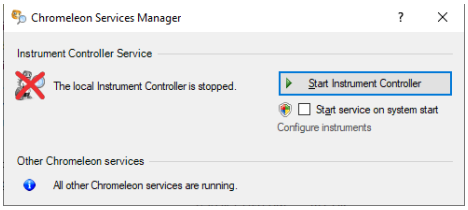


4.5.6 Checking driver installation

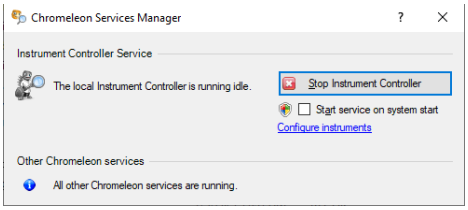
The Evosep One driver integrates into Chromeleon’s Installation Qualification, by registering its own inventory file. This means you can simply run “Station IQ” from the “Thermo Chromeleon 7” start menu folder, to verify the installation.

4.5.7 Create Chromeleon hardware configuration for Evosep One

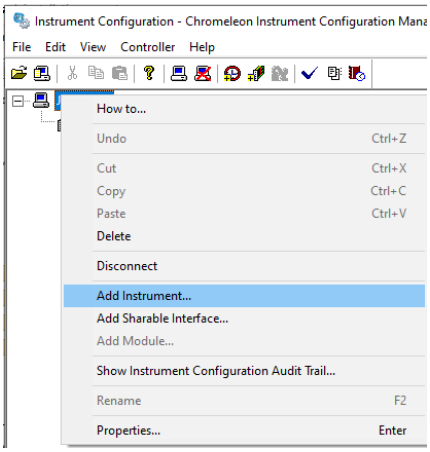
10. From the Chromeleon Services Manager, click “Start Instrument Controller”.



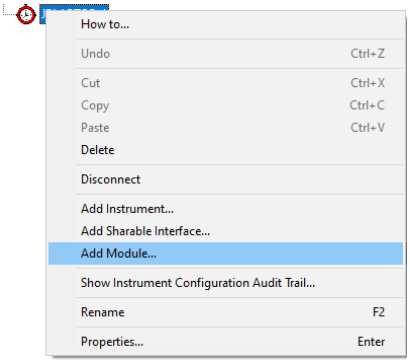
11. Click “Configure instruments”



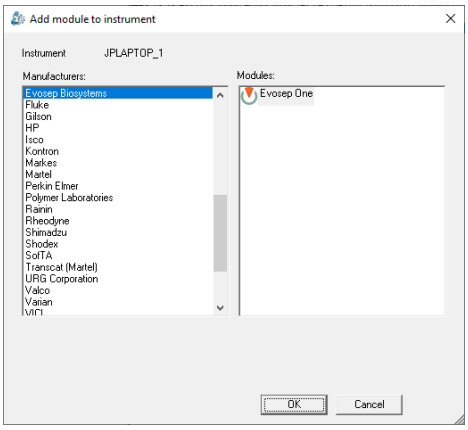
12. Right-click the controller instance and click “Add Instrument…”.



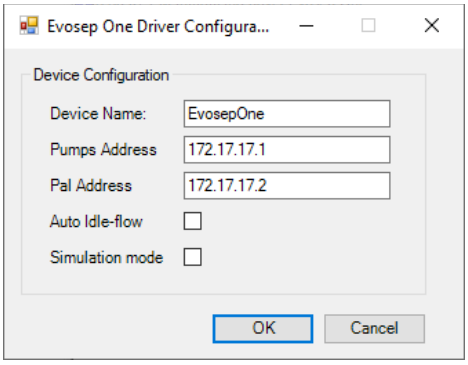
13. Enter desired name, or leave at default, then click “OK”.
14. Right-click the added instrument and click “Add Module…”.



15. Select “Evosep Biosystems” in the “Manufacturers” list and “Evosep One” in “Modules”. Then click “OK”.



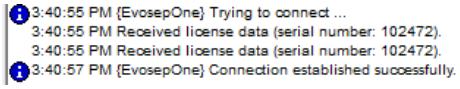
16. On the Evosep One Driver Configuration dialog, you can set the basic instrument settings:



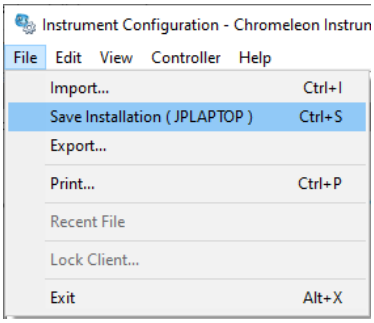
a. Name: Used for display, leave at default.
b. Pumps address: Communication setting, leave at default.
c. Pal address: Communication setting, leave at default.
d. Auto idle-flow: Start idle-flow after a few minutes of inactivity.
e. Simulation mode: **Use for testing without an Evosep One device present.** This will offer some very basic methods for testing, including emitting generated pump trace data.

17. When satisfied with the configuration, click "OK" to close the dialog.

The message window should show something like this:



18. Click "File", then "Save Installation".

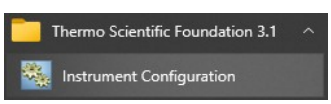


19. Upon successful configuration, you may close the window.

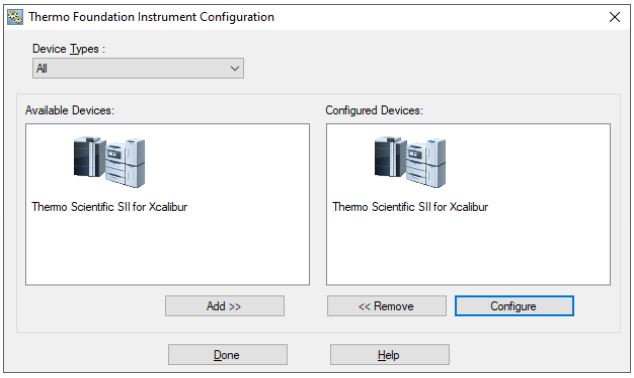
4.5.8 Configuring Thermo Scientific SII for Xcalibur

Note: This is only necessary if using the Evosep One driver in Xcalibur, through SII. If using the driver directly in Chromeleon, you do not need to perform this instrument configuration.

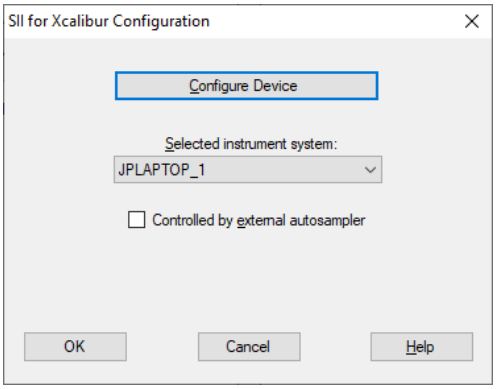
6. Open the Thermo Foundation Instrument Configuration from the Windows Start Menu.



7. In the “Available Devices” section(left), click “Thermo Scientific SII for Xcalibur” and click “Add >>”.



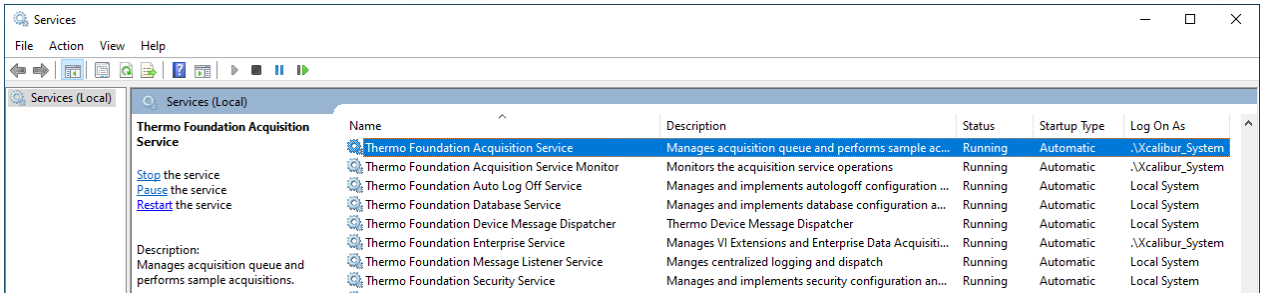
8. In the “Configured Devices” section(right), click “Thermo Scientific SII for Xcalibur” and click “Configure”.
9. In the “SII for Xcalibur Configuration” window, click “Configure Device”.



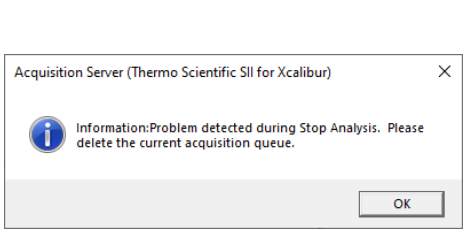
10. Close the window that opened, then click “OK”.
    (This may seem unnecessary, but if this step is not performed, the configuration will not be initialized correctly!)
11. Back in the “Thermo Foundation Instrument Configuration” window, click “Done”.

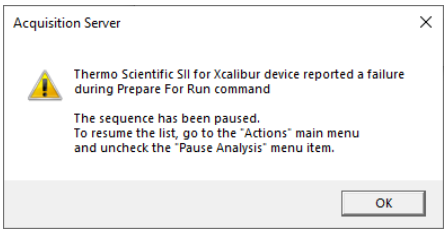
4.5.9 Important - When upgrading from earlier driver version

If you upgraded the Evosep One Chromeleon Driver from an earlier version, you may need to restart the Thermo Foundation Acquisition Service, before starting Xcalibur.



Failing to do so, may result in below errors when starting a sample or sequence:



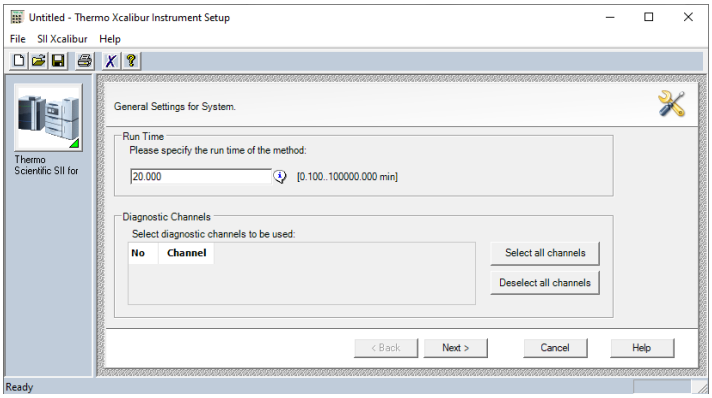


4.5.10 Create LC methods for Evosep One

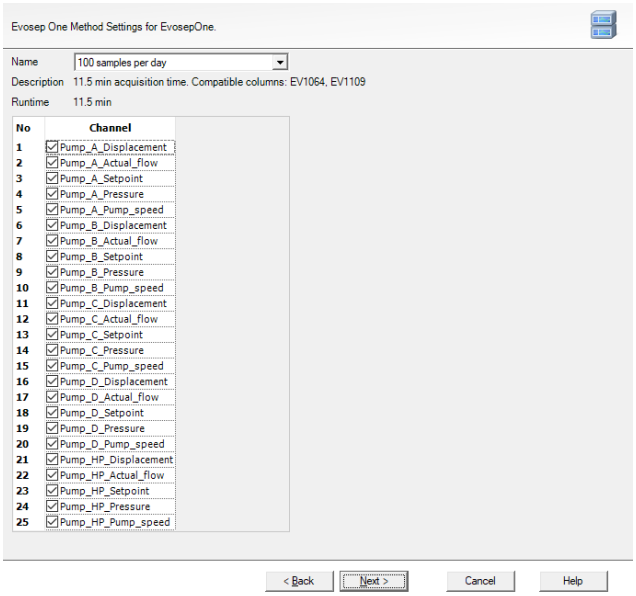
This section describes how to create LC methods through Xcalibur.

1. In Xcalibur, click “Instrument Setup”.

2. Click “Next”
   Note: The run time can be ignored here, it is set automatically when selecting the Evosep One method next.



3. Select the desired method in the drop-down and click “Next”.



4. Click “Finish”
5. Save the method using File>Save As…
   Note: Name the method file the same as the method, e.g. “100 samples per day.meth”
6. Click “Save”
7. To create the remaining methods, simply repeat steps 3, 5 and 6 only.

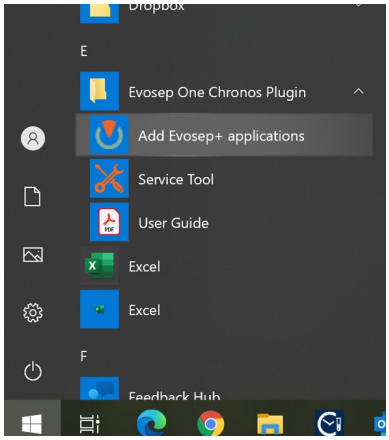
4.6 Adding specialized applications to the Evosep One

The Evosep One is preconfigured with 5 standard methods with throughput ranging from 300 to 30 samples per day. In addition, it is now possible to add a specialized long method with a gradient duration of 88 minutes. The method must be manually enabled by:

1. Close the Evosep One control software (Chronos/HyStar)

2. Depending on what software is being used on the Evosep One, choose the appropriate folder from the Windows Start menu. I will be one of:

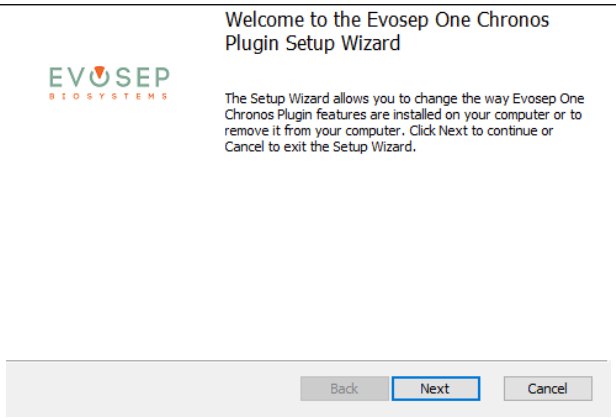
   - Evosep One Chronos Plugin
   - Evosep One HyStar Driver
   - Evosep One SCIEX OS Driver
   - Evosep One MassHunter Driver
   - Evosep One Chromeleon Driver



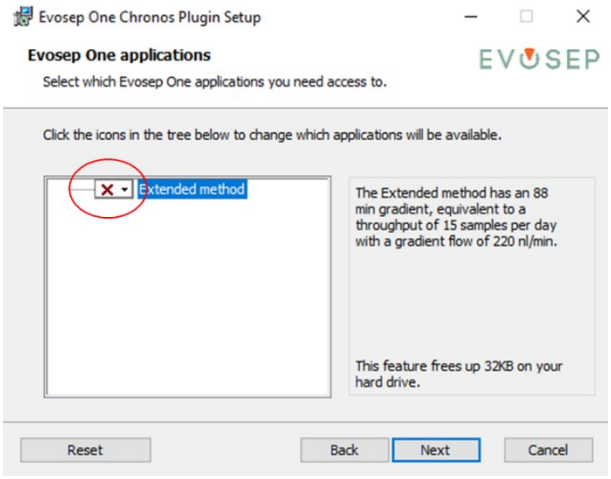
3. Expand the folder and click the “Add Evosep+ applications”.

4. Click next in the Evosep One Setup window.

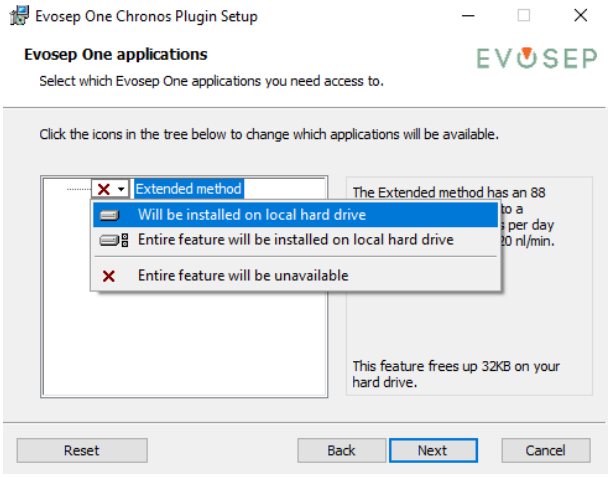
Following example is for the extended method, when other methods become available the procedure will be the same.



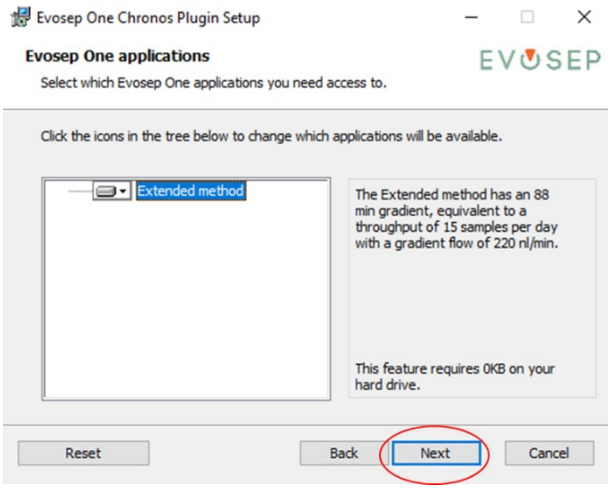
5. Click the Extended method icon.



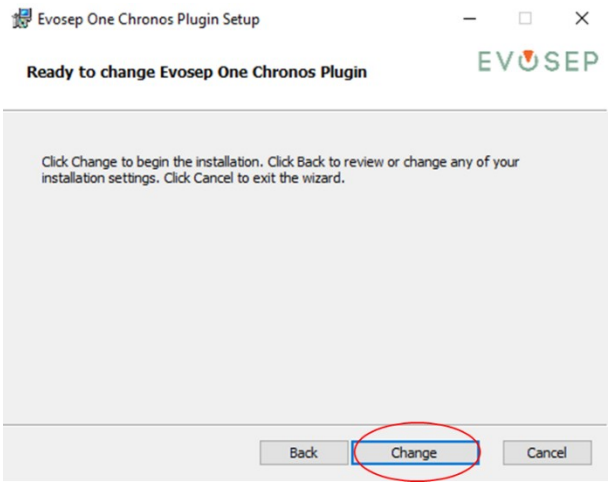
6. Choose “Will be installed on local hard drive”.



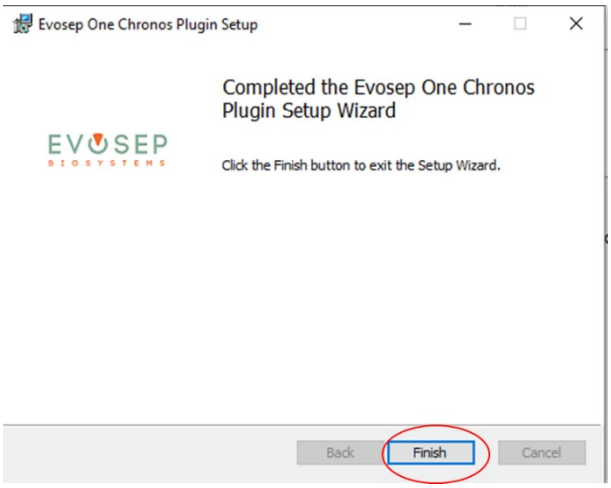
7. Click Next.



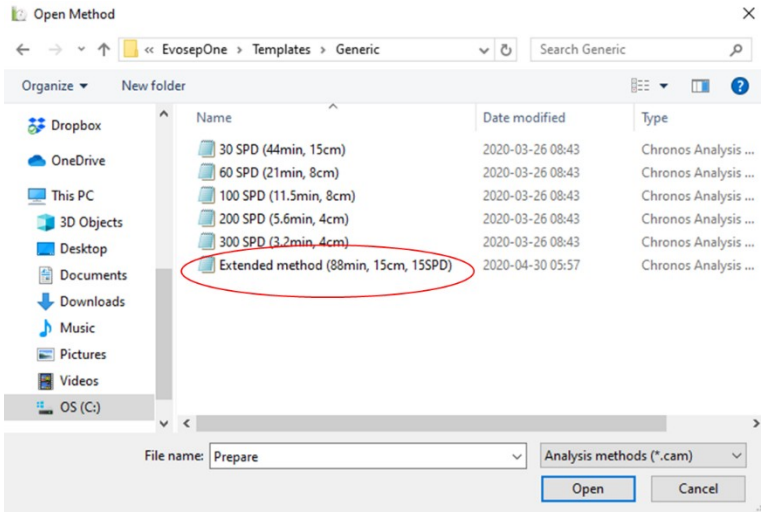
8. Click Change.



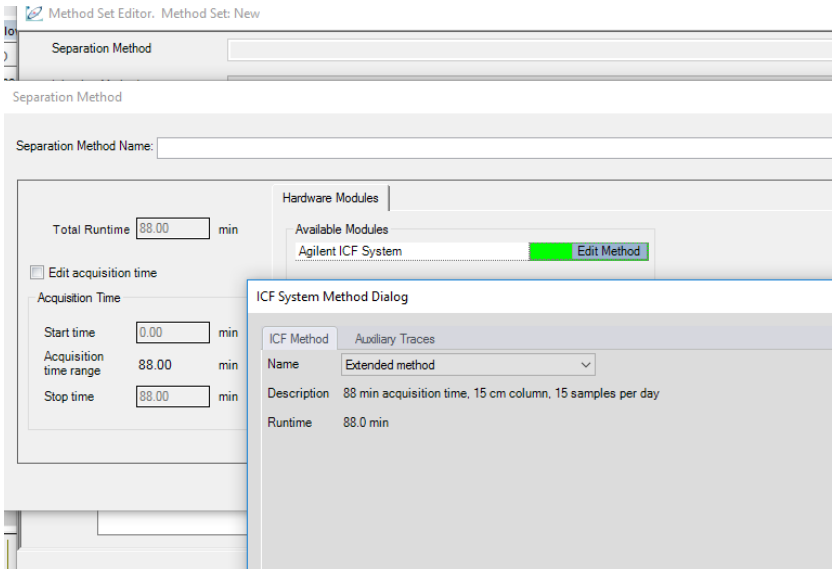
9. Click Finish.



10. Allow the installation procedure to finalize.
11. Start the Evosep One control software.
12. The Extended method is now available in the method directory in Chronos.



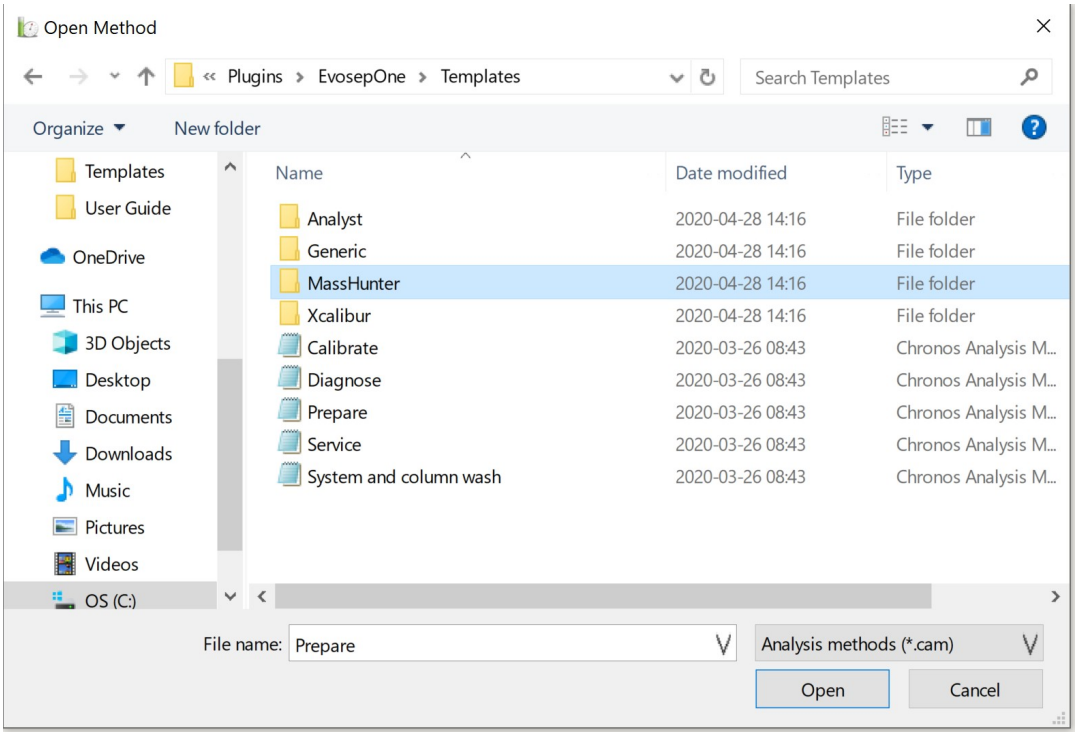
13. For Compass HyStar go to the previous section of the User guide “Create evosep separation methods” and follow the description for creating a separation method for the 88 min Extended method.



5 Instrument Software Control

5.1 Chronos for control of Thermo, Analyst (Sciex), Agilent and Waters MS

The Evosep One instrument is controlled through the “Chronos” sample acquisition software via a plugin. Chronos can control some of the common mass spectrometry vendors such as Chromatographic Data Systems (CDS) like Xcalibur (Thermo), MassHunter (Agilent), Analyst (Sciex) and MassLynx (Waters) – and hence start both the Evosep One and the mass spectrometer using one sample list.



In this section, Evosep One specific topics in relation to running samples and viewing pump graphs will be covered, whereas a complete overview of Chronos software features can be found in the Chronos User Manual (stored on the USB license stick).

5.1.1 Running samples

All tasks on the instrument, from running diagnostics procedures to sample acquisition are executed in a similar fashion from a user point of view.

1. A method is selected
2. A sample list is composed (method(s) and sample position(s))

3. The sample list is scheduled for run
4. The schedule is executed

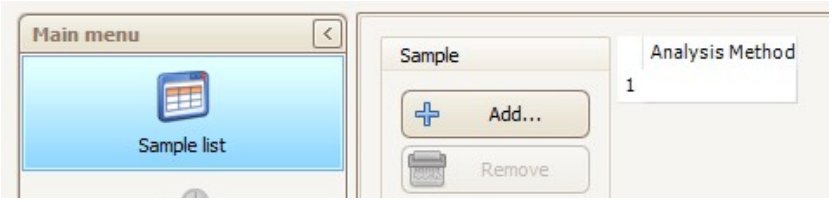
5.1.2 Methods

The Evosep One instrument software is configured with several predefined methods for maintenance, diagnostics, service tasks and sample acquisition. All methods are specifically optimized for the instrument hardware and cannot be modified by the end-user. The individual methods are described in detail in the following sections.

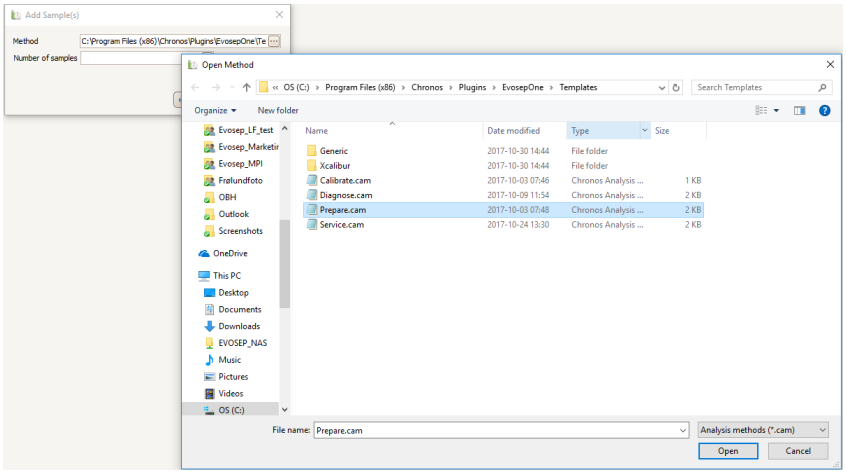
5.1.3 Sample lists

The creation of a new sample list is done in the “Sample list” section of the “Main menu”.

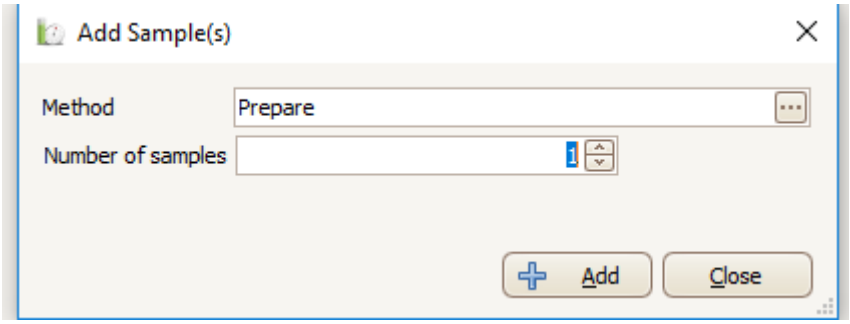
1. Click the Add button



2. Select the method of choice from the Evosep template folder (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\



3. Specify the number of samples to be run – and press the Add button



4. The method file will be entered in the sample list. Depending upon which method was chosen, a range of columns will be displayed.

   For Sample methods, Source tray (1-6), Source vial (1-96), MS file name and MS output directory must be specified. Processing file is optional

	Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur Processing	Xcalibur Output Dir
1	C:\Pro...n).cam	EvoSlot1	1	C:\Pro...\Plugins			

   For Prepare methods; select one or more methods to run using the checkbox

	Analysis Method	Pump preparation	Align solvents	Flow to column / idle flow
1	...\Prepare.cam	none	☐	none

   For System and column wash method, Source tray (1-6), Source vial (1-96) must be specified.

Analysis Method	Source Tray	Source Vial
C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\System and column wash.cam	EvoSlot 1	1

   For Diagnose methods, select subsystem test, e.g. “Pump HP” and “HP system” method by using the checkboxes .

Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☐	☐	☐	☐	☐

   For Calibrate method; select “Flow sensor” using the check box

	Analysis Method	Flow sensor ABCD	Flow sensor HP	Loop volume*
1	C:\Pro...te.cam	☐	☐	☐

- The flow sensor ABCD calibration script performs a multipoint flow sensor calibration of the pump A, B, C and D.
- The flow sensor HP calibration script performs a multipoint flow sensor calibration of the pump HP.

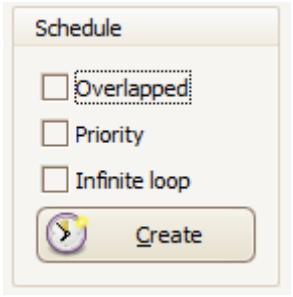
- The Loop volume calibration script measures the exact volume of the sample loop. The calculated volume is used in the sample runs to ensure higher analyte retention time accuracy. If the loop is replaced, the calibrate/loop volume script must be re-run.

For Service methods, select one of the options.

5. Additional methods and samples can now be added to the sample list

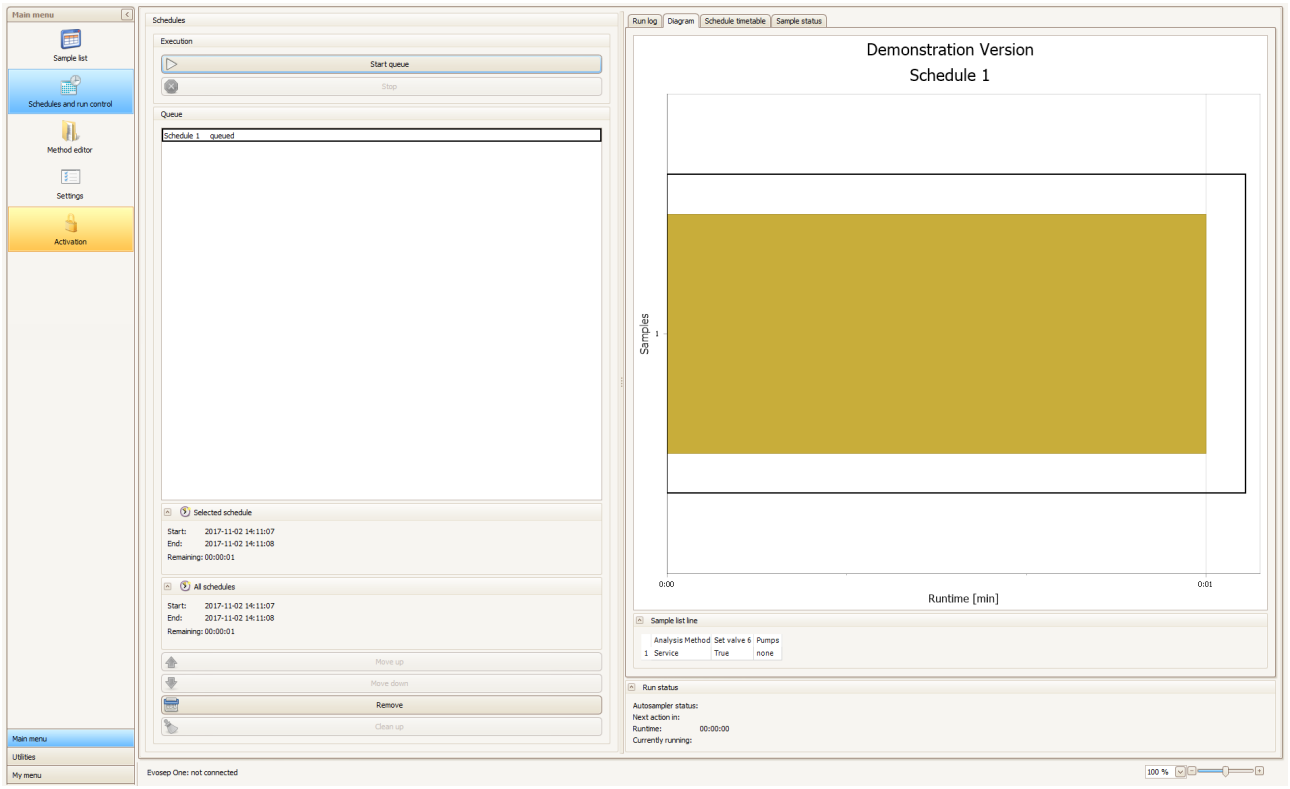
5.1.4 Creating a schedule

Next, the user must create a schedule based on the sample list. Make sure that the “Overlapped” check box is cleared and press “Create”.



5.1.5 Running a schedule

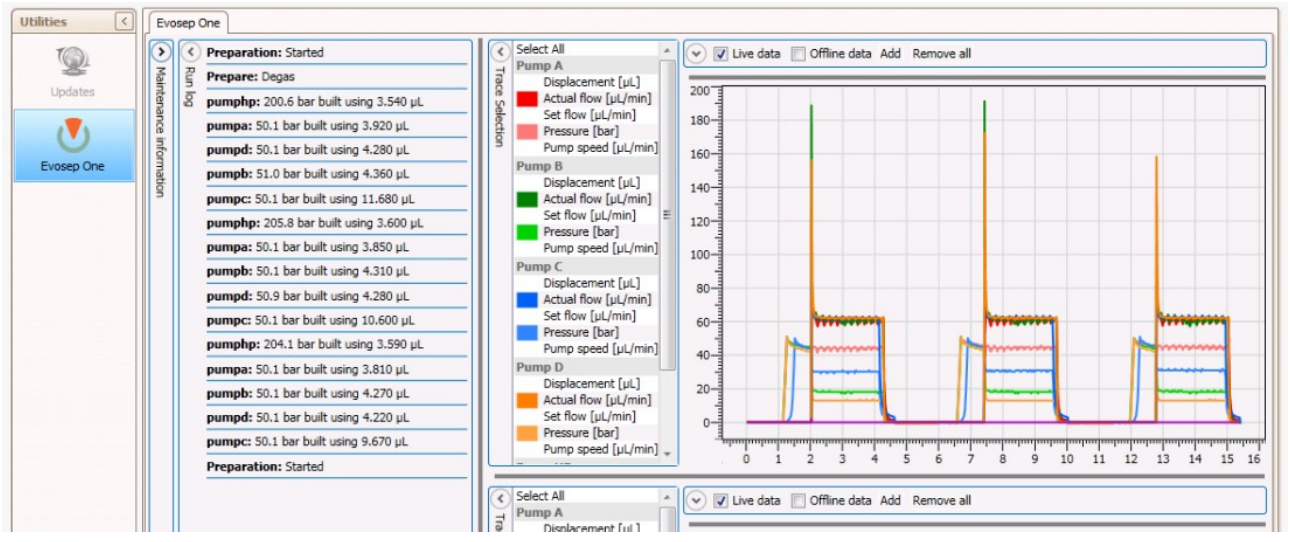
The schedule is now transferred to the schedule queue in the “Schedules and run control” section of the main menu.



The sample queue is started by pressing the “Start queue” button.

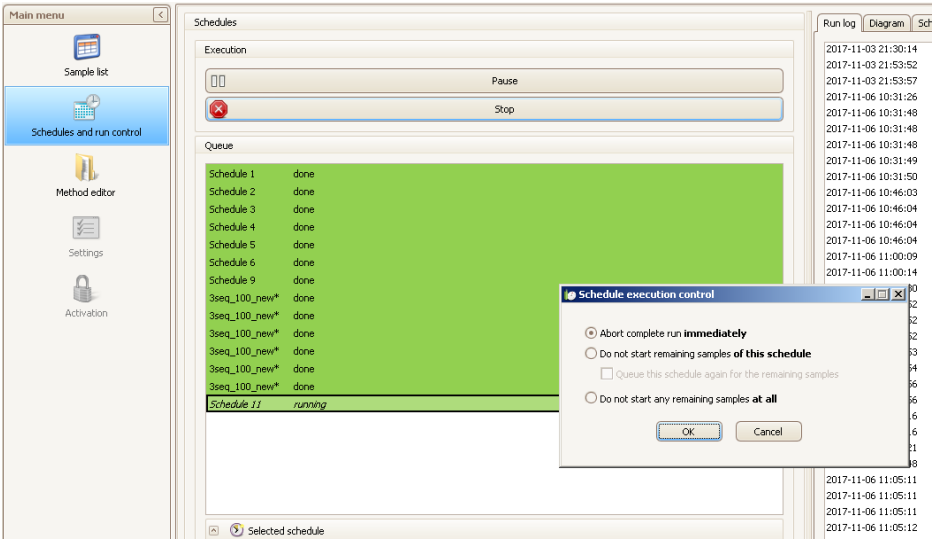
Additional schedules can be entered in the sample queue and the execution order of the schedules can be shuffled up and down using the arrow-buttons.

Information about the previous and currently running analysis, such as diagnostic leak rates etc., is printed in the Evosep One Run log:

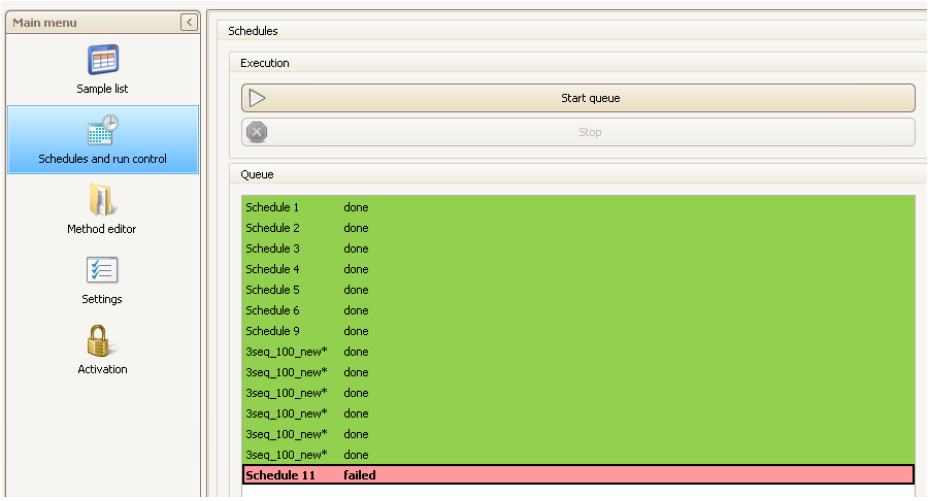


5.1.6 Aborting samples

The user can stop running schedules by pressing the “Stop” button, which opens a “Schedule execution control” dialog box. The user can select between three abort options.



An aborted schedule will be categorized as “failed” in the schedule queue.



The user can choose to restart the failed schedule by pressing the “Start” button – or remove the schedule from the list using the “Remove” button.

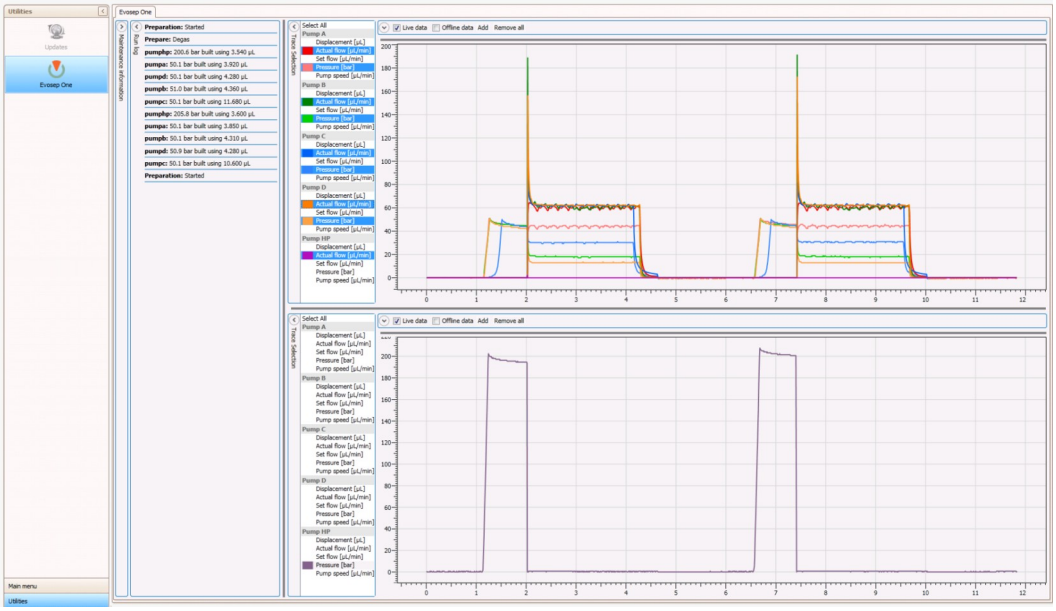
NOTE: Please be aware of the following:

- If a failed schedule is not removed from the queue and new ones are entered below, Chronos will start by running the failed schedule before proceeding with the newly entered schedules.

- Aborting a schedule in Chronos will not abort the MS acquisition. If sample runs are stopped, pay special attention to stopping and clearing the MS CDS sample queue.

5.1.7 Looking at graphs

Pump pressure, flow and speed graphs for the current analysis are plotted in the “Evosep One” section of the Utilities menu.



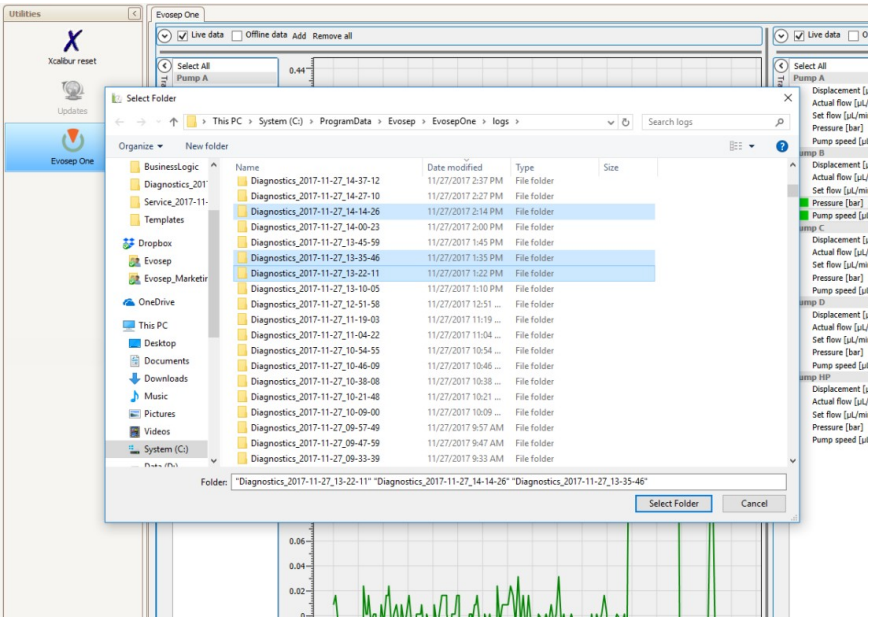
the following traces can be selected for each pump by a single left click on the side bar

Name	Description
Displacement [µl]	Pump piston position. Pumps are full when Displacement is 0 µl and empty when displacement is 55 µl and 138 µl for Pump HP and Pump A/B/C/D respectively.
Actual flow [µl/min]	The actual flow is measured in the liquid stream for each pump using an individual flow sensor.
Setpoint	The Setpoint graph shows the intended flow (µL/min) or pressure (decibar), set by the software for each pump.
Pressure [bar]	The Pressure is measured in the liquid stream for each pump using individual pressure sensors. The pressure limits are 500 bar and 100 bar for the Pump HP and Pump A/B/C/D respectively.

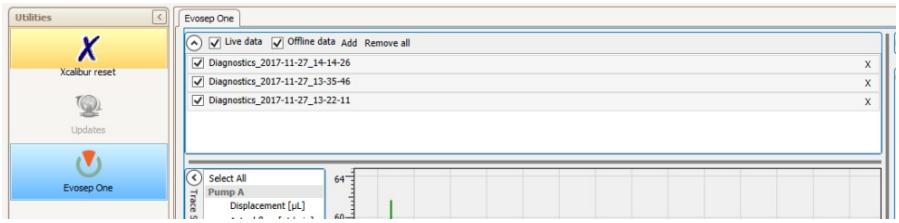
Pump speed [µl/min]	The Pump speed graph shows the actual pump piston movement velocity. It may differ from the set flow when the flow feedback control is utilized, e.g. during the gradient formation.

Tips and tricks

1. The graph viewing area is divided into two plots, which can be resized by left mouse clicking the intersection and draw. Double left clicking a graph type on the side bar will enable or disable the specific graph type for all pumps.
2. The graphs can be zoomed by “mousing over” the area of interest while holding down the “shift” button + left mouse button.
3. It is possible to view “historic” pump traces by clicking the “Offline data” check box. Select one or more sample folders and click “Select Folder”.



4. The opened sample folders are visible in the Offline data list and individual samples can be selected/unselected using their respective checkboxes.

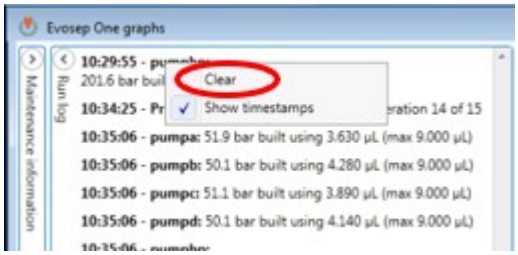


It is also possible to use drag-n-drop from Windows File Explorer to the Offline data section, as an alternative way to load the data.

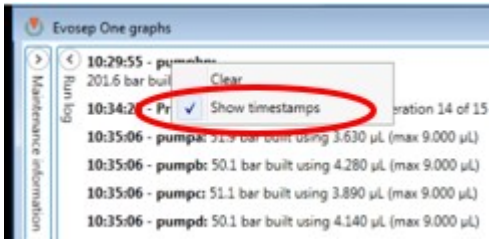
5.1.8 Run log

The run log displays information to the user regarding the sample methods, Calibration, Diagnostics and Preparation programs being executed. The log will show which and when each program was started and finalized including pass/fail criteria.

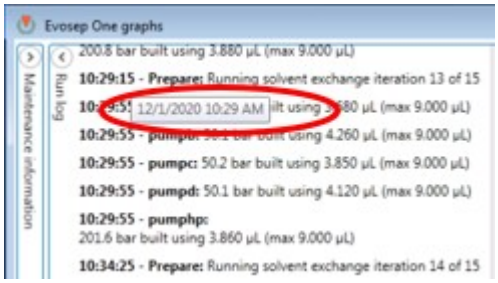
The run log can be flushed, by right clicking the log and pressing “clear”.



The user can choose to enable/disable timestamps in the log by right clicking the log and select/deselect the “show timestamps” box



If timestamps are selected, the complete date string can be shown for each timestamp by mousing-over the log entry.



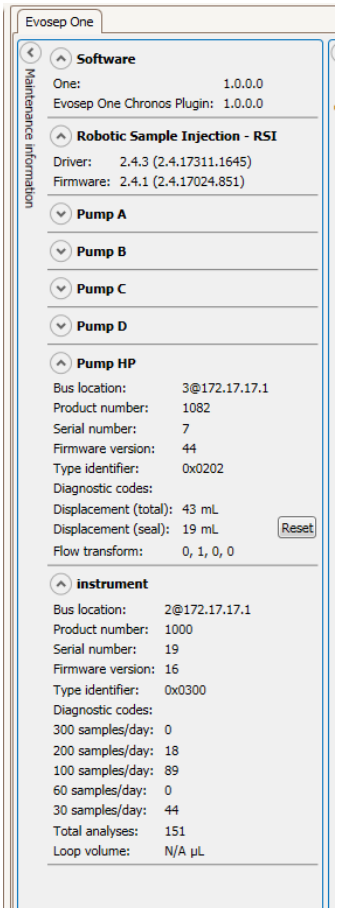
5.1.9 Maintenance information

The software version, autosampler, pump and other instrument specific data is displayed in the “Evosep One” section of the “utilities” tab. The information is found in the “Maintenance information” section and can be expanded/hidden by left mouse clicking the arrow in the upper left corner. A subset of the most important information is explained below:

- Software:
  - Plugin software version
- Pump
  - Serial number

- Firmware version
- Displacement (total): Total pumped volume
- Displacement (seal). Pumped volume since reset (if a pump seal was replaced)

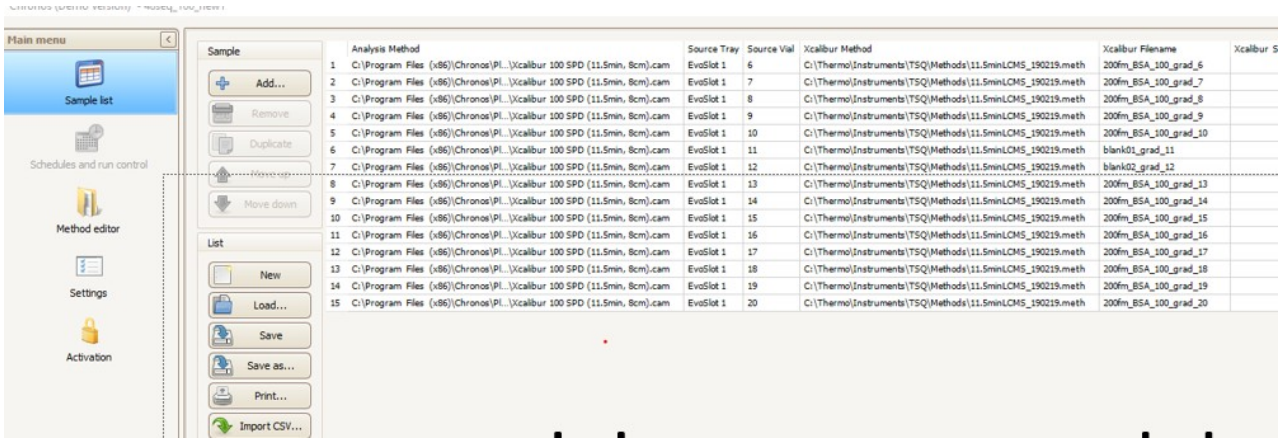
- Instrument
  - Serial number
  - Firmware version
  - Analysis completed (by type)



5.1.10 How to import CSV files into Chronos

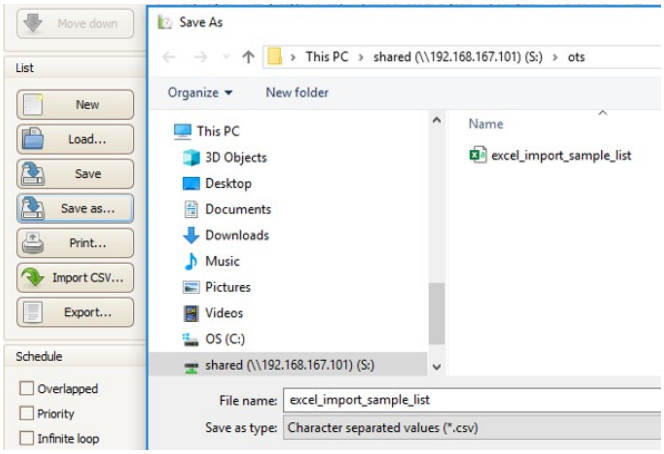
The general idea here is to use a mapping template to define the column matching between the CSV file you want to import, and the Chronos sample list columns. This example uses a CSV file saved from Chronos, but you can use any CSV file as source, as long as it contains the values to fill-in the columns described in the Chronos .cam file.

1. Create a sample list in Chronos (this can then be edited in e.g. Excel)

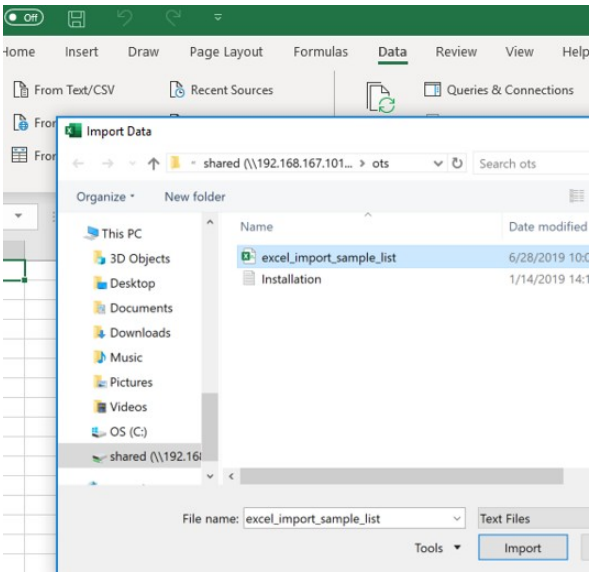


2. Then Click “Save as…” and choose “Character separated values” as type.

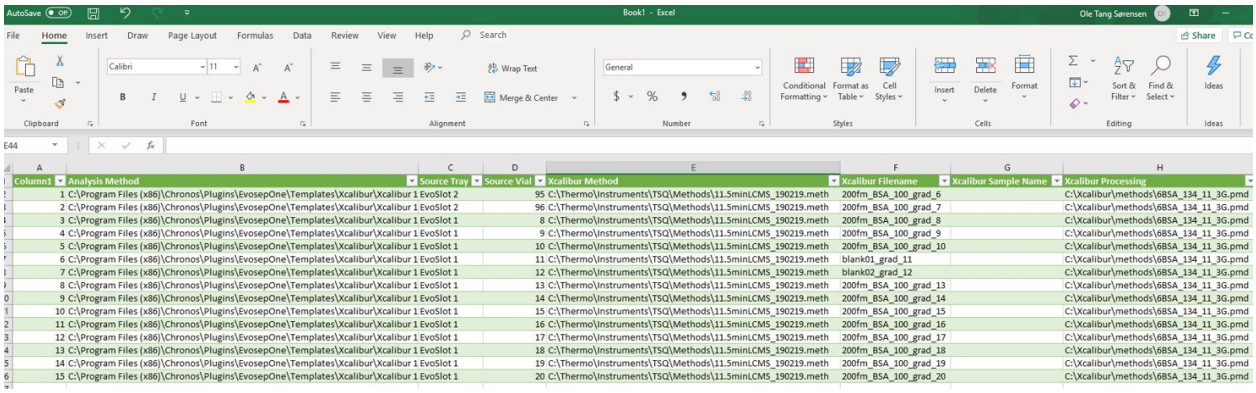
   IMPORTANT: The extension CSV typically (and by some standards) denotes “Comma Separated Values”. However, depending on Windows regional settings, another character may be used as separator, e.g. semicolon or tab. Keep this in mind, when you import the data later!



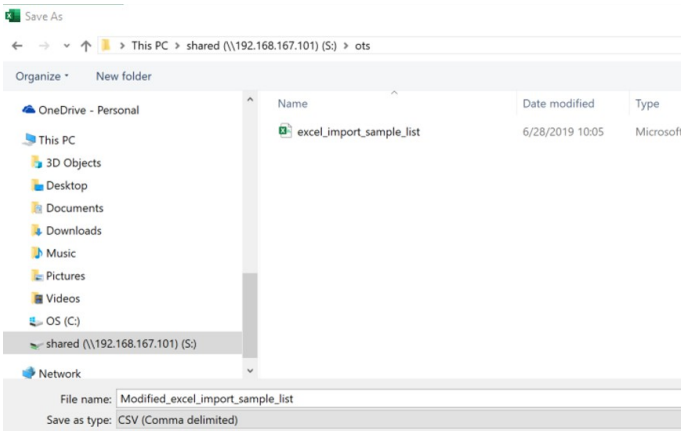
3. In Excel click “Data” and then choose to open “From Text/CSV”



4. Now changes can be made to the sample list in Excel



5. To save the modified sample list click “Save As” and select type “CSV (comma delimited)

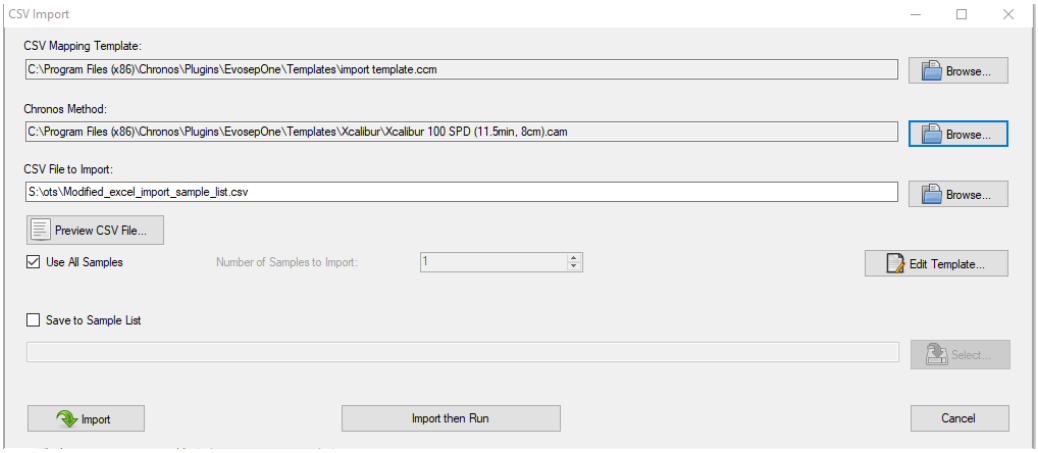


6. To import sample list into Chronos, click “Import CSV…”

NOTE: The first time you perform this operation in Chronos, you will be taken directly to step 7a.

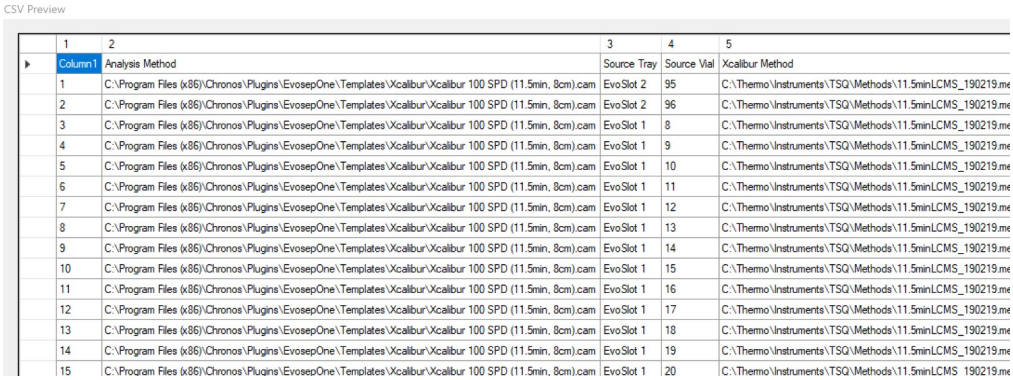
7. In the CSV import window do following

a) Choose “Browse…” to select the desired “Chonos Method”, this will ensure correct format of sample list columns, (E.g. for Xcalibur, use one of the Evosep Xcalibur methods)



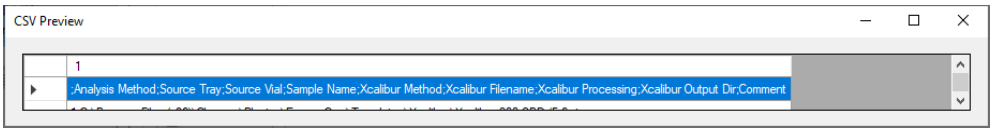
b) Choose “Browse…” to select the “CSV File to Import”.

c) Click “Preview CSV File…” to see the correct column numbers in the sample list.

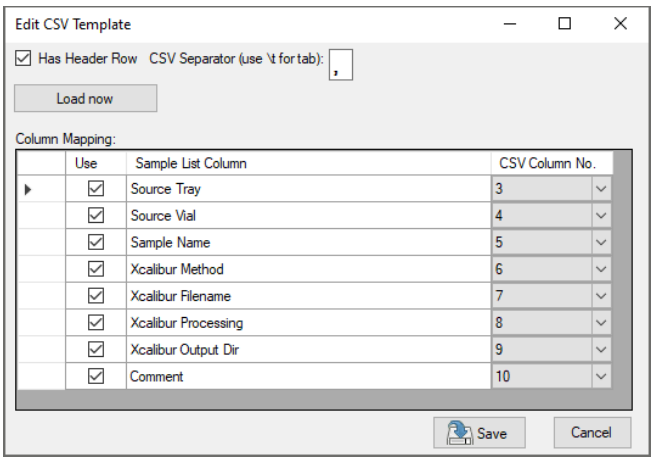


	1	2	3	4	5
▸	Column1	Analysis Method	Source Tray	Source Vial	Xcalibur Method
	1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 2	95	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	2	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 2	96	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	3	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	8	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	4	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	9	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	5	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	10	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	6	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	11	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	7	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	12	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	8	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	13	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	9	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	14	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	10	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	15	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	11	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	16	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	12	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	17	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	13	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	18	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	14	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	19	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me
	15	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	20	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.me

IMPORTANT: If all columns are shown merged together as a single column, you probably must adjust the CSV Separator as per next step, then save the template and preview the file again.



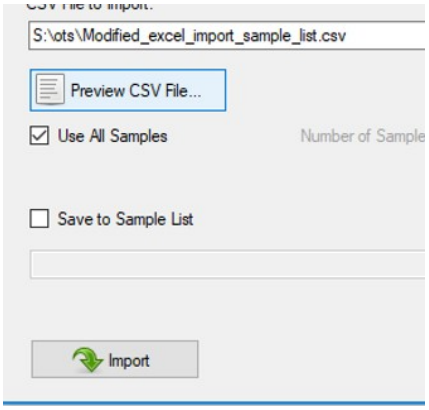
d) Click “Edit Template…” and make sure the CSV Separator is set to the character used in your CSV file.



e) Match sample list columns to headers. Make sure to check the “Use” column, or that data will not be included!

f) Click “Save” to save the template, which can then be re-used for other imports.

g) Click “Import” to add sample list to Chronos.

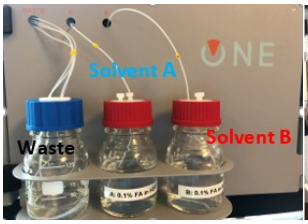


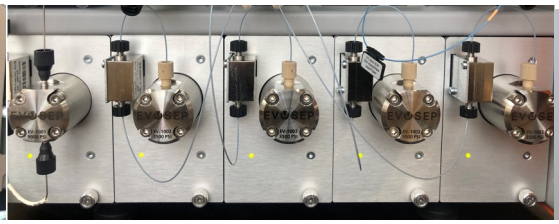
5.2 Evosep driver for control of Bruker MS

5.2.1 Instrument Preparation with Compass HyStar

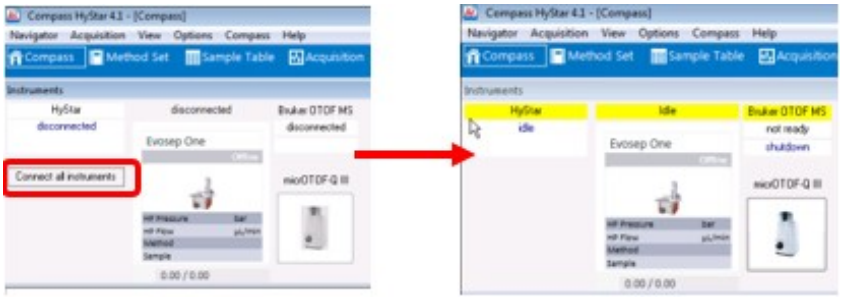
Please see chapter 7, “Preparing the Evosep One for use” for information about instrument automatic assessment of need for preparative actions.

1. Check Evosep solvent levels, Waste, Solvent A, Solvent B. (left picture) Check LEDs for power on pumps (middle) and autosampler (right picture).






2. Open Compass HyStar, click “Connect all instruments”



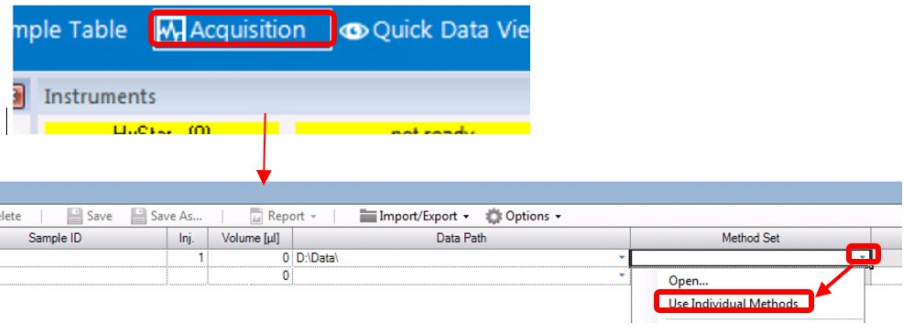
3. Check that the column and emitter are connected to the MS ion source, if not connect column and emitter and run the Preparation “flow to column” script to check spray.

5.2.2 Sample Acquisition with Compass HyStar

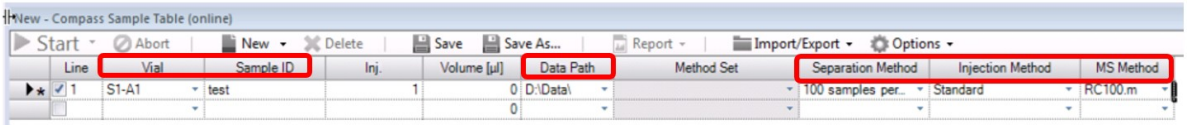
1. Make sure that Instrument Preparation has been performed.

2. Prepare samples according to SOP for sample loading. Remove lid from Evotip box and place box in position 1.

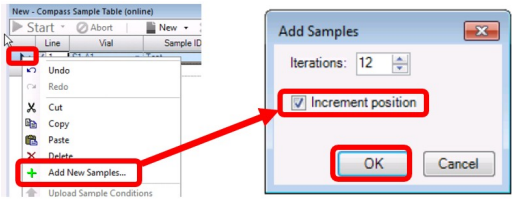
3. In HyStar, click the “Acquisition” Icon to open the HyStar Sample Table, then click “New” and click the small arrow in the first line under “Method Set” and choose “Use Individual Methods”



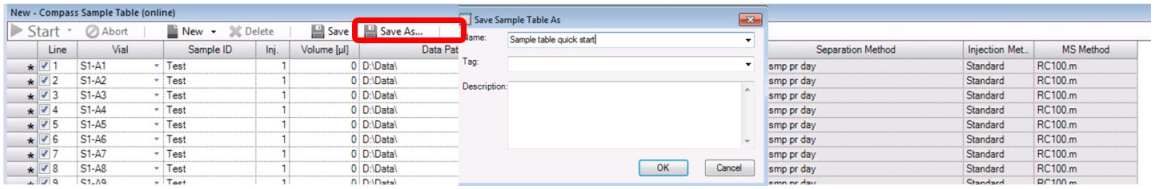
4. In the Sample Table, populate the following: “Vial”, “Sample ID”, “Data Path”, “Separation Method”, Injection Met” and “MS Method” for line 1



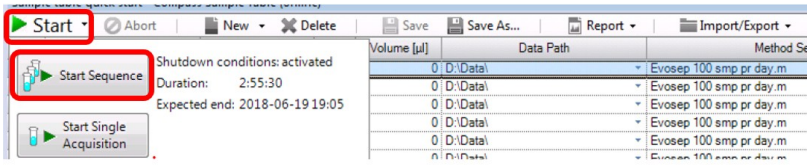
5. Right click black triangle to the left most side of sample line 1 and choose “Add New Samples”, in the pop-up box. Set # of “Iterations” (samples) to add and select the increment position option (automatically increment Vial position) then click “OK”



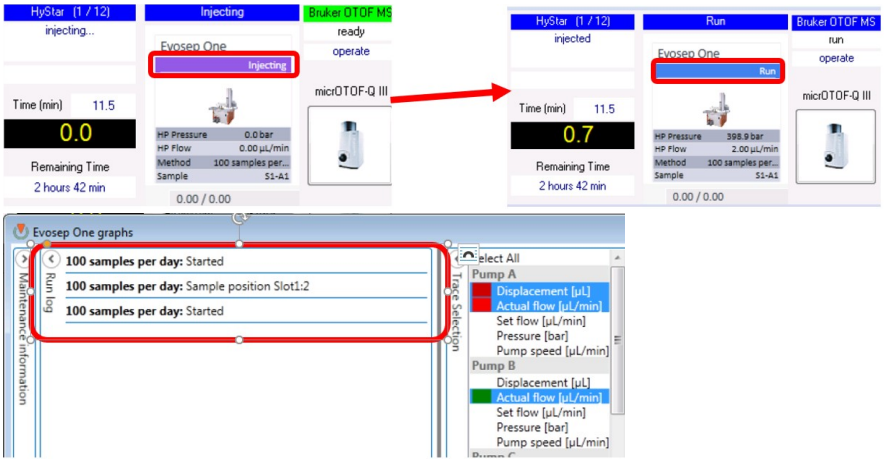
6. Now click “Save As” and save the Sample Table with an appropriate name



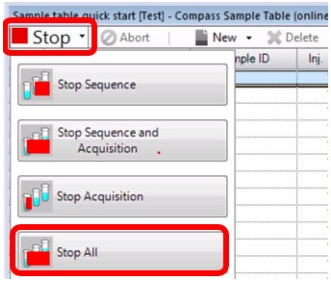
7. Mark first line in the sample table by clicking the black triangle in line 1, click “Start” and “Start Sequence”



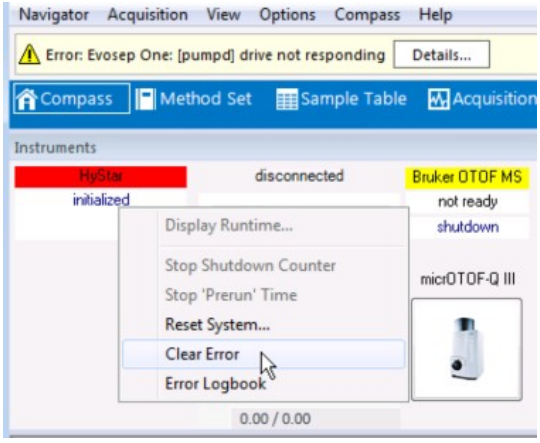
8. After a short while, Evosep One status will change from “Idle” to “Prerun” to “Injecting” and to “Run” when the gradient starts. More info during the run can be found in the “Run log” of the Graphs window



9. To stop an acquisition, click “Stop” in the sample table and click “Stop All”



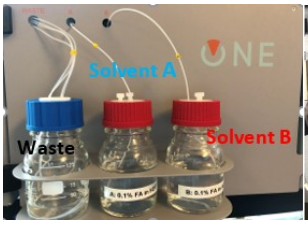
10. If something unforeseen happens during a maintenance script or a method any error messages can be cleared by right clicking the HyStar status view and choose “Clear Error”

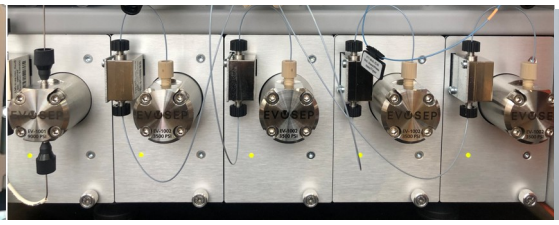


5.3 Evosep driver for control of SCIEX OS

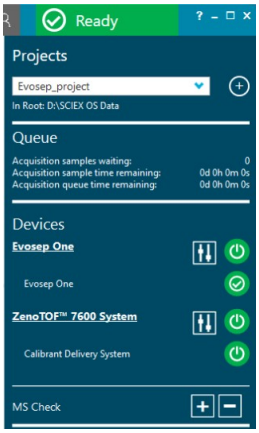
5.3.1 Instrument Preparation with SCIEX OS

1. Check Evosep solvent levels, Waste, Solvent A, Solvent B. (left picture) Check LEDs for power on pumps (middle) and autosampler (right picture).






2. Open SCIEX OS. Check status ribbon if Evosep and MS are configured and ready.



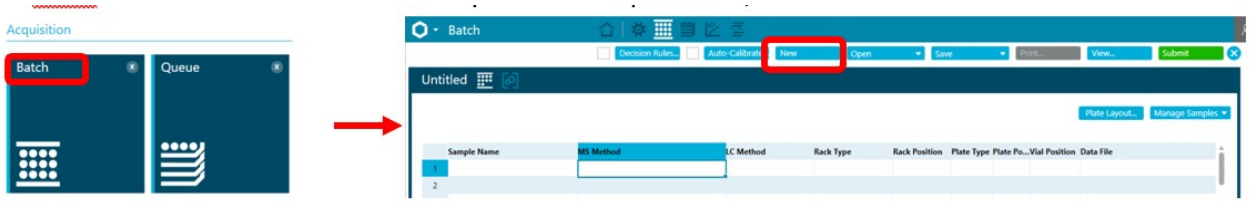
3. Check that the column is connected to the micro-probe on the Optiflow ion source. Then connect the transfer line from Evosep to column. Close the column oven.


Note that if the column oven is not used, the “high voltage enable switch” needs to be held down as shown to the right. For low flow applications the Nanoprobe configuration can be selected (see Optiflow Operator Guide for details).

5.3.2 Sample Acquisition with SCIEX OS

1. Make sure that Instrument Preparation has been performed
2. Prepare samples according to SOP for sample loading. Remove lid from Evotip box and place box in Slot 1.

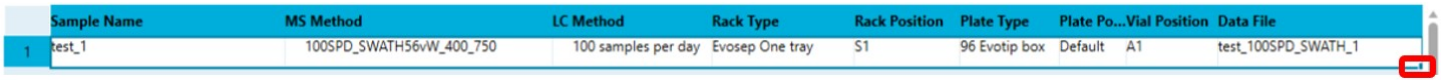
3. In SCIEX OS click the “Batch” Icon to open the Sample Table, then click “New”



4. In the Sample table populate: “Sample Name”, “MS Method”, “LC Method”, “Rack Type”, “Rack Position”, “Plate Type”, “Plate Position”, ”Vial Position” and “Data File” for line 1. Note that the MS Method length should match the LC Method.

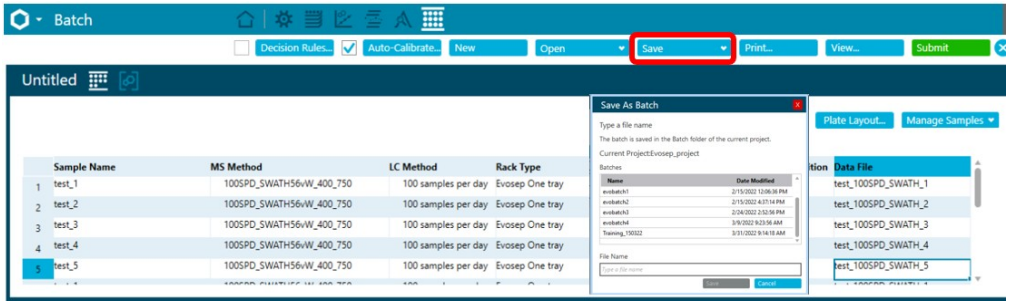
	Sample Name	MS Method	LC Method	Rack Type	Rack Position	Plate Type	Plate Po...	Vial Position	Data File
1	test_1	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	S1	96 Evotip box	Default	A1	test_100SPD_SWATH_1

5. Select line and drag down to add samples. Adjust sample names, vial positions and data file names.

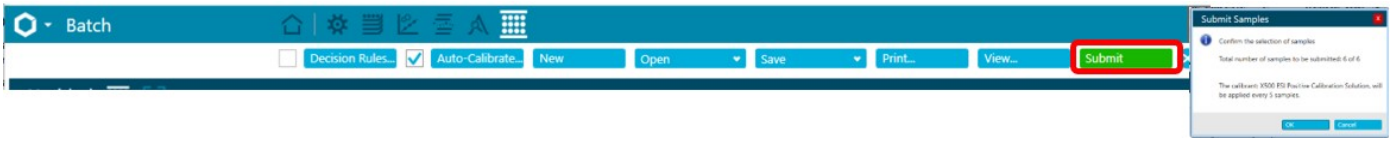


	Sample Name	MS Method	LC Method	Rack Type	Rack Position	Plate Type	Plate Po...	Vial Position	Data File
1	test_1	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	S1	96 Evotip box	Default	A1	test_100SPD_SWATH_1

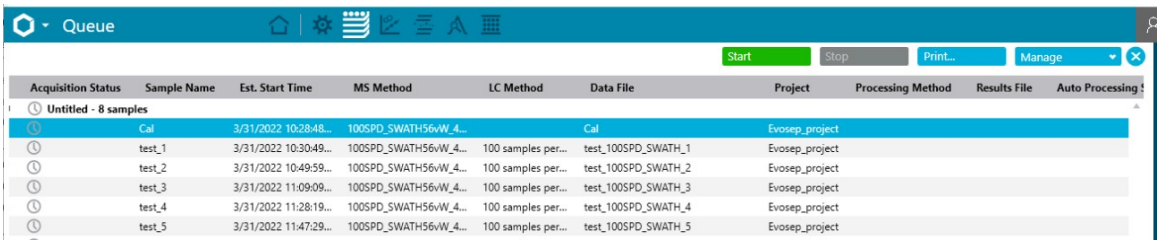
6. Now click “Save” and save the Batch Table with an appropriate name



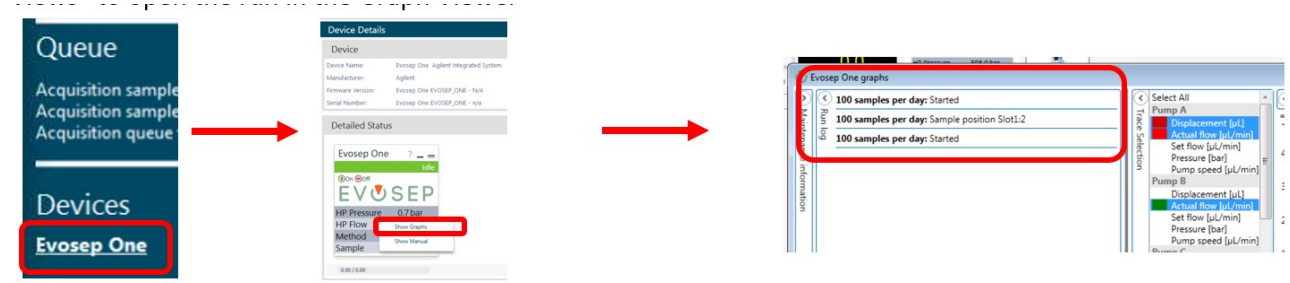
7. Press Submit to submit samples to sample queue.



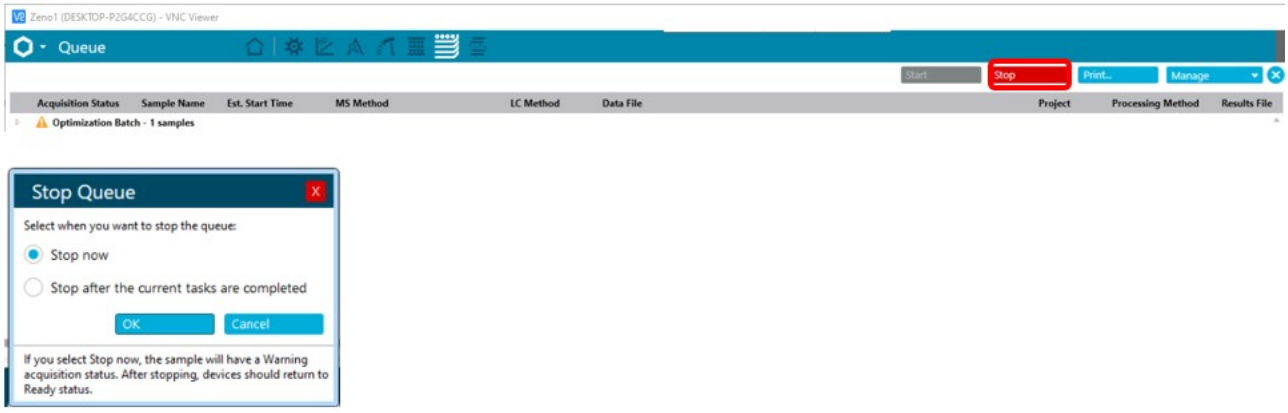
8. Go to the “Queue” and click “Start”



9. After a short while Evosep One status will change from “Idle” to “Prerun” to “Injecting” and to “Run” when the gradient starts. More information during the run can be found by clicking the Evosep One link to open the status window. Right click and select “Graph Views” to open the run in the Graph Viewer.



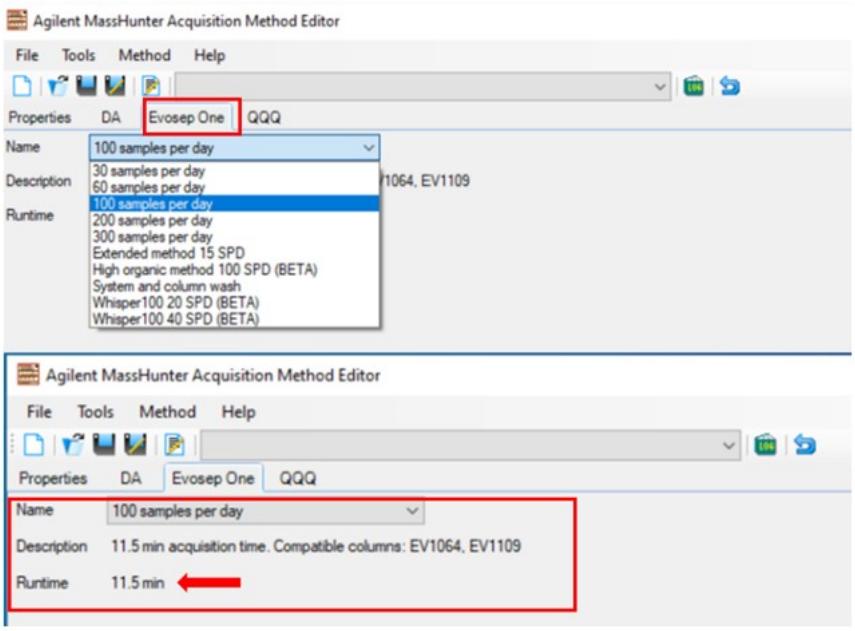
10. To stop an acquisition, click "Stop" in the sample table and select option:



5.4 Evosep driver and sample Acquisition with MassHunter Software

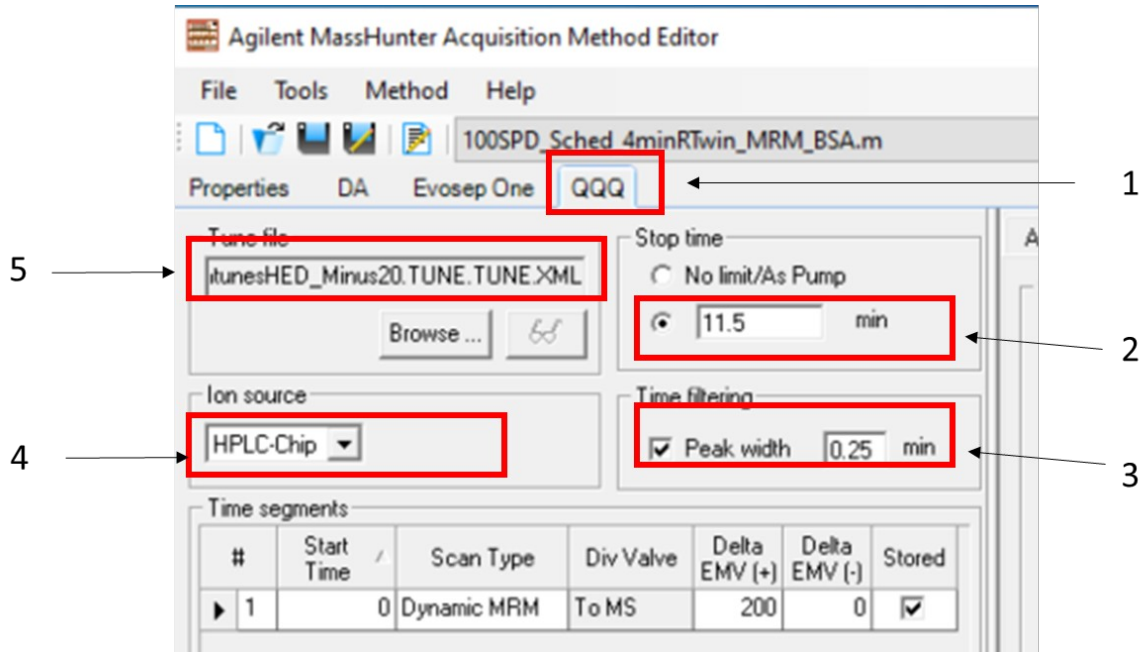
5.4.1 Create MS method with MassHunter

1. To create a method, open **"Offline Method Editor"**, and on the Evosep One tab select the desired chromatography method.



Note the LC method run time for MS method timing.

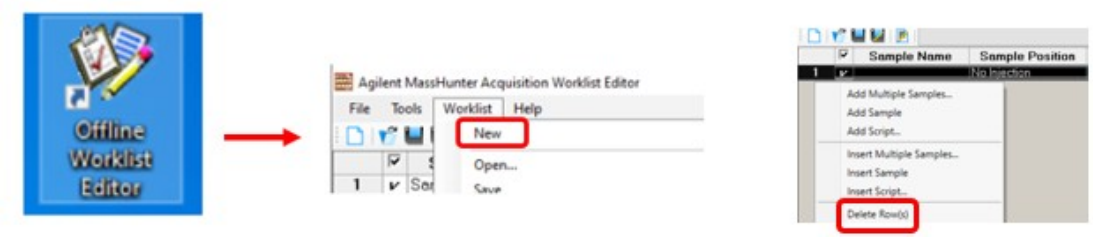
2. Select MS tab (1) – here QQQ. The MS method should equal the time of LC method (2). Enter estimated peak width e.g. for 15 second peaks provide 0.25 min (3). Select the ion source appropriate for use with Evosep one- **HPLC chip** (4). Ensure current mass spec tune file is incorporated into the method (5).



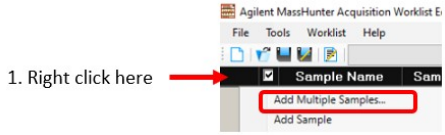
3. Save method by selecting “Save As Method” and navigate to D:\MassHunter\Methods :

5.4.2 Sample Acquisition with MassHunter

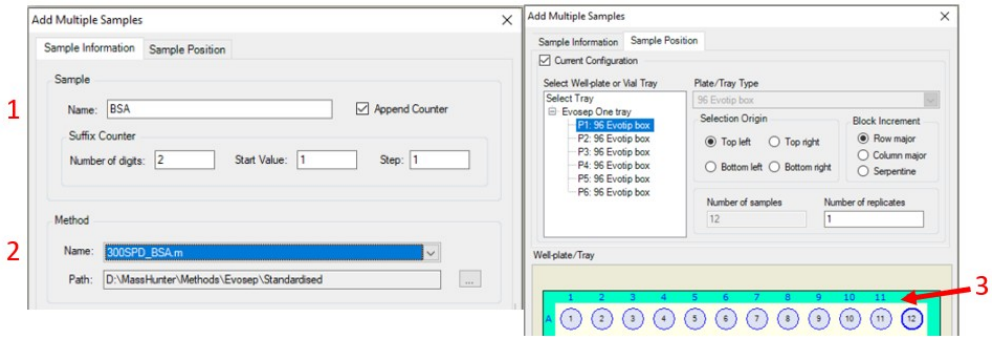
1. To prepare an MS run list. In MassHunter Acquisition Worklist Editor click on the “Worklist” pane and select “New”. Delete first row.



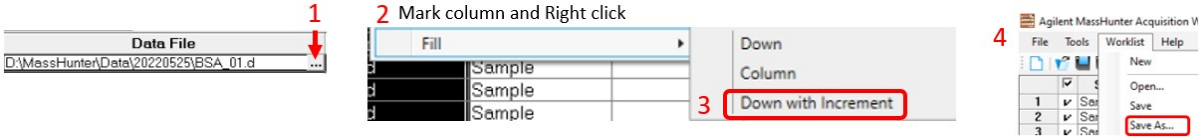
2. Right click and select “Add Multiple Samples…”.



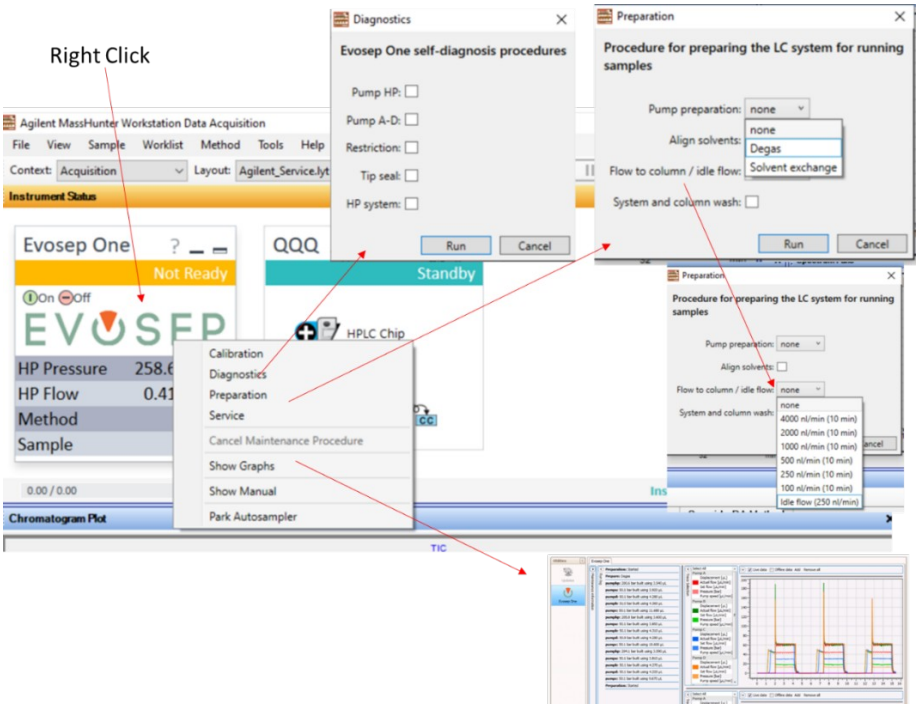
3. Enter Sample Name (1) and choose method (2). Select sample positions (3). Click “OK”.



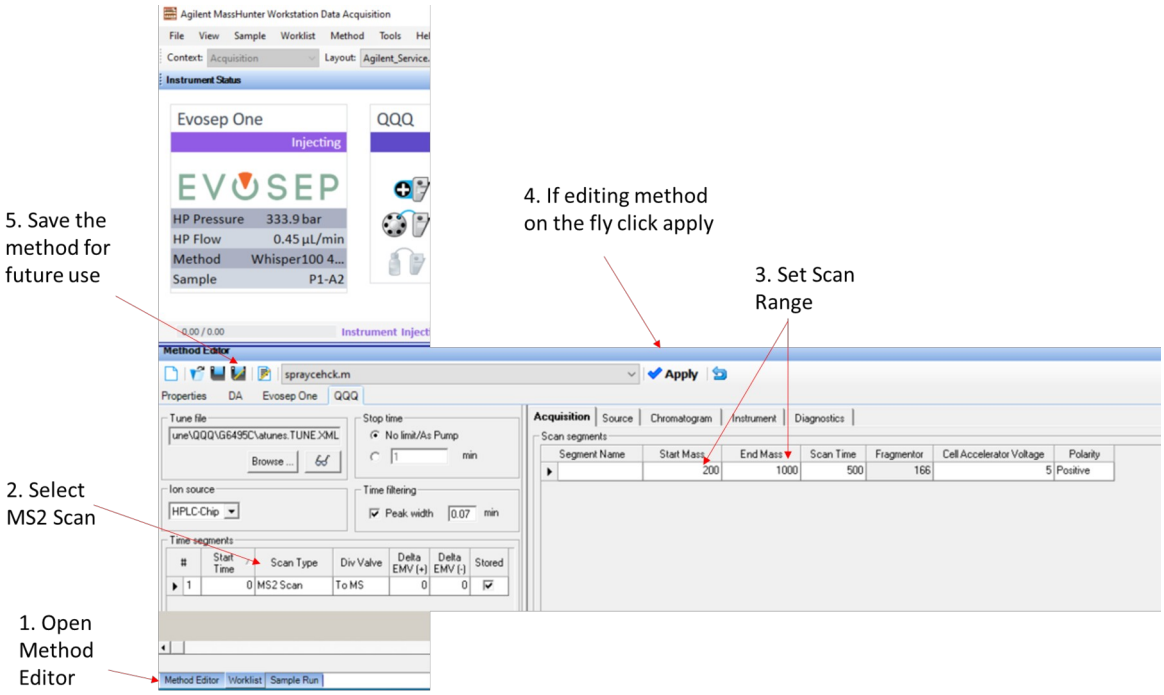
4. Create Data File folder and enter file name for first sample (1). Mark “Data File” column (2), right click and select “Fill”. Choose ”Down with Increment” (3). Save Worklist (4).

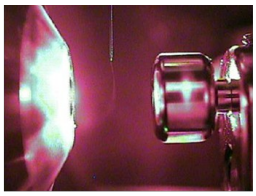


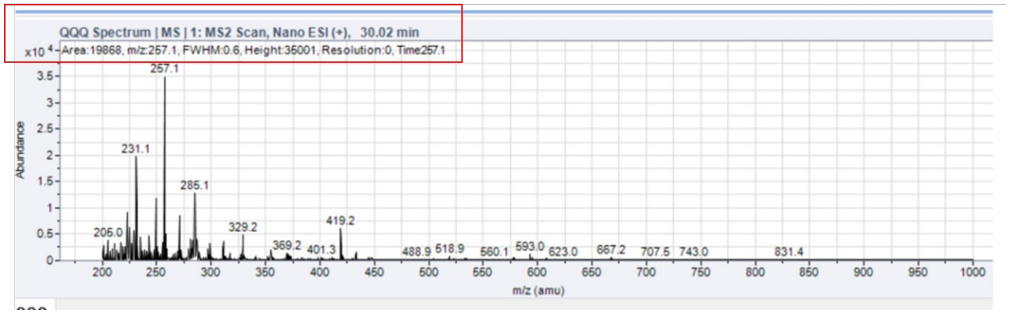
5. To run LC checks/preparations prior to MS run. Right click for drop down method. E.g. use" flow-to-column" at 1000nl/min to check spray for 100 SPD LC method.



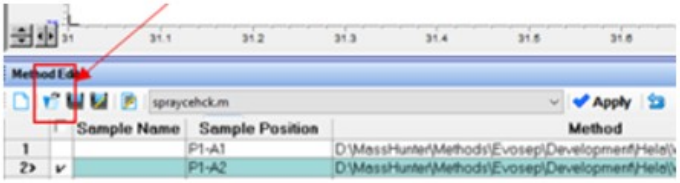
6. Check emitter position. With flow running at a stable pressure, switch on the MS and perform an MS 2 scan to check stability and intensity of the spray.



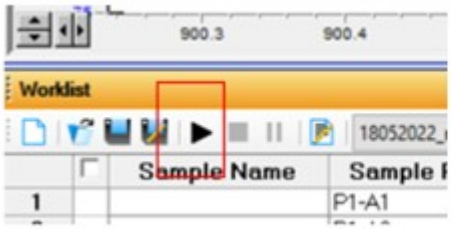


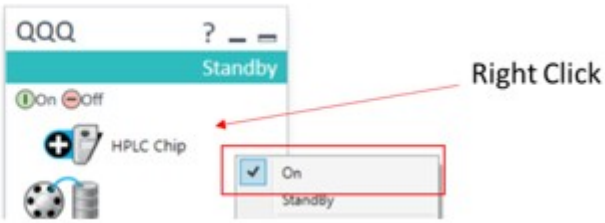


7. To run the worklist. Upload worklist in MassHunter Data Acquisition.

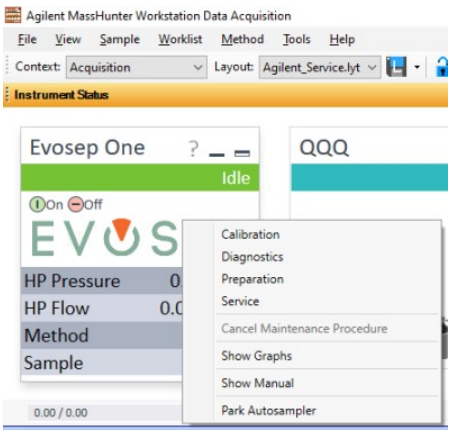


8. Please note the MS must be “On” to launch a run.





9. After a short while Evosep One status will change from "Idle" to "Prerun" to "Injecting" and to "Run" when the gradient starts. More information during the run can be found by clicking the Evosep One link to open the status window. Right click and select "Graph Views" to open the run in the Graph Viewer. Right click and select to initiate Calibration, Diagnostic tests etc.

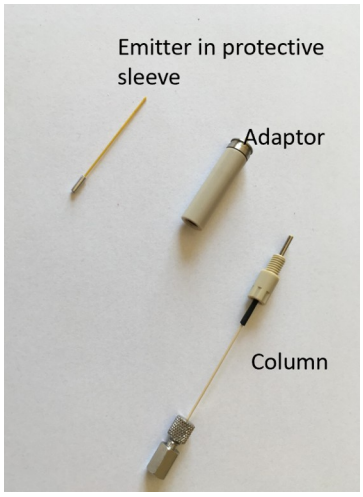


6 Configuration, source, column, and emitter

The following sections describe how we recommend connecting the Evosep One LC to various mass spectrometers and ion source configurations.

6.1 Thermo Scientific EASY-Spray source

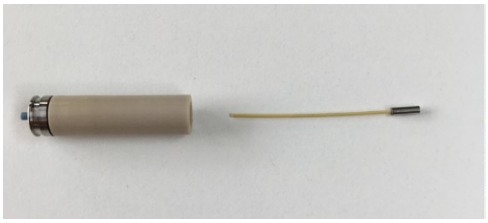
Column, spray adaptor and emitter used to run the Evosep One together with an EASY-Spray source.



How to set up

1. Carefully remove emitter from the box, use fingers or a pair of tweezers.

2. Slide the emitter with the sleeve into the spray adapter .



3. Now connect the column to the adapter to push the emitter into correct position.

4. When the column is connected, and the emitter is in position, the protective sleeve can be pulled of the emitter.

5. After removing the sleeve from the emitter, the spray adapter can be pushed gently into the Easy-Spray source. **To avoid damage to the emitter, move the Easy-Spray Z-axis back using the manipulator before inserting the adaptor.**

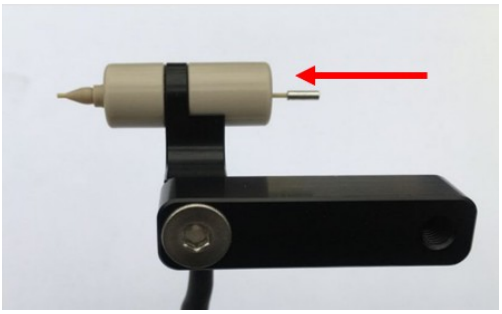
6. Connect Evosep One transfer line to the column and adjust emitter position with the manipulator.
7. To remove the adaptor loosen it gently with fingers or a fingernail and slide it outwards, do not pull in the connecting union to remove the adaptor.

6.2 Thermo Scientific Flex source

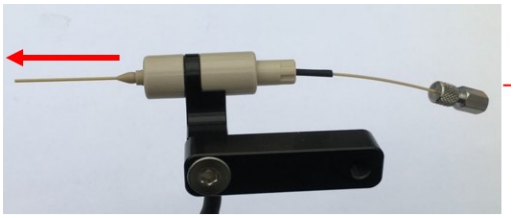
How to set up

1. Carefully remove emitter from the box, use fingers or pair of tweezers.

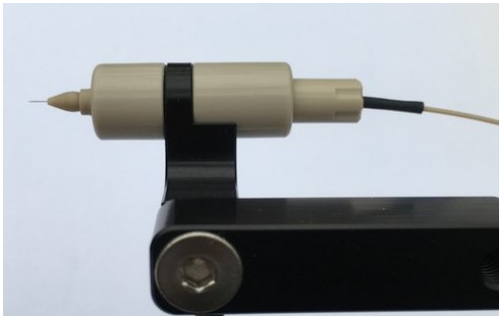
2. Slide the emitter with the sleeve into the flex source spray adaptor.



3. Connect the column to push the emitter into the correct position.

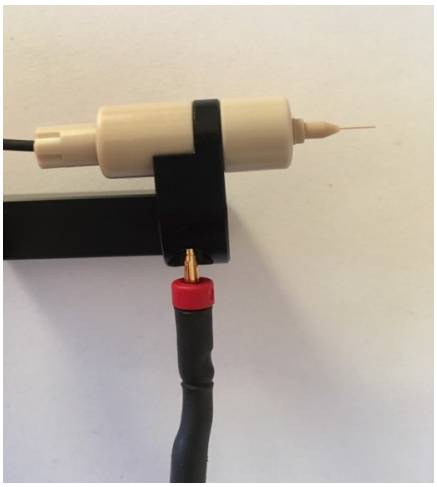


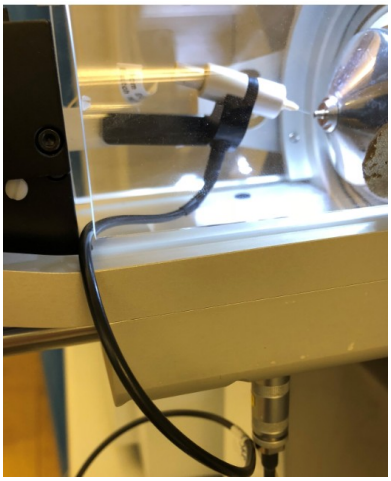
4. Remove the protecting sleeve, connect the transfer line and position the sprayer in the source with the flex source manipulator.



Note:

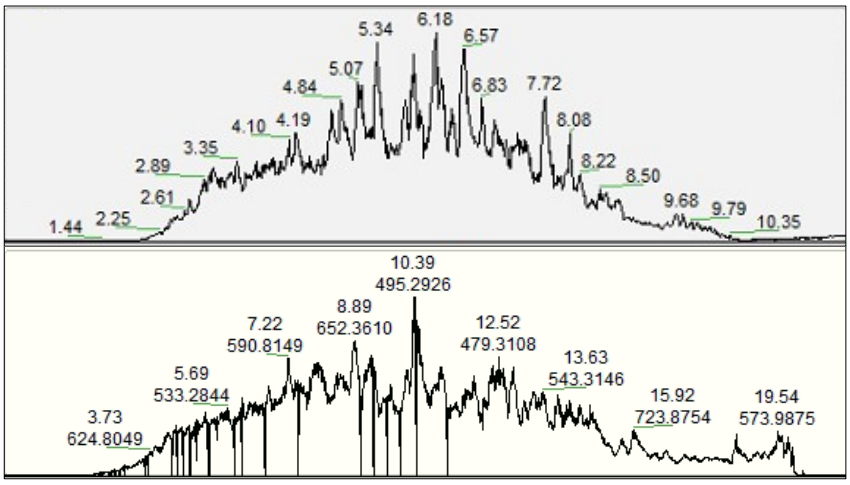
Please use the Nanospray Flex HV cable P/N# EV1092 for supplying voltage to the spray adapter





6.3 Thermo Scientific FAIMS Pro interface

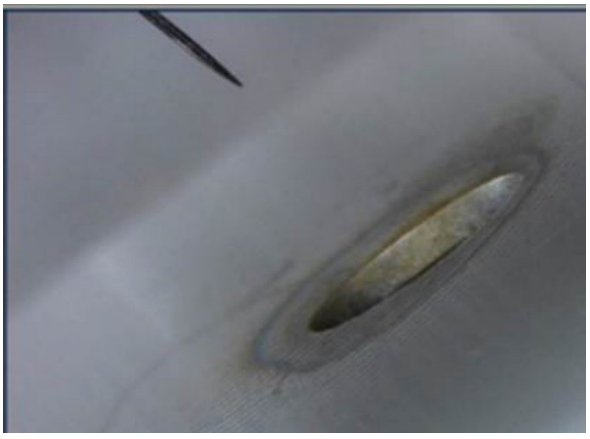
Optimal spray stability is a critical parameter in LC/MS and becomes even more crucial in combination with the FAIMS Pro Interface. This is challenged after continuous analysis of hundreds of samples and the electrospray becomes unstable leading to loss of signal and lower identifications. The spray stability and performance can be stabilized after cleaning of the FAIMS Pro Interface.



Chromatogram (upper part) obtained with clean FAIMS compared to chromatogram obtained with dirty FAIMS (lower part) that has many dropouts and instable spray.

The following set of recommendations is devised to help you use your Evosep One in combination with FAIMS Pro Interface.

1. Position the emitter away from the orifice of FAIMS Pro Interface (positioning emitter closer leads to faster accumulation of dirt and spray instability). We found that for most of standard applications 4-5 mm away is optimal, however, that may vary depending on the methods used and type of sample and it can be further optimized. Both fused silica and stainless-steel emitters work, however we recommend the latter.

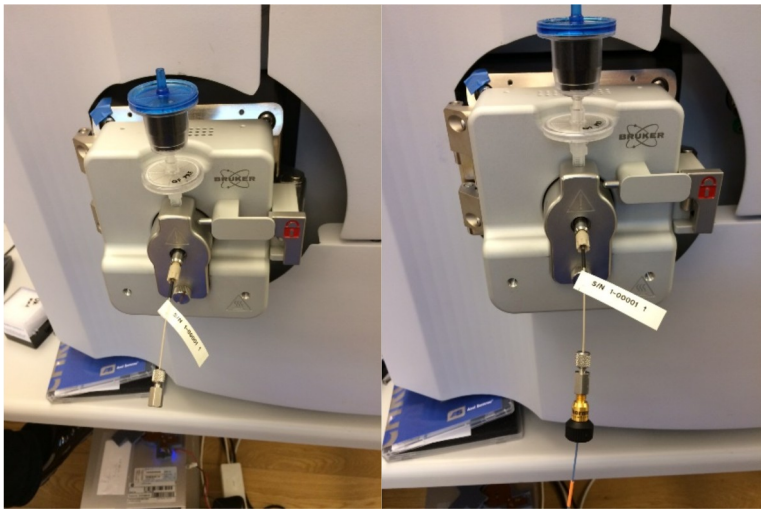


Before cleaning

After cleaning

2. We recommend cleaning of the entrance plate and the inner and outer electrodes of the FAIMS Pro Interface as soon as the spray instability occurs. As a guideline, cleaning after every 350 samples, or once a week is recommended, but more or less frequent cleaning might be needed, depending on the type of samples analyzed.
3. Ionization voltage should be ≈300V higher when FAIMS Pro Interface is used.

6.4 Bruker Daltonics CaptiveSpray source.



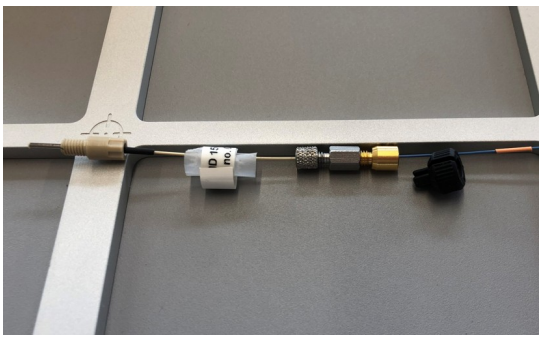
Connect the column to the CaptiveSpray source and connect the transfer line.

6.5 Agilent Nanospray source.

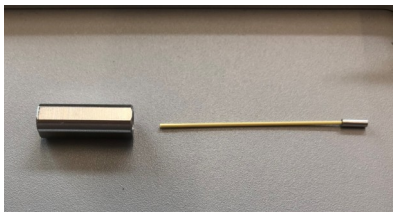
Evosep Column, Agilent Needle emitter EV1117, Agilent sleeve adapter EV1116 and Agilent Needle sleeve assembly

How to set up

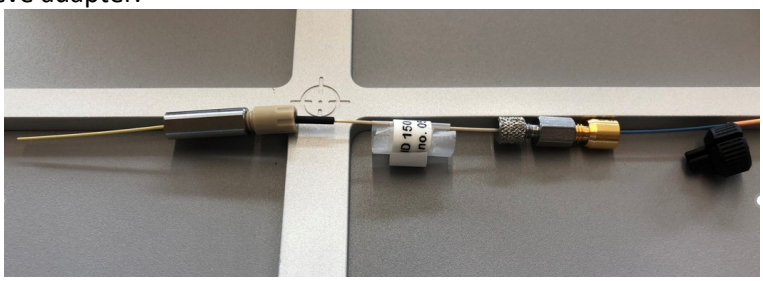
1. Connect the column to the Evosep One transferline and remove the black removable knurl from the nanoViper fitting.



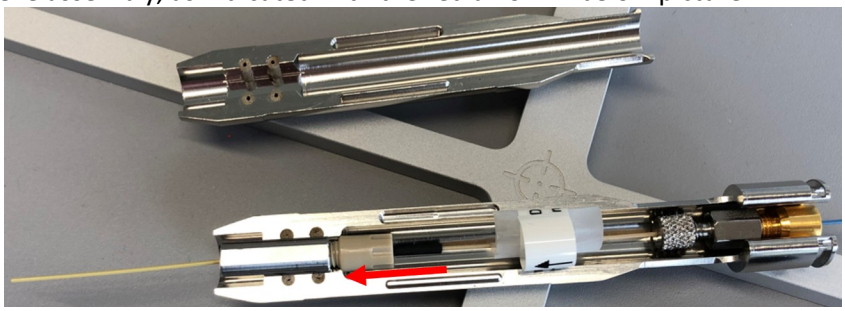
2. Slide the emitter with the sleeve into and through the sleeve adapter.



3. Connect the column to the sleeve adapter, creating a zdv connection between emitter and column inside the sleeve adapter.

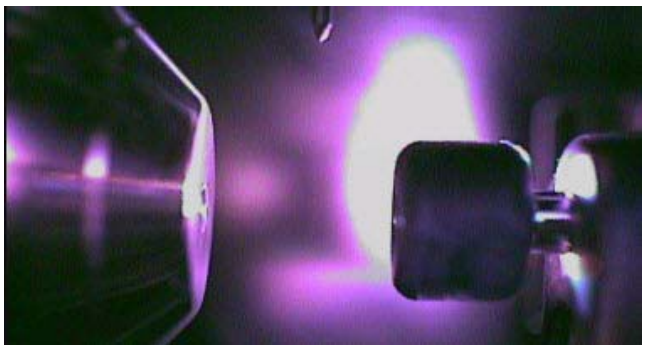


4. Insert the sleeve adapter with the column into the needle sleeve assembly. To ensure the correct position of the sleeve adapter and emitter, make sure to push the sleeve adapter towards the end of the needle sleeve assembly until the column peek nut is stopped by the small narrowing in the needle sleeve assembly, as indicated with the red arrow in below picture.

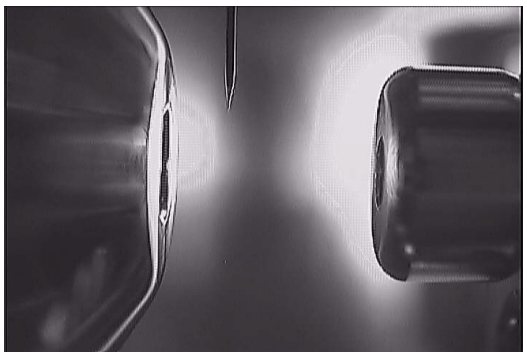


5. Now assemble the needle sleeve assembly and remove the protective sleeve from the emitter.

6. The needle sleeve assembly can now be inserted into the Nanospray slide assembly of the Nanospray source. When doing this be careful to keep the two parts of the needle sleeve assembly pushed together and not to stress the nanoViper transferline. View the video capture screen to make sure that the emitter appears at the top of the screen roughly midway between the counter electrode and spray shield.



7. Use the adjustment knobs on the source to position the needle as in below picture.

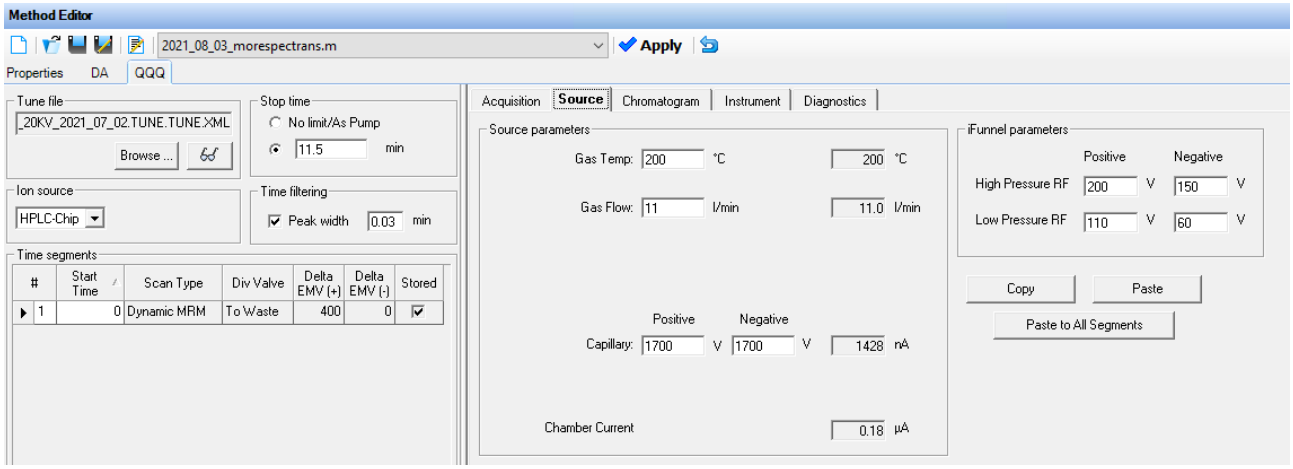


8. Run the flow to column from the Evosep One and adjust needle position, ionization voltage etc. to get stable spray.

Recommended start source conditions:

- Gas temperature 200C.
- Gas flow 11L/min.

- Capillary voltage 1700V



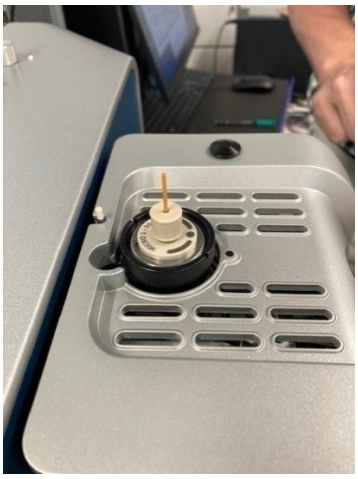
6.6 Sciex Optiflow ion source in microflow regime.

To run samples with the microflow probe on the Optiflow ion source and the Evosep One.

How to set up

1) Carefully insert a “1-10µL/min” electrode into the Micro probe.

2) Insert probe in top hole and fasten the lower PEEK fitting.



3) Put on the steel upper fitting with a PEEK ferrule on the electrode end. Put on a Peek NanoTight Union for 1/16” (P-779).

4) Connect the Evosep Column to the PEEK union. Connect the Evosep One transferline to the end of the column and remove the black removable knurl from the nanoViper fitting. Put on the column oven and close the oven compartment to activate the “High-voltage enable switch” on the ion source.

6.7

7 Preparing the Evosep One for use

Before running samples on the Evosep One system check solvent and waste levels:

Item	Details
Solvents	• Solvent-A: 0.1% formic acid in water • Solvent-B: 0.1% formic acid in Acetonitrile • Only LC-MS grade solvents must be used • Solvents must be exchanged on a weekly basis
Waste	• Check the waste bottle solvent level and empty if necessary • Check the Evotip waste bin and empty if necessary

The Evosep One is preconfigured with preparation programs which can be used to prepare the system for running samples and help to maintain the system performance.

Program	Usage
Pump preparation	Degas: To prepare the pumps for sample running if the instrument has been idle for more than 6 hours Solvent exchange: To purge pumps after solvent exchange
Align solvents	To prepare the system flowlines if the instrument has been idle for more than 6 hours
Flow to column	To set a flow to the column e.g. when setting up the MS spray conditions or when idle flow is required
System and column wash	To clean up the system and analytical column if they are contaminated, e.g. from running a dirty sample

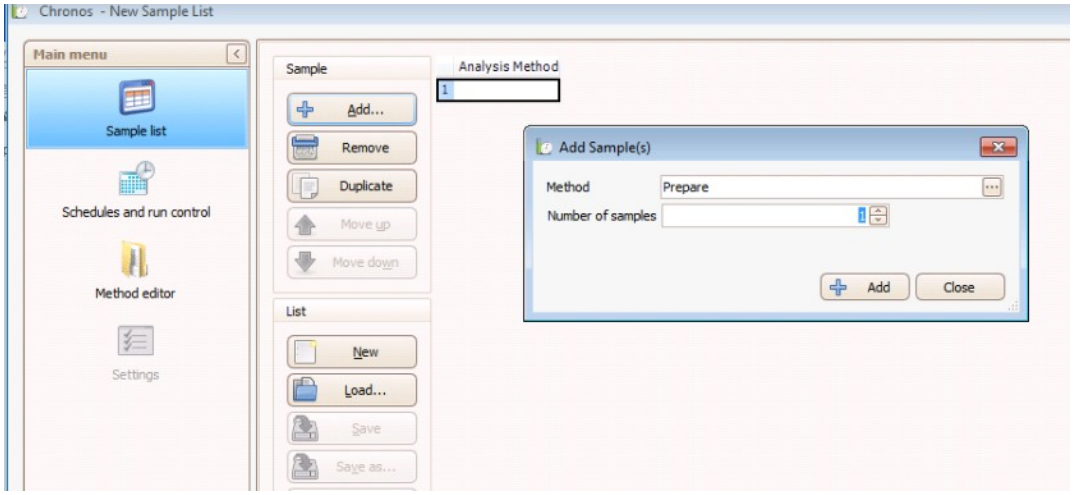
The Evosep One automatically assesses the need for doing preparative actions, to make sure each analysis is successful. Under these circumstances the system autonomously initiates the proper system preparation tasks immediately preceding the actual sample analysis.

1. Degas is initiated if the systems has been idle for more than 6 hours

2. Align solvents is initiated
    a. if the system has been idle for more than 6 hours
    b. The previous procedure was aborted
    c. The previous procedure caused the solvents in the flowlines and the ceramic needle to be unaligned.

This means, the user can start sample runs without considering the instrument state, and the system will by itself run the appropriate preparation protocols to ensure maximum performance.

In addition, all the preparation programs can be manually run by adding a sample using the “Prepare” method (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Prepare.cam) in the “Sample list” panel.



The programs can be selected individually or be run in succession using the dropdown and check boxes:

	Analysis Method	Pump preparation	Align solvents	Flow to column / idle flow
1	...\Prepare.cam	none	☐	none

Start queue in the “Schedules and run control” panel.

7.1 Degas pumps

During instrument idle periods gas penetrates the instrument pumps and tubing’s. The increased solvent gas level has a negative impact upon pump responsiveness and mass spectrometer electrospray stability. Consequently, peak retention time and area reproducibility are lowered.

The program automatically runs a loop of aspirate, degas and dispense procedures on all the system pumps (HP, A, B, C, D) until the volume required to reach 200 bar (HP) and 50 bar (A, B, C, D) of pressure on each pump is less than 9 µl (HP, A,B,C,D). If this target is not reached within 15 iterations, the program will abort.

If one or more of the pumps fail to reach 50 bar, within the maximum volume, proceed to the "Troubleshooting" section for guidance.

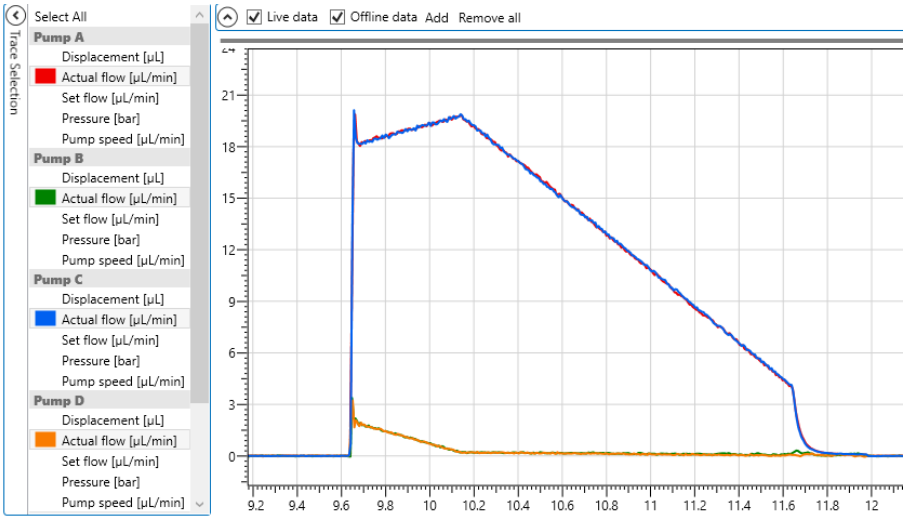
7.2 Solvent exchange of pumps

The "Solvent exchange" program is an automated pump purge procedure. It runs 15 cycles of complete pump solvent exchanges and it should be used following the weekly solvent exchange or if the instrument has been idle for an even longer period.

7.3 Align solvents

When the instrument is standing idle for longer periods, the solvent will be subject to diffusion and evaporation in areas with contact to the ambient air. This causes an unintended solvent mixing in the flow lines that will influence the chromatography in the following sample. The "Align solvents" program flushes the flow paths with solvent to re-create the expected starting conditions and hence improve the chromatography. It is recommended to run the "Align solvents" program if the instrument has been standing idle for more than two hours since the last sample was analyzed.

The AB and CD pumps run two identical but separate gradients. The AB gradient goes through the Autosampler needle and ends up in the wash station, whereas the CD gradient flushes the flowlines going to the tip cross.



7.4 Flow to column

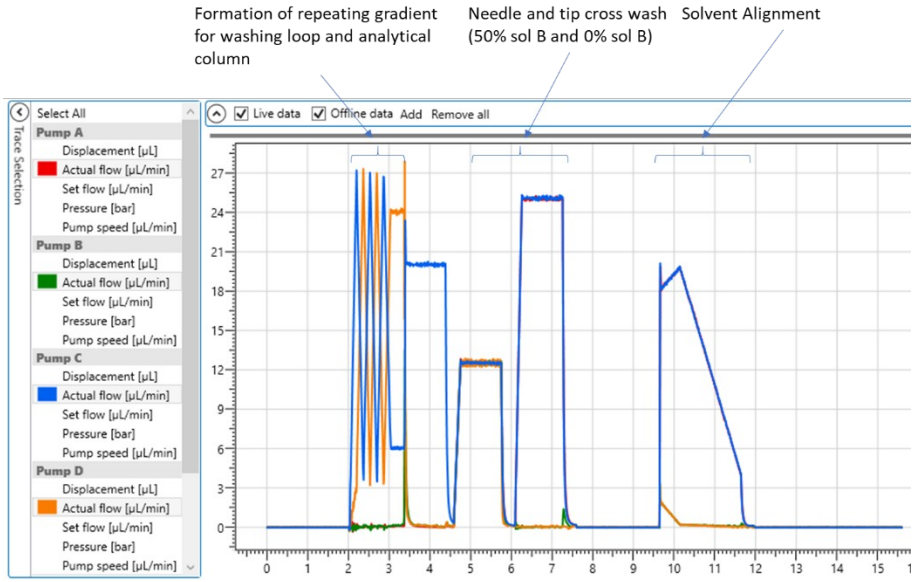
Before running the first sample, it is recommended to tune the MS ion source conditions, according to MS manufacturer guidelines to secure a stable electrospray. For this purpose, use the “Flow to column” program, which encodes an automated procedure for delivering solvent-A to the analytical column at a flow rate of 0.5, 1, 2 or 4 µl /min for 10 min. Make sure that flow and pressure are stable before starting to tune the MS.

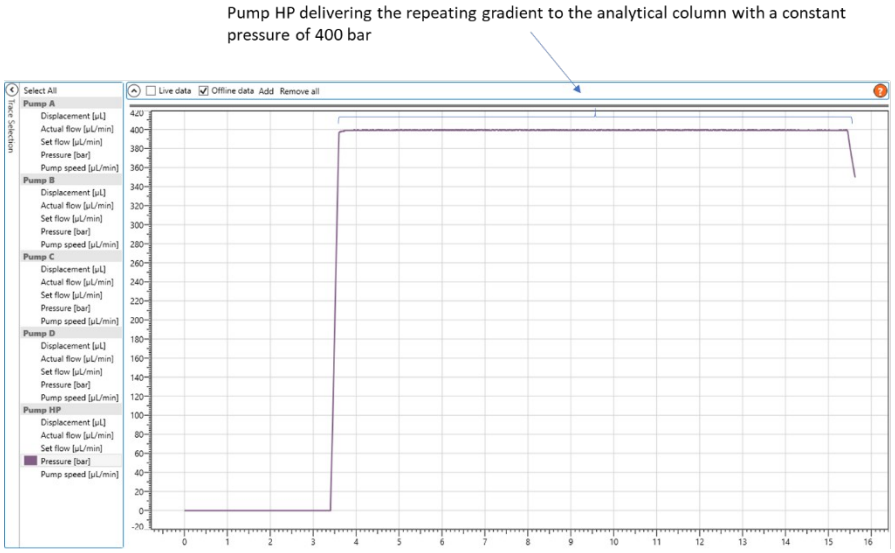
The last option, “Idle flow”, enables a continuous flow of 0.25 µl/min to the column. It is typically used when the instrument is expected to be idle for a longer period, e.g. following the last sample in a sequence. The Idle flow must be manually stopped from the Schedules and run control window, before other preparation or sample method can be started.

7.5 System and column wash

The “System and column wash” program can be used if the Evosep One autosampler, loop or analytical column has been heavily contaminated from running a dirty sample. The duration of the program will vary according to the backpressure of the connected analytical column (typically 10-15 min.)

1. The program automatically creates a gradient of repeated increments from 10-90% solvent-B, which is deposited in the loop.
2. The HP pump delivers the gradient to the analytical column, at a constant pressure of 400 bar.
3. The autosampler Needle and Tip cross are washed in two steps; 50% solvent-B and 0% solvent B.
4. The system is prepared for the next sample by re-aligning the solvent in the low-pressure pump flow lines and the autosampler needle.



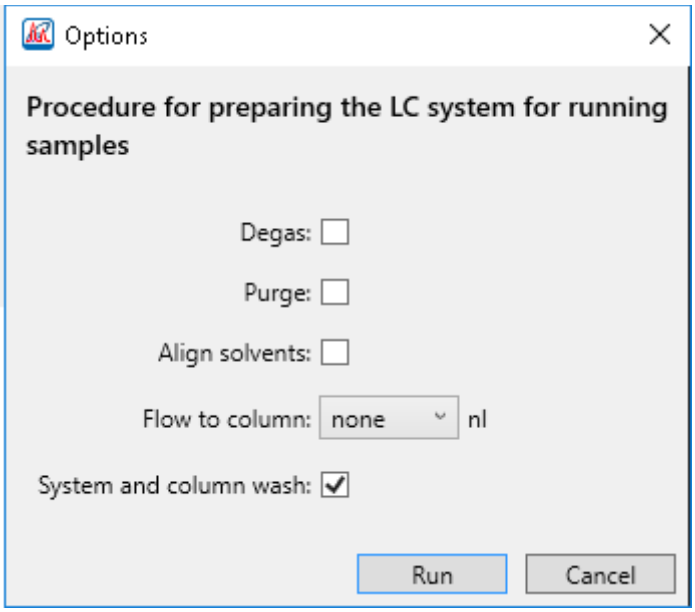


The “System and column wash” procedure can be started in two different ways:

1. In Chronos and HyStar, the program can be started from the sample table using the “System and column wash” method. Here, the position of the blank Evotip position can be freely chosen.

	Analysis Method	Source Tray	Source Vial
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\System and column wash.cam	EvoSlot 1	1

2. In HyStar, the program can also be started by right-clicking the Evosep One status view window and choosing the “System and column wash” option. Here it is required, that a blank Evotip is present in the autosampler tray 1, pos 1 (A1).

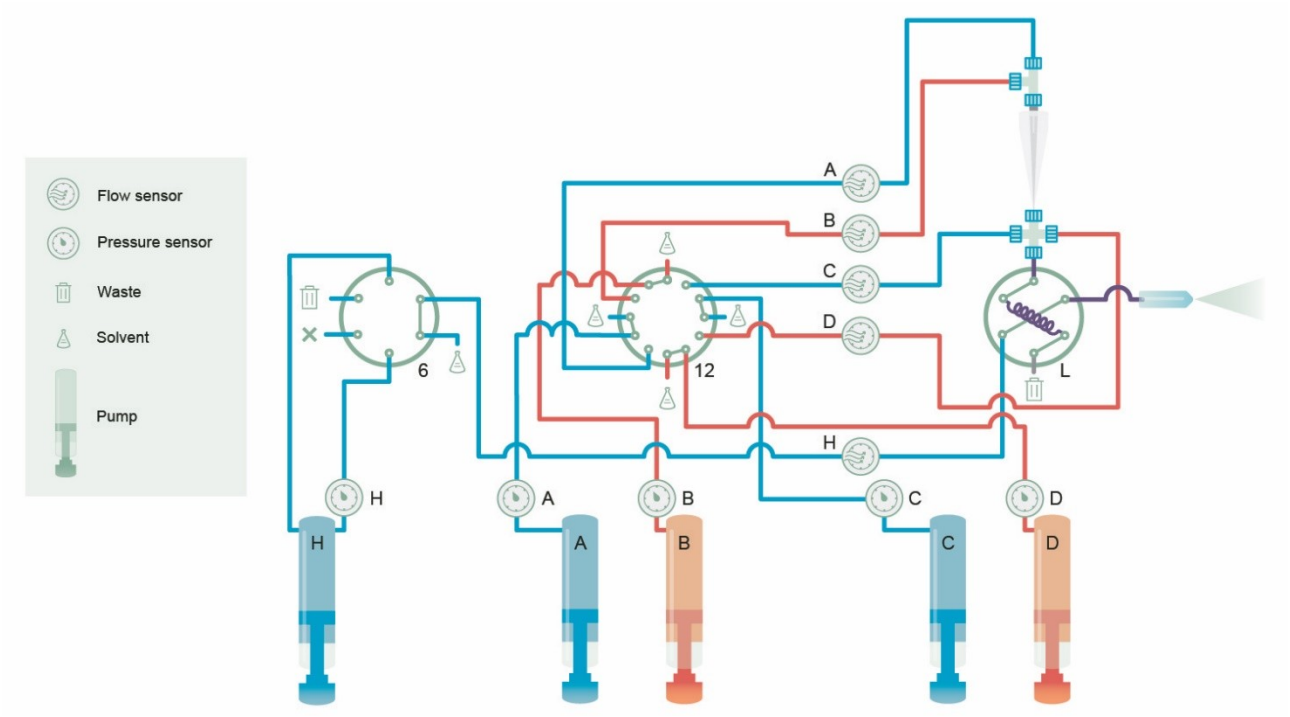


8 Running Samples using Evosep One

The Evosep One chromatographic system is designed to minimize the sample overhead time and improve the instrument duty cycle. All the traditional HPLC household steps and execution sequences such as pump refilling, column equilibration, sample loading etc. has been re-thought with the aim of maximizing the time spent on the analyte elution.

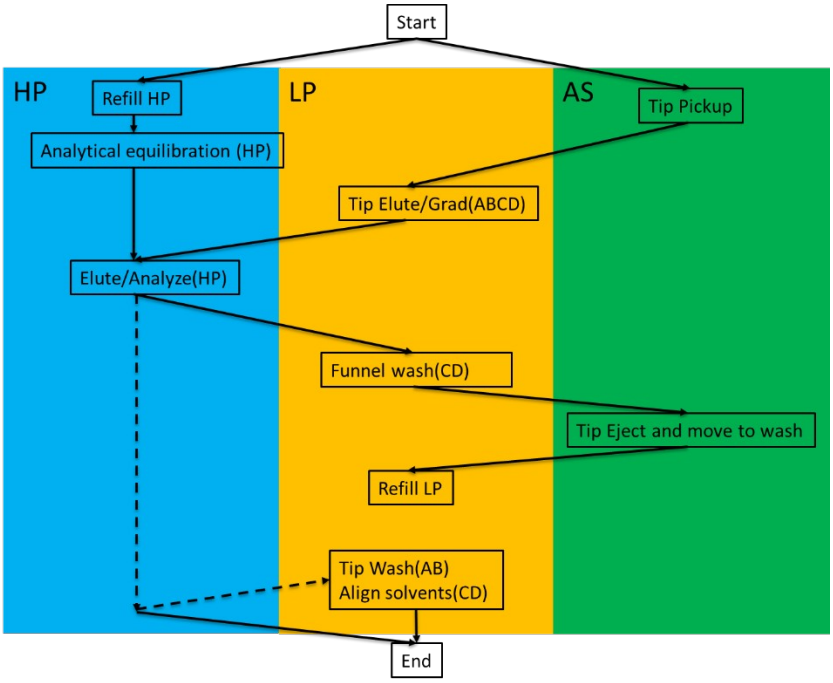
8.1 Separation principle

The Evosep One technology is centered around the Evotip and integrates sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with a small plug of C18 stationary phase at the bottom of the tip. The Evotips are used to de-salt and clean up the samples prior to LC-MS analysis, however, the traditional subsequent steps of eluting, drying down, re-suspending the samples from tips are completely omitted, and instead the tips are loaded directly into Evosep One for analysis. This new process leads to significantly less sample loss and variation as well as much simpler and faster workflows. The Evosep One sample tray accommodates up to 6 racks of 96 tips, i.e. 576 rinsed samples may be lined up for fast analysis.



Evosep One plumbing diagram. H: High-pressure Pump, A/B/C/D: Low-Pressure pumps, 6: 6 port high-pressure solvent valve, 12: 12 port low-pressure solvent valve, L: Loop Valve.

Upon starting an analysis, the autosampler places one tip at the time (with the pre-loaded sample) in-line with the solvent system (Tip Pickup).



High level Sample acquisition process diagram. The acquisition cycle can be divided into three sections; Blue: High pressure – pump HP, Yellow: low pressure – pump A,B,C,D and Green: autosampler actions.

Once the Evotip is sealed in-line with the solvent system, a gradient from pumps A and B runs through the Evotip and sequentially elutes the adsorbed analytes (Tip Elute/Grad(ABCD)). While the gradient, with the embedded and pre-separated analytes, elutes from the Evotip, a secondary gradient from pumps C and D continuously modify the composition of the initial A/B gradient to generate an offset gradient that ensures optimal chromatographic performance at the analytical column, see figure 3. Pumps A+B deliver a partial gradient which is sufficient to sequentially elute the analytes of interest but still leave all the high-molecular contaminants behind which are then discarded with the Evotip after the analysis. A high organic wash (80% ACN) volume is introduced just after the gradient using Pump D, bypassing the Evotip, to efficiently wash the analytical column. It takes approximately one minute at 20-40 µl/min (<20 bar) to create the preformed and offset gradient with the embedded analytes and position it precisely in the storage loop (ID100µm, 30µl), see figure 3.

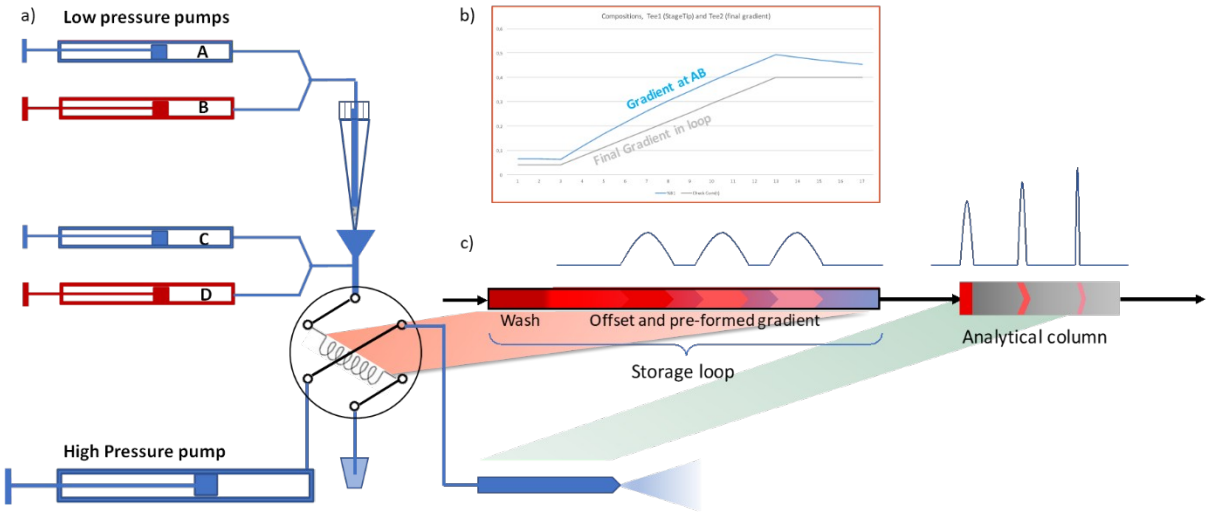


Figure 3: Evosep One a) Simplified plumbing diagram of Evosep One. b) Illustration of the A/B gradient running through the Evotip and the following C/D modified gradient resulting in an offset gradient for optimal focusing at the analytical column. c) Illustration of the preformed and offset gradient stored in the storage-loop containing the pre-separated analytes. The gradient offset helps to focus and significantly increase the capacity and chromatographic performance of the analytical column.

After generation of the gradient, the loop-valve switches the storage-loop in-line with the high-pressure pump and analytical column. The high-pressure pump can now push the pre-formed and offset gradient with the pre-separated analytes to the analytical column (Elute/Analyze (HP)). The gradient offset lowers the organic contents, such that the analytes are initially retained on the analytical column. This allows each analyte to refocus on the analytical column and hereby significantly increases the capacity and chromatographic performance, see figure 3c.

The instrument comes with pre-set methods, optimized for separation performance, see table 1. This always gives the user the best separation quality, for a given throughput requirement, for a particular experiment.

Throughput	Cycle time	Gradient length	Flow rate	Column (length/ID/C18 bead size)
Samples/day	Minutes	Minutes	µl/min	cm/um/um
300	4.8	3.2	4	4/150/1.9
200	7.2	5.6	2	4/150/1.9
100	14.4	11.5	1.5	8/100/3
60	24	21	1	8/100/3
30	48	44	0.5	15/150/1.9

Table 1: Evosep One Methods.

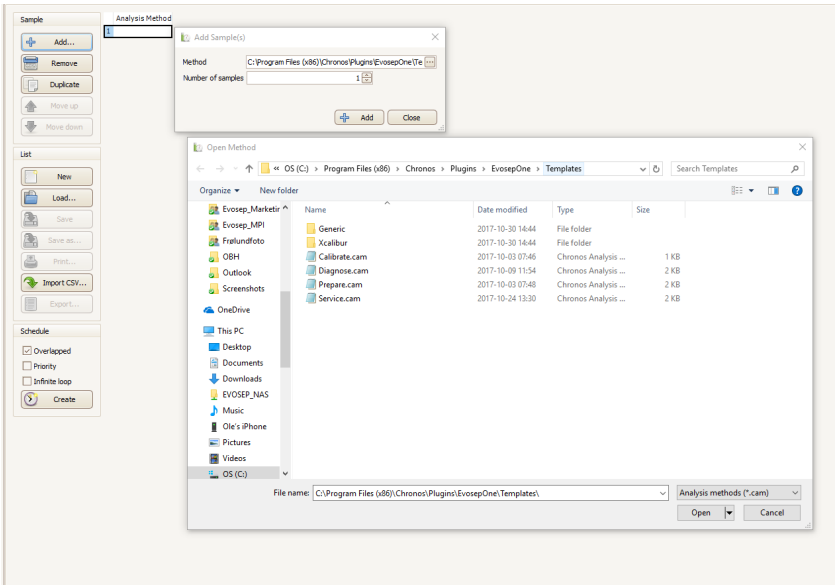
8.2 Sample acquisition

Chromatographic Data system (CDS) and generic methods

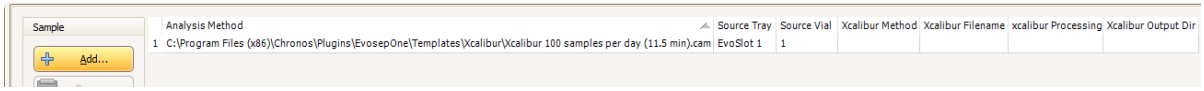
The sample acquisition methods are executed using the Chronos software, as described in the SW section. The instrument can be operated in two distinct fashions.

1. Integrated LC-MS mode; Chronos controls both the Evosep One instrument and the mass spectrometer, using one sample list.
2. Standalone LC mode; Chronos only controls the LC – which means two sample lists are required for sample acquisition - one for the Evosep One (in Chronos) and one for the mass spectrometer (in the MS CDS).

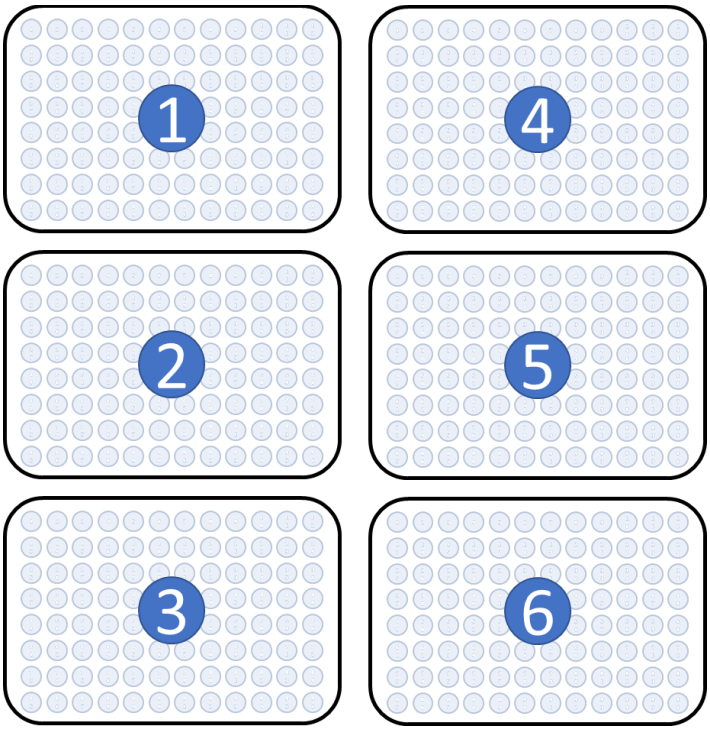
The sample methods for both modes are stored in the: C:\Program Files “(x86)\Chronos\Plugins\ EvosepOne\Templates” folder. Standalone mode methods are in the “Generic” folder whereas LC-MS integrated methods are stored in a MS CDS specific folder, e.g. methods for Thermo mass spectrometers are saved in the “Xcalibur” folder.



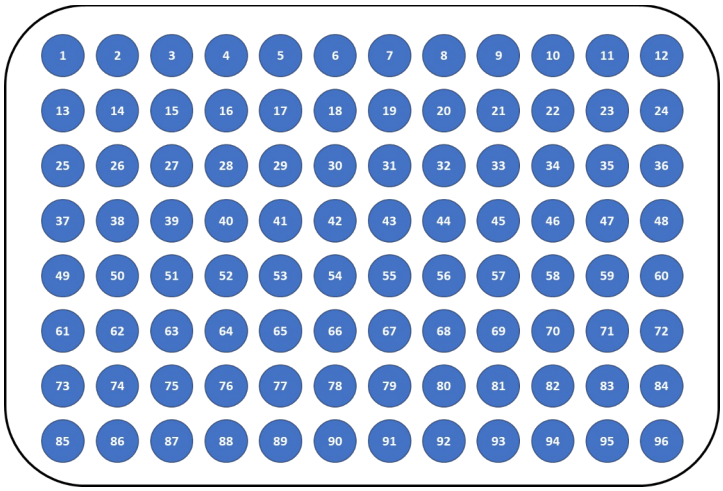
- Integrated LC-MS mode
    a. The appropriate sample method is chosen firstly based upon MS CDS vendor and secondly required sample/day throughput (Table 1)



    b. Source Tray (Slot 1-6) must be specified using the dropdown menu



    c. Source vial position (1-96) must be specified using the dropdown menu,



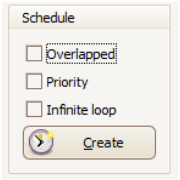
d. For Thermo MS; “Xcalibur Method” – the MS acquisition method must be specified. MS acquisition time must be correlate with the individual LC-MS methods, as specified in table 1.

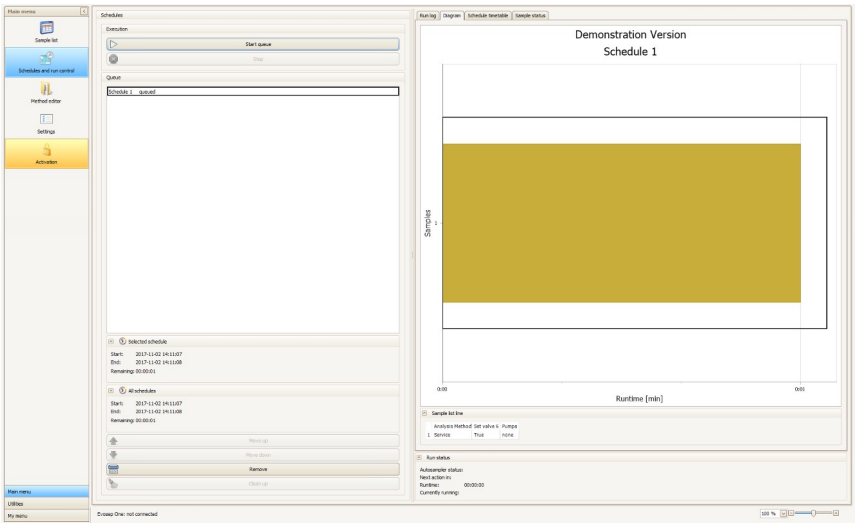
e. For Thermo MS; “Xcalibur Filename” – the MS data filename(s) must be specified.

f. For Thermo MS; “Xcalibur Processing” – the MS data post processing method *can* be specified.

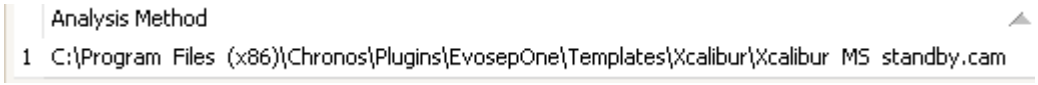
g. For Thermo MS; “Xcalibur Output Dir” – the MS data directory must be specified.

h. When the sample list is completed, create a schedule and start the analysis. Chronos will send the sample information to Xcalibur and once the MS is in “waiting for contact closure status”, the Evosep One sample separation will start.

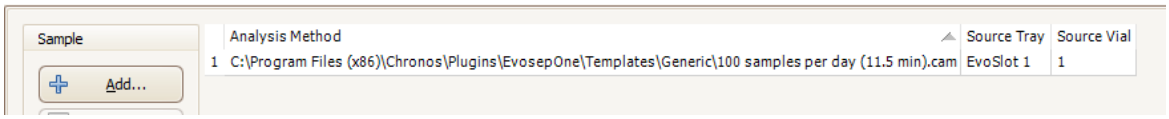




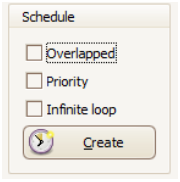
i. For Thermo MS. Running the Xcalibur MS standby program, will set the mass spectrometer in standby. The program will force the MS into standby, even if time remain in the chosen MS method. Typically this method is chosen as the last sample in a batch.



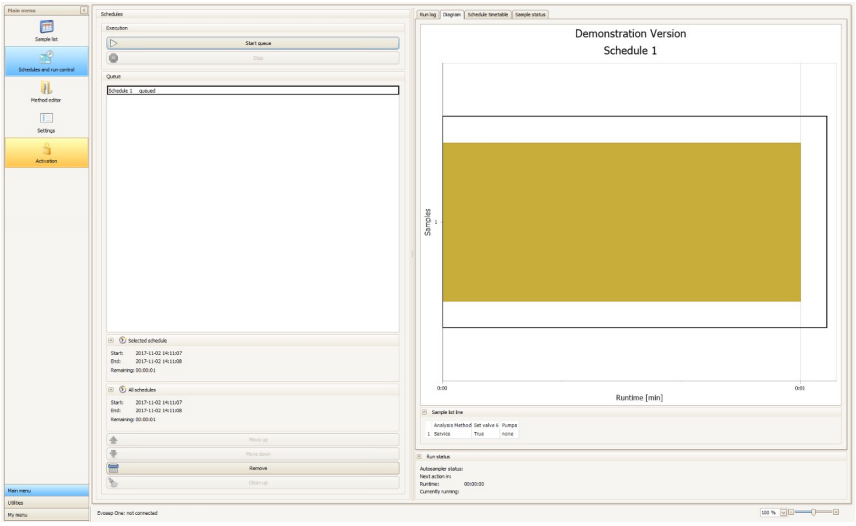
- Standalone LC mode
  a. The appropriate sample method is chosen based on the required sample/day throughput (Table 1)



  b. "Source Tray and "Source Vial" must be specified as above.
  c. When the sample list is completed, create an Evosep One schedule.



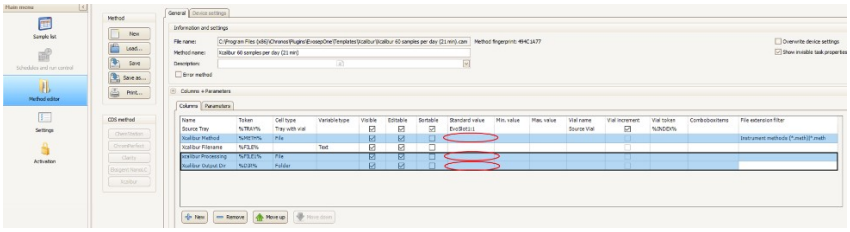
  d. in the MS CDS; create a synchronous sample list, queue the sample list for acquistion and wait for the MS to be in "wait for contact closure" mode.
  e. Start the Evosep One shedule queue.



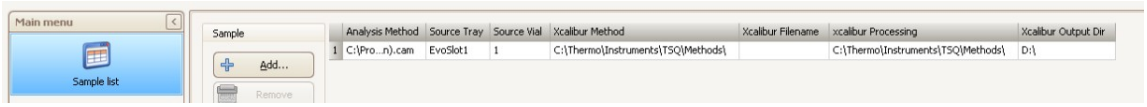
  f. The Evosep One starts the sample separation produce and sends a contact closure signal to the MS CDS, when the sample elution begins.

Tips and tricks:

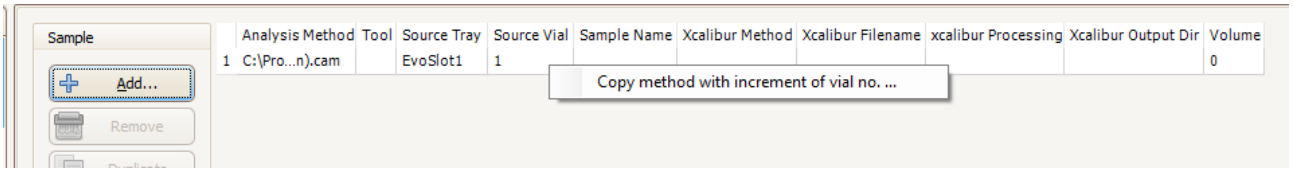
- When running integrated LC-MS methods, e.g. for Thermo mass spectrometers, the user can set up default values for:
    - "Xcalibur method": folder or file names
    - "Xcalibur processing method": folder or file names
    - "Xcalibur Output Dir": folder
  - These values are set in the "Method editor" section. Load the Evosep One method and paste the path of MS method, processing method and MS data output directory.

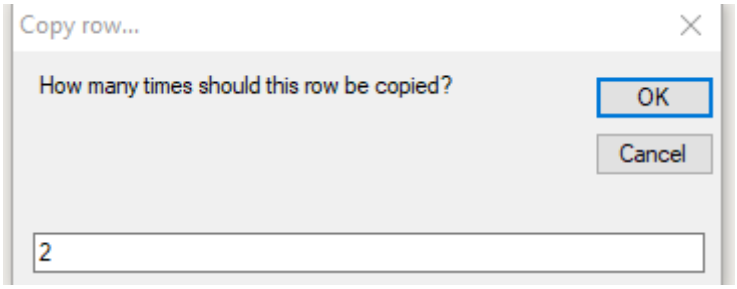


  - Save the method in the same name.
  - When submitting new samples with the updated method, the user can now browse from the specified default directory.

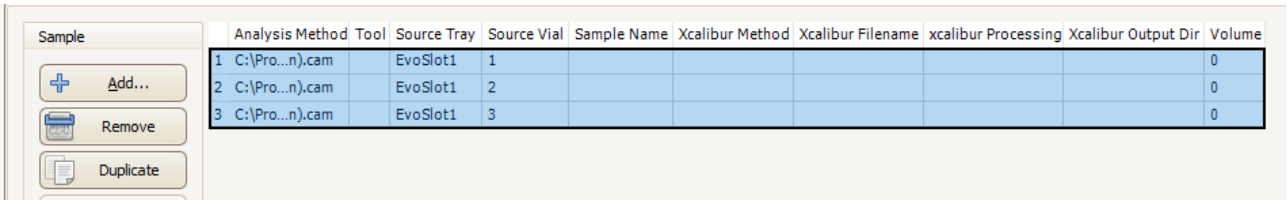


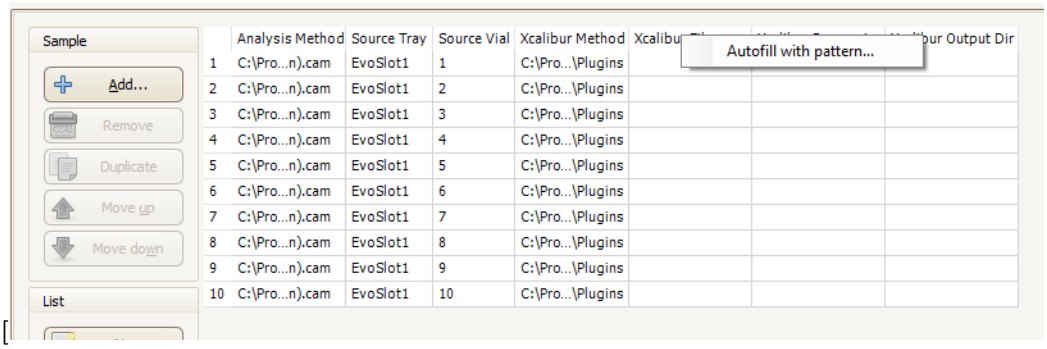
- If several samples in consecutive autosampler positions are to be processed using the method, right-clicking the "Source Vial" column will open a copy row dialog. Define how many samples must be added to the sample list and press OK.

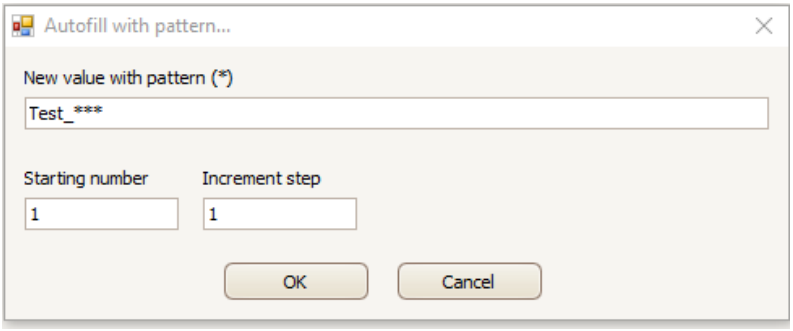


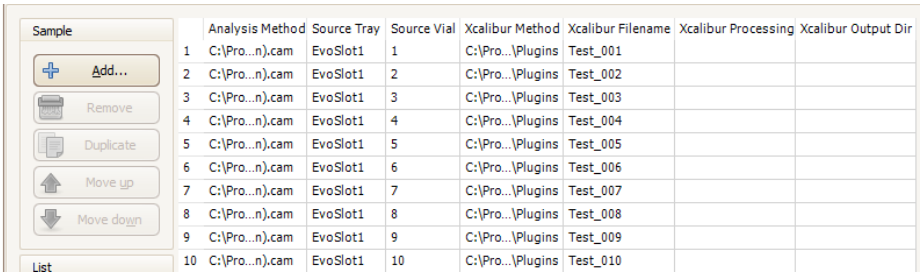


- If the sample name of several samples only should vary by a suffix, right clicking the “MS Filename” will open a “Autofill with pattern…” dialog. Fill in the sample “base name” followed by one or more asterixes (*). When you press OK the sample names will be filled down in the sample list with the starting number and incremental step chosen (here Test_001-Test_010).





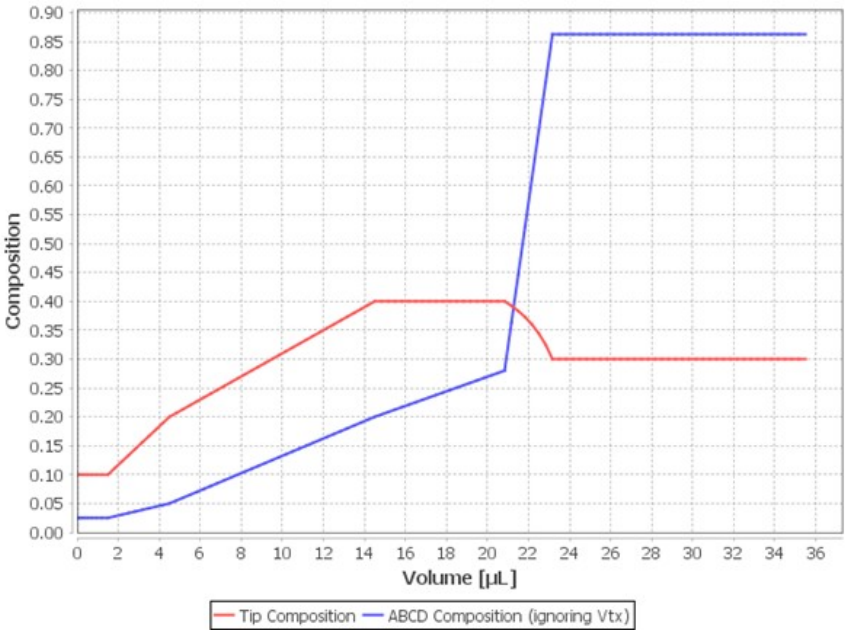




- If you often run the same analysis using the same methods, the sample list can be saved and reloaded instead of created from scratch.

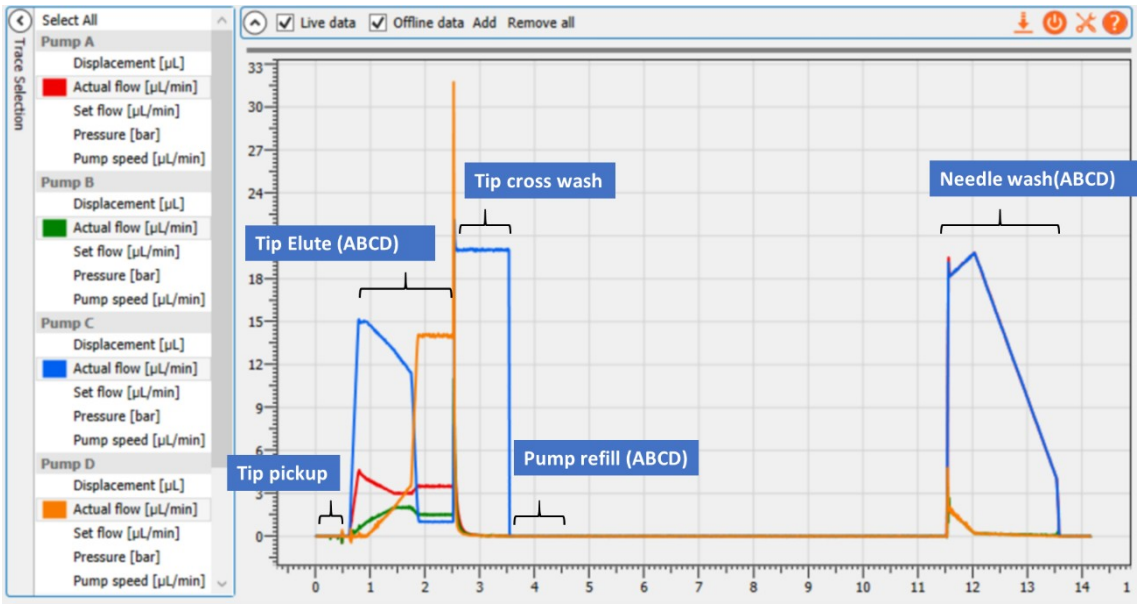
8.3 Example pump data

The pump graphs shown below are from a standard “100 SPD” chromatographic method on an Evosep One system using the installation analytical column (length 8 cm, ID 100 µm, 3 µm C18 beads)



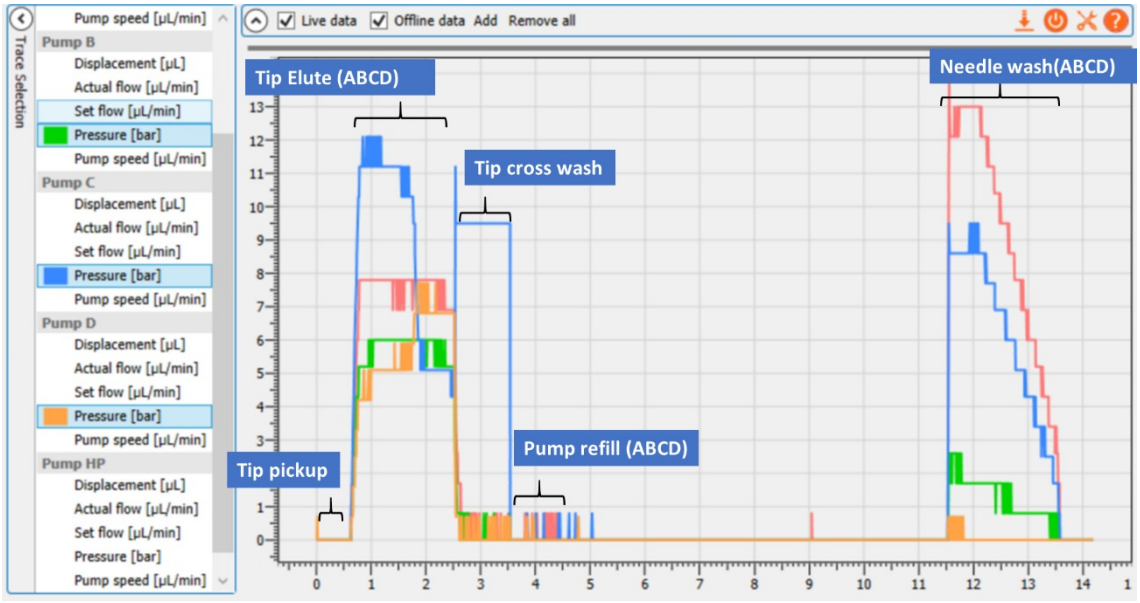
ABCD pump

Actual flow rates [µl/min]

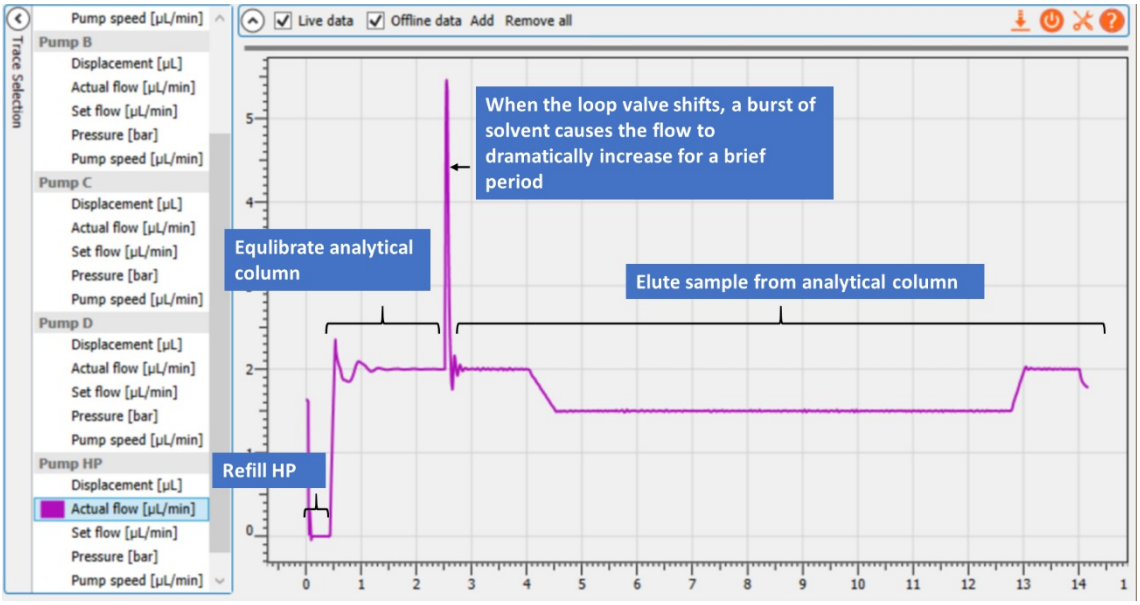


ABCD pump

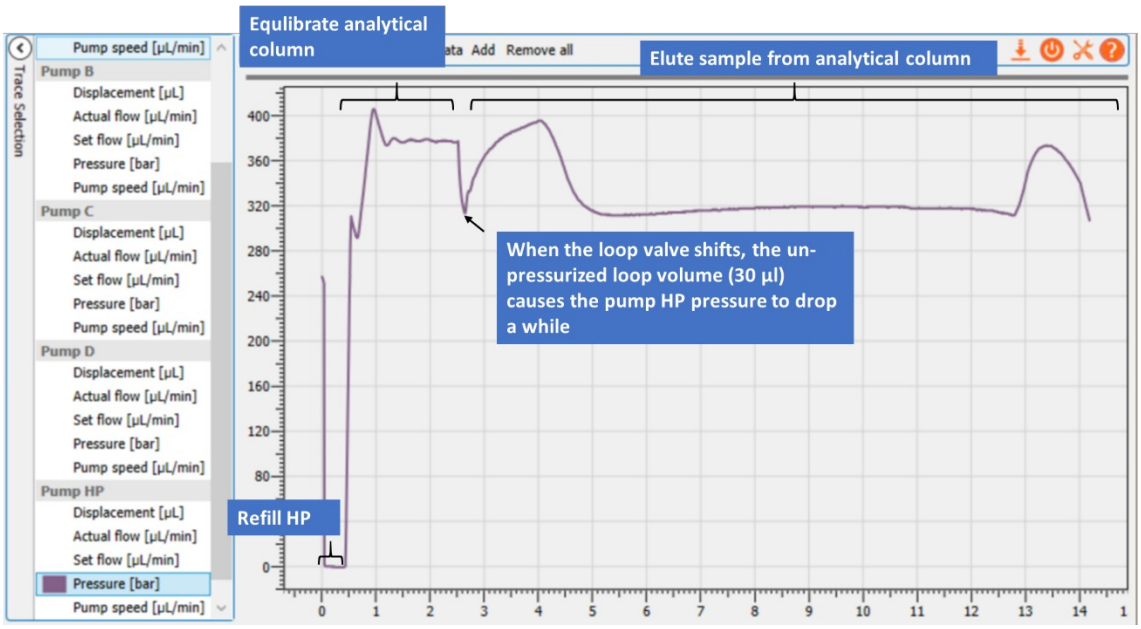
Pressure [bar]



HP pump Actual flow rate [µl/min]



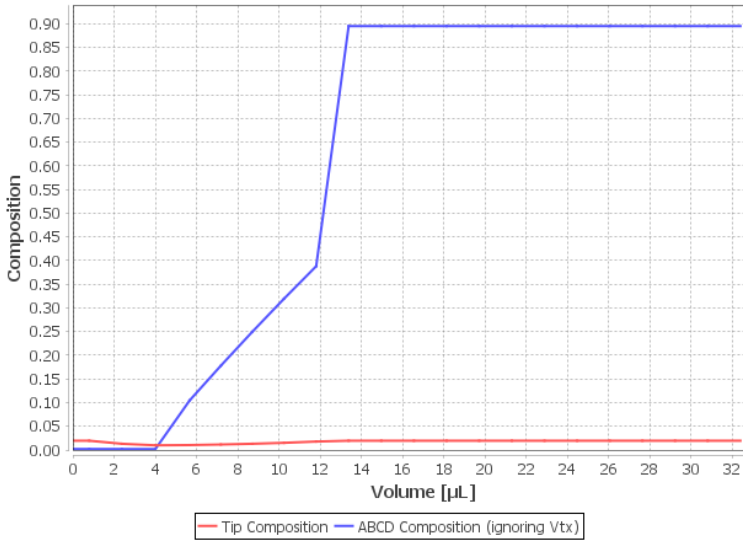
HP pump Pressure [bar]



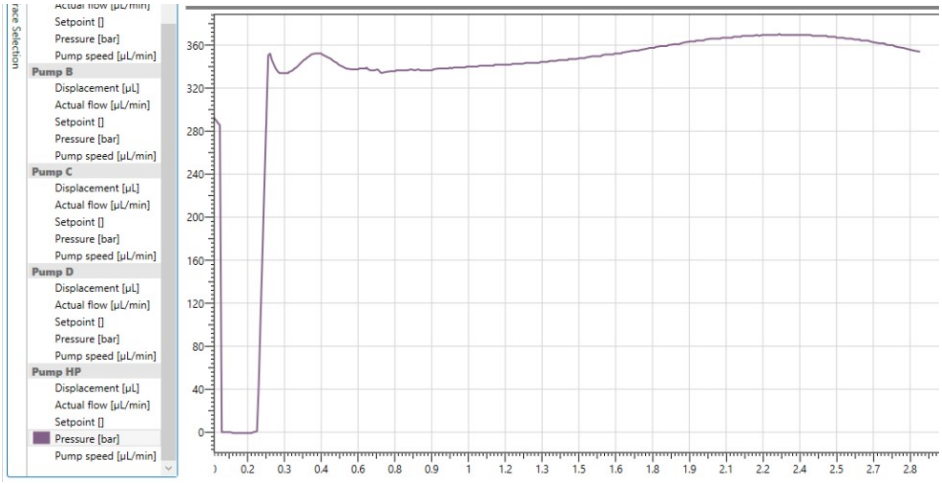
Below is a collection of the gradients and sample HP pump pressure and flow profiles for the different Evosep One methods

- 500 SPD method
- The 500 SPD method is optimized for speed and hence deviates from the other method in several ways:
    - The ABCD flow rate is higher during the gradient formation
    - The Pump refill speed is 200/100 µl/min for HP and A/B/C/D pumps respectively
    - The HP pump moves more rapidly when starting the column equilibration
    - The tip cross wash and the needle wash is faster
    - The HP pump is pressure controlled when the loop valve shifts to elute position

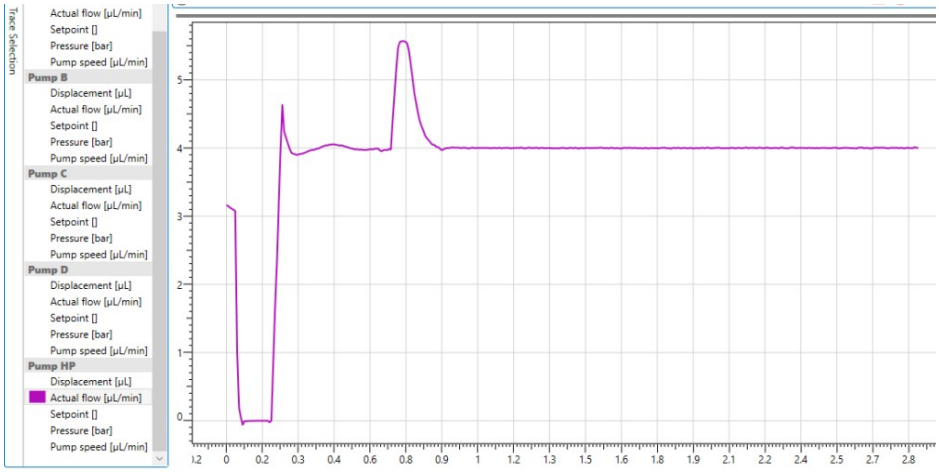
    - Gradient



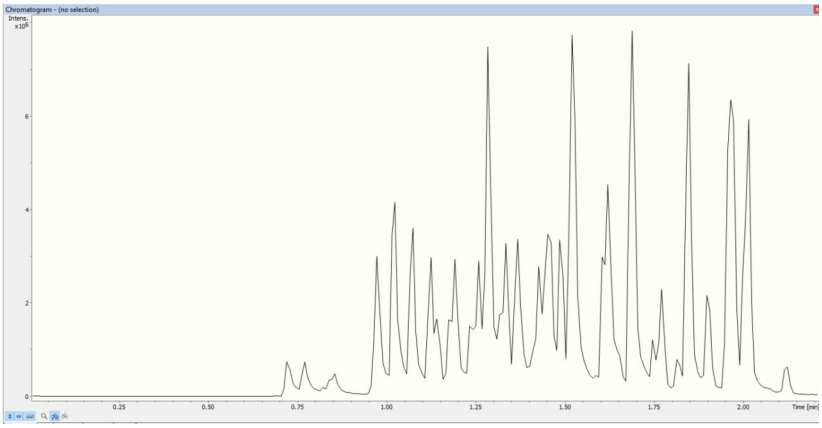
- Pump HP pressure



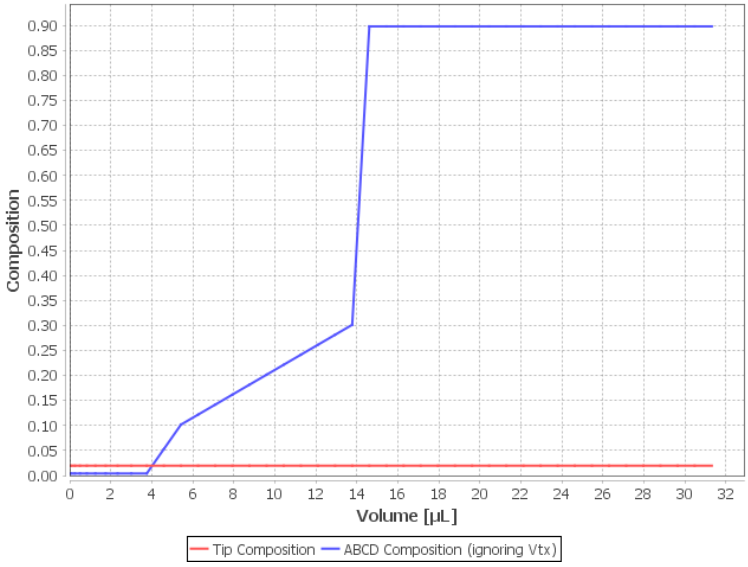
- Pump HP flow



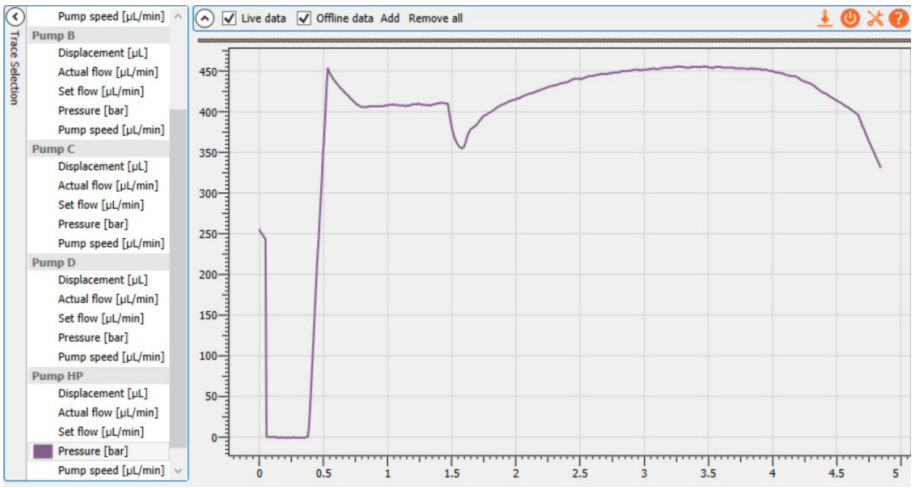
- Example base peak chromatogram, tryptic BSA digest



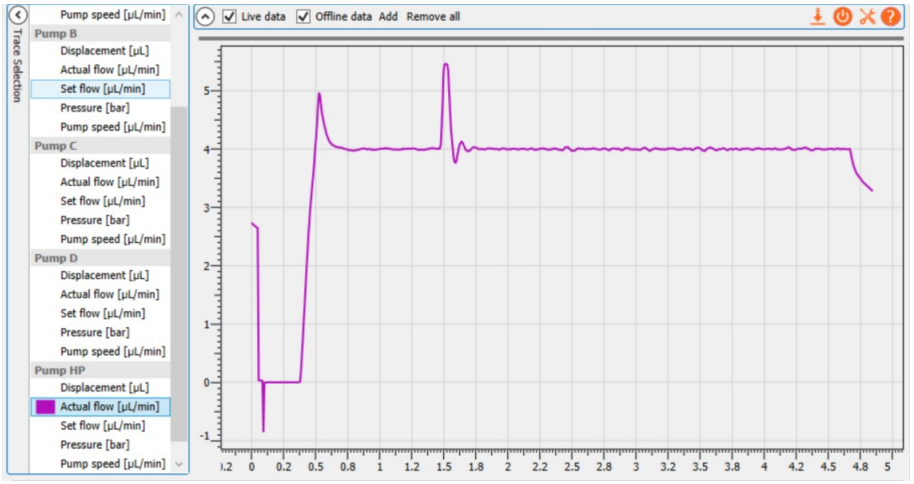
- 300 SPD method
    - Gradient:



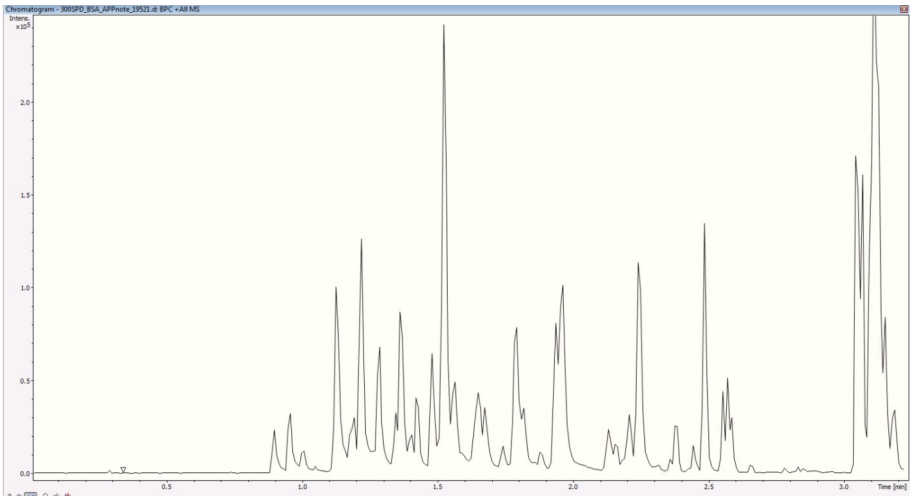
    - Pump HP pressure



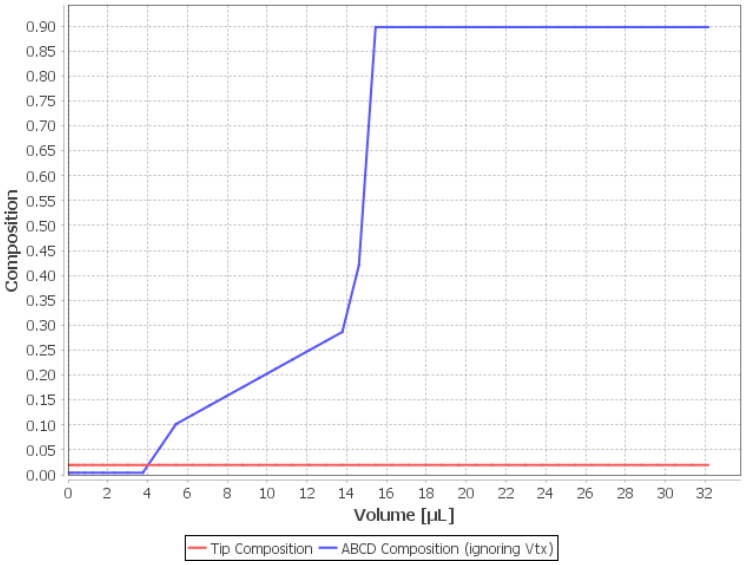
- Pump HP flow



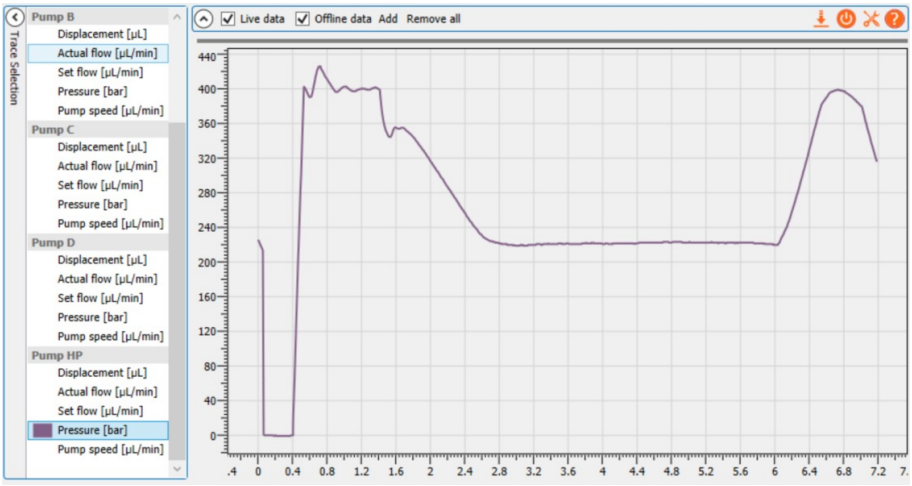
- Example base peak chromatogram, tryptic BSA digest



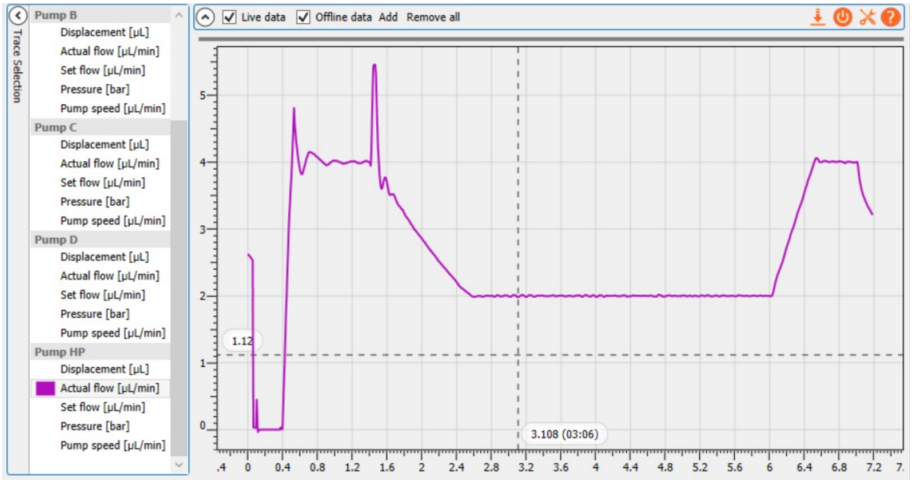
- 200 SPD
  - Gradient:



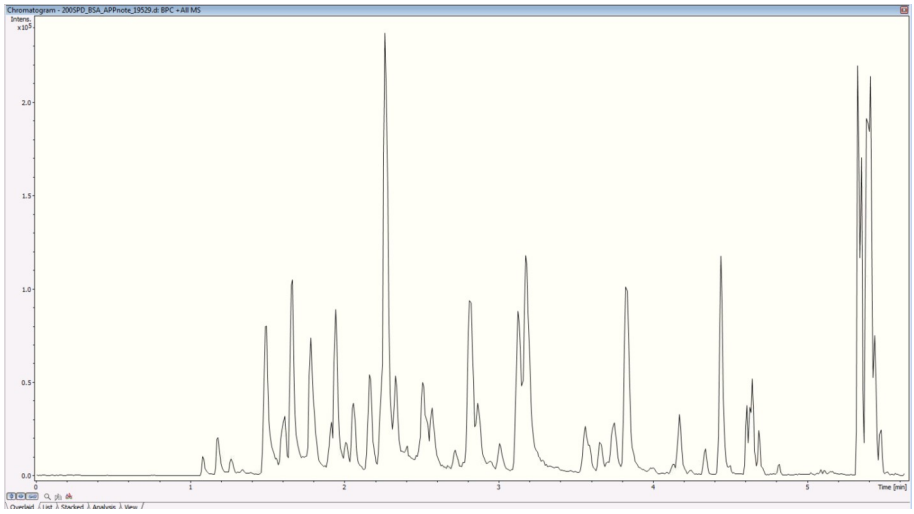
- Pump HP pressure:



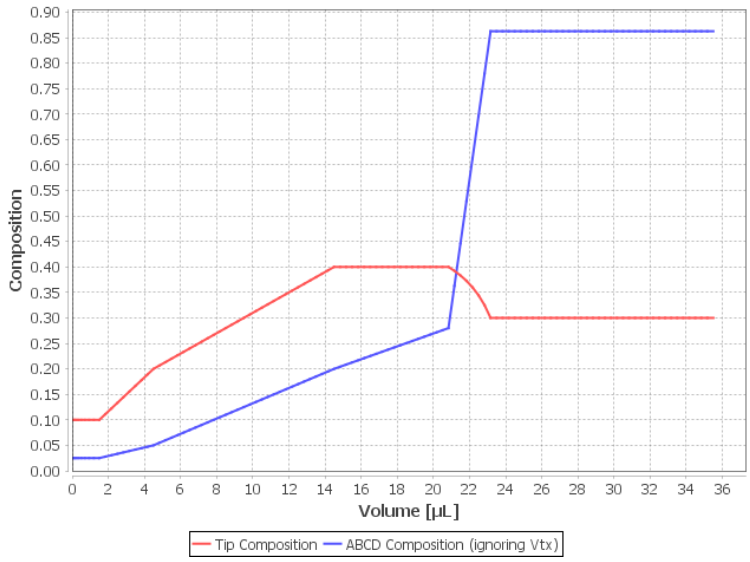
- Pump HP flow:



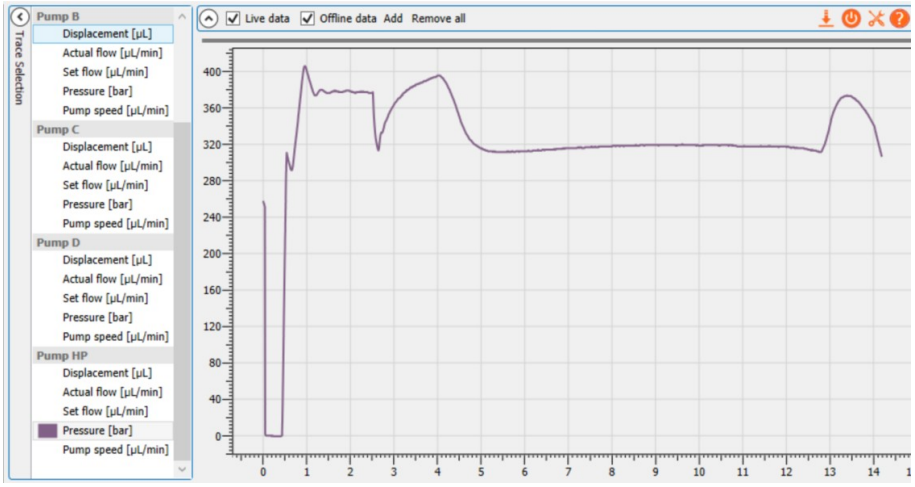
- Example base peak chromatogram, tryptic BSA digest


Chromatogram - 200SPD_BSA_APPnote_19529.d: BPC + All MS
Intens.
x10^5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
Time [min]

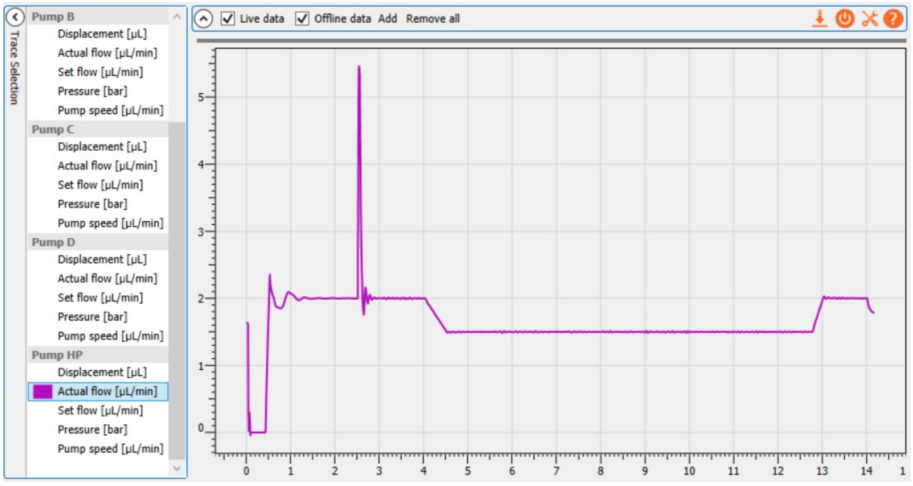
- 100 SPD method
  - Gradient:



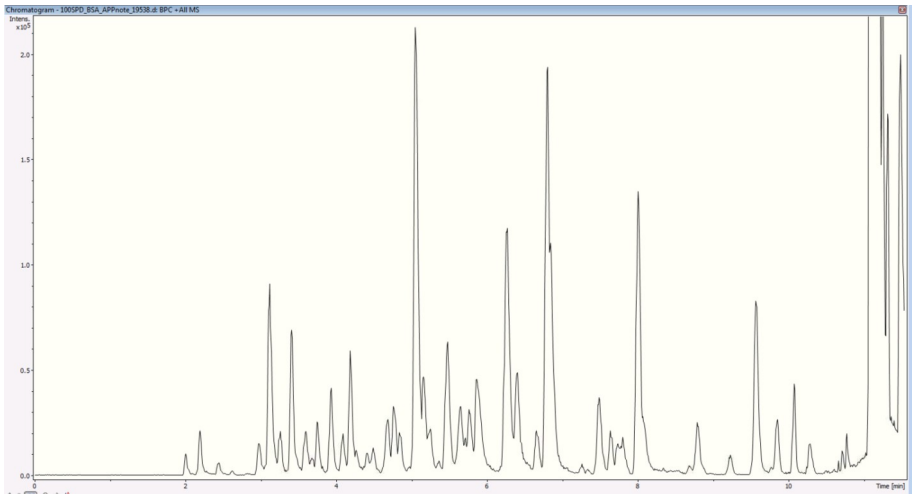
  - Pump HP pressure:



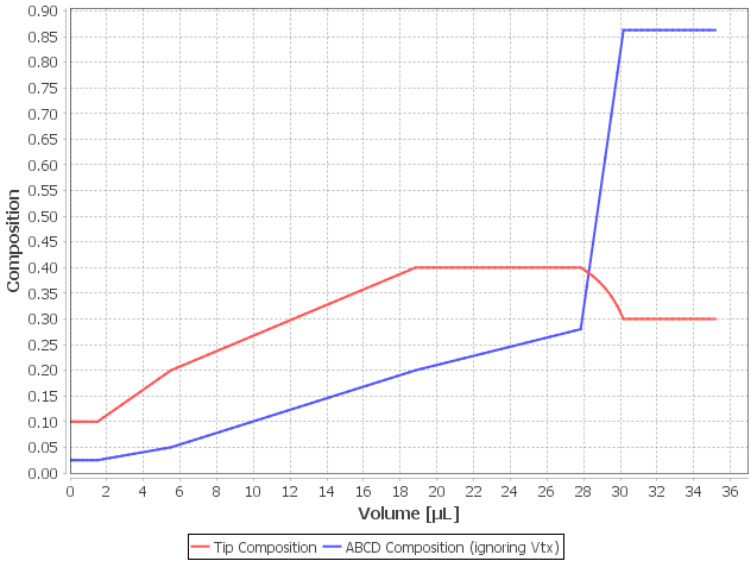
  - Pump HP flow:



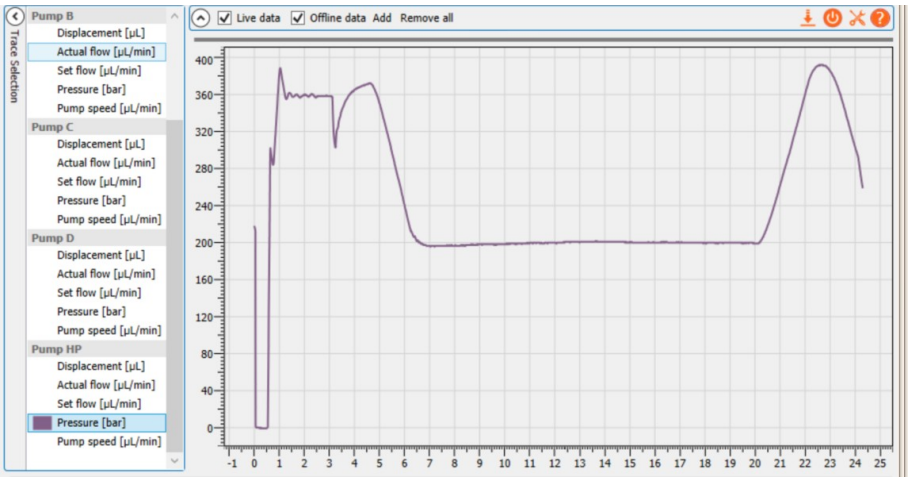
- Example base peak chromatogram, tryptic BSA digest



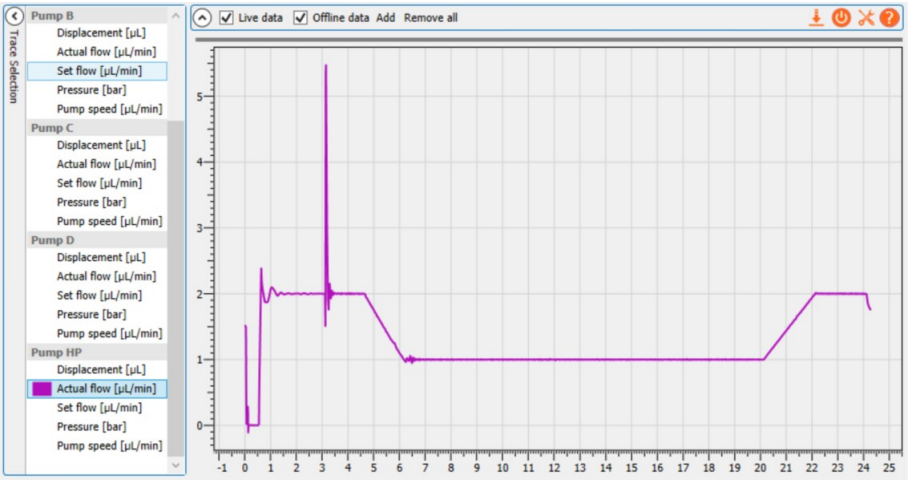
- 60 SPD method
  - Gradient:



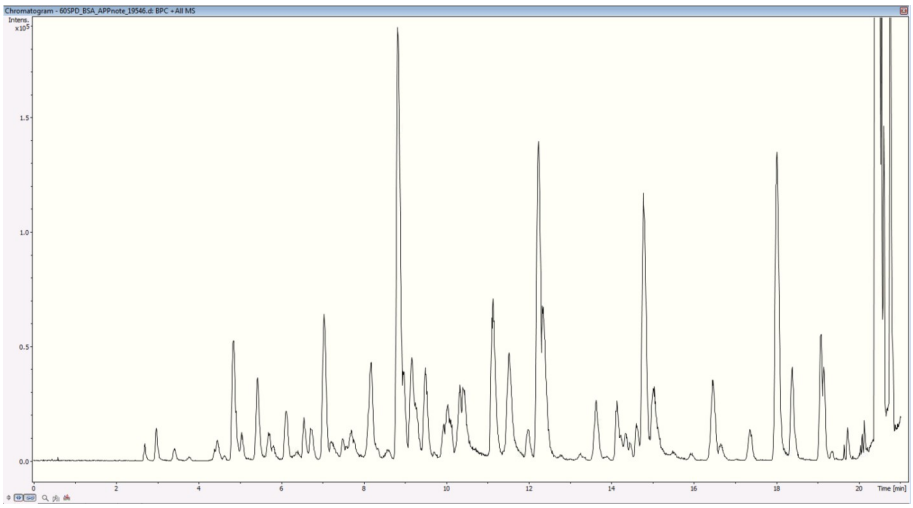
  - Pump HP pressure:



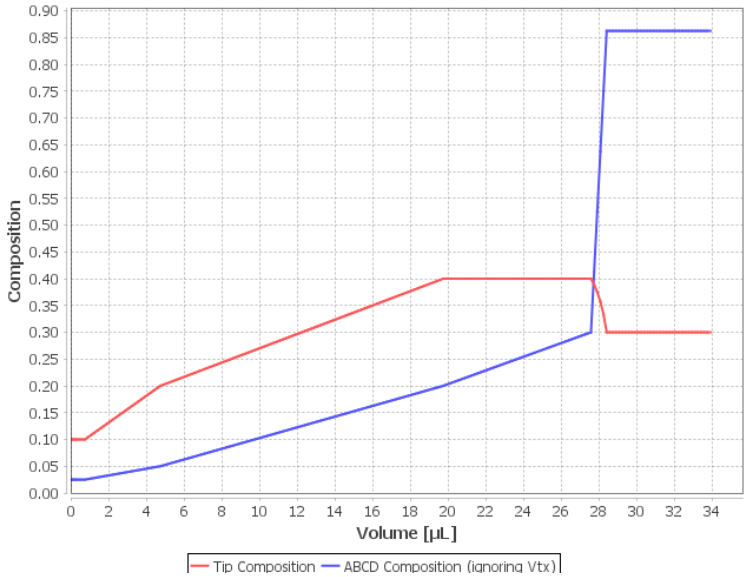
- Pump HP flow:



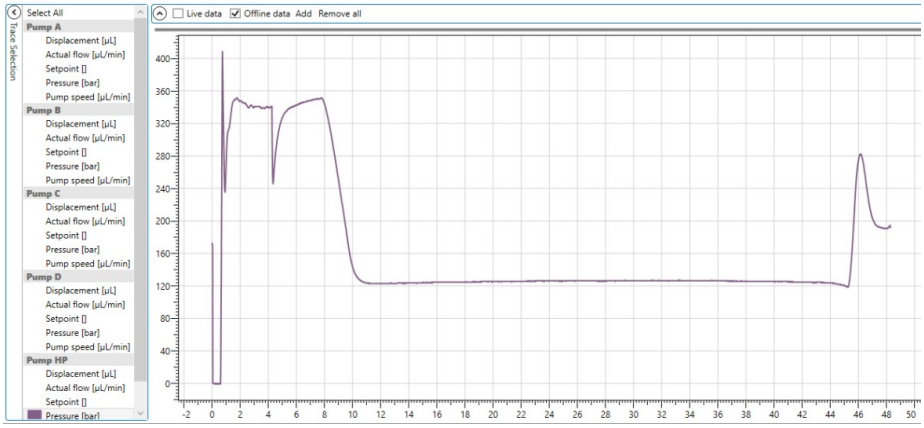
- Example base peak chromatogram, tryptic BSA digest



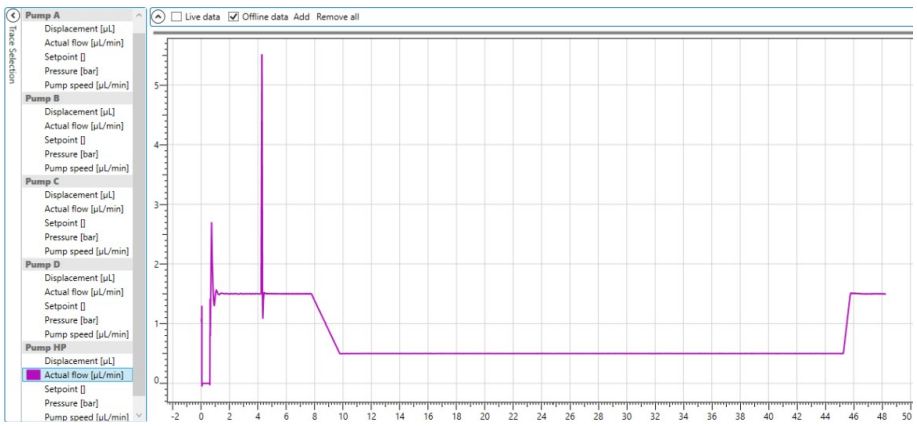
- 30 SPD method
  - Gradient:



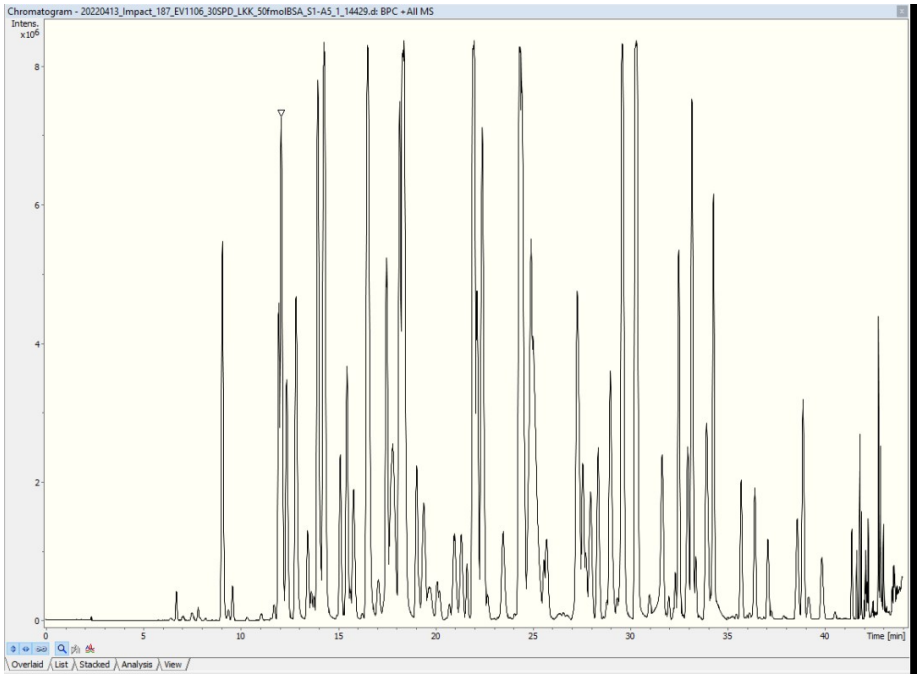
  - Pump HP pressure:



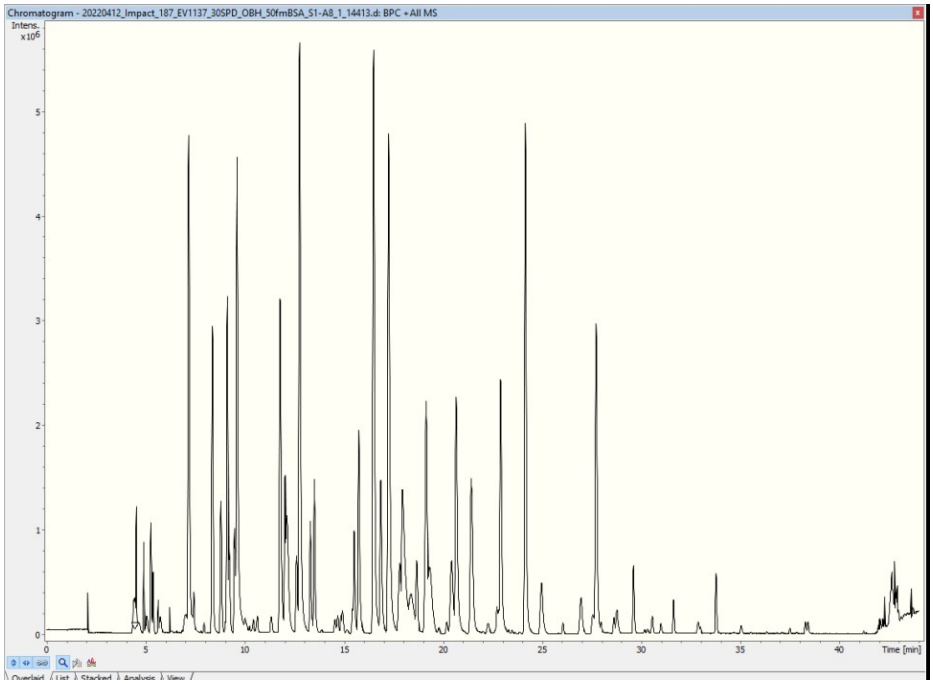
  - Pump HP flow:



- Example base peak chromatogram, EV1106, tryptic BSA digest



- Example base peak chromatogram, EV1137, tryptic BSA digest



9 Troubleshooting

9.1 Evotip troubleshooting.

If the Sample loading onto the Evotips is not done correctly, it can have a negative influence on the chromatography and hence the results.

This can happen if the chromatographic material in the Evotip dries out, either before or after loading a sample. Or if the equilibration / washing protocol is not followed completely.

This is illustrated below with some BSA runs, compared with a correctly loaded tip.

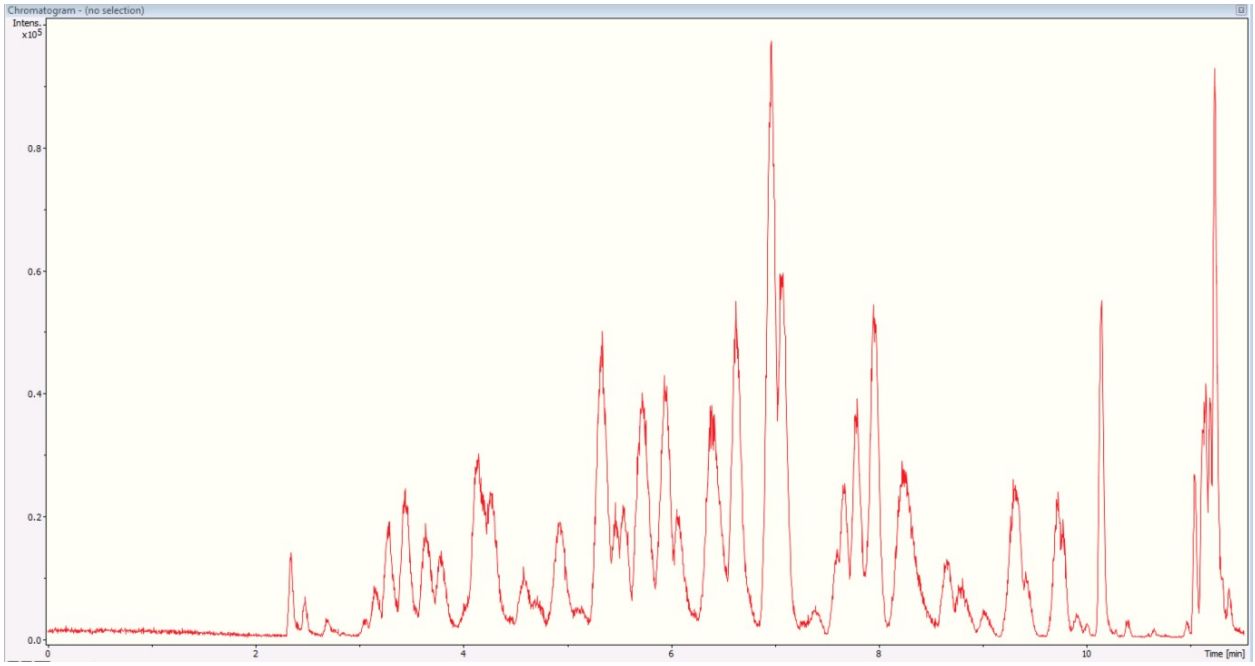


Figure 2 200 fmol BSA correctly loaded with the SOP

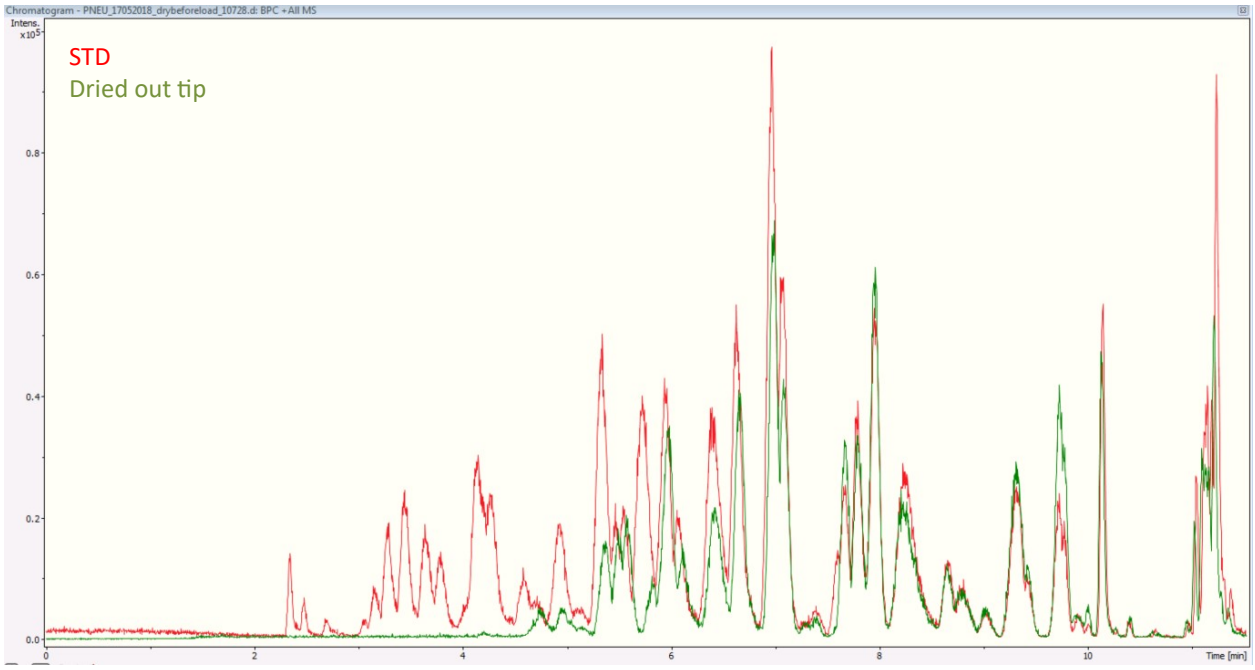


Figure 3 Tip dried out before load

200 fmol BSA loaded with the SOP vs. a tip which was dried out before loading. In the beginning of the gradient the hydrophilic peptides are missing or weaker than normal.

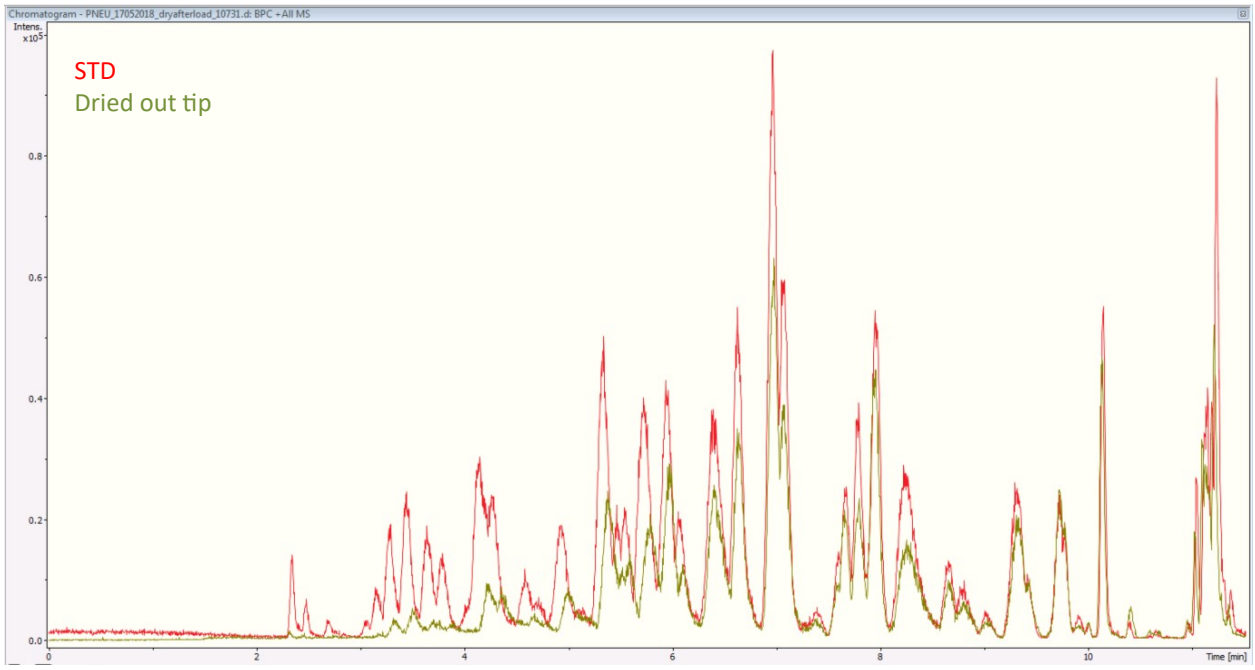


Figure 4 Tip dried out after load

200 fmol BSA loaded with the SOP vs. a tip which was dried out after loading. In the beginning of the gradient the hydrophilic peptides are missing or weaker than normal.

200 fmol BSA loaded with the SOP vs. tip which has not been equilibrated correct before loading of the sample. This error can occur if equilibration of the chromatographic material is not done correctly or sufficiently.

Typically, this can happen if activation accidentally is done with water and not 1-propanol. Weak binding of the peptides leads to poor chromatography seen as poor separation and low intensity.

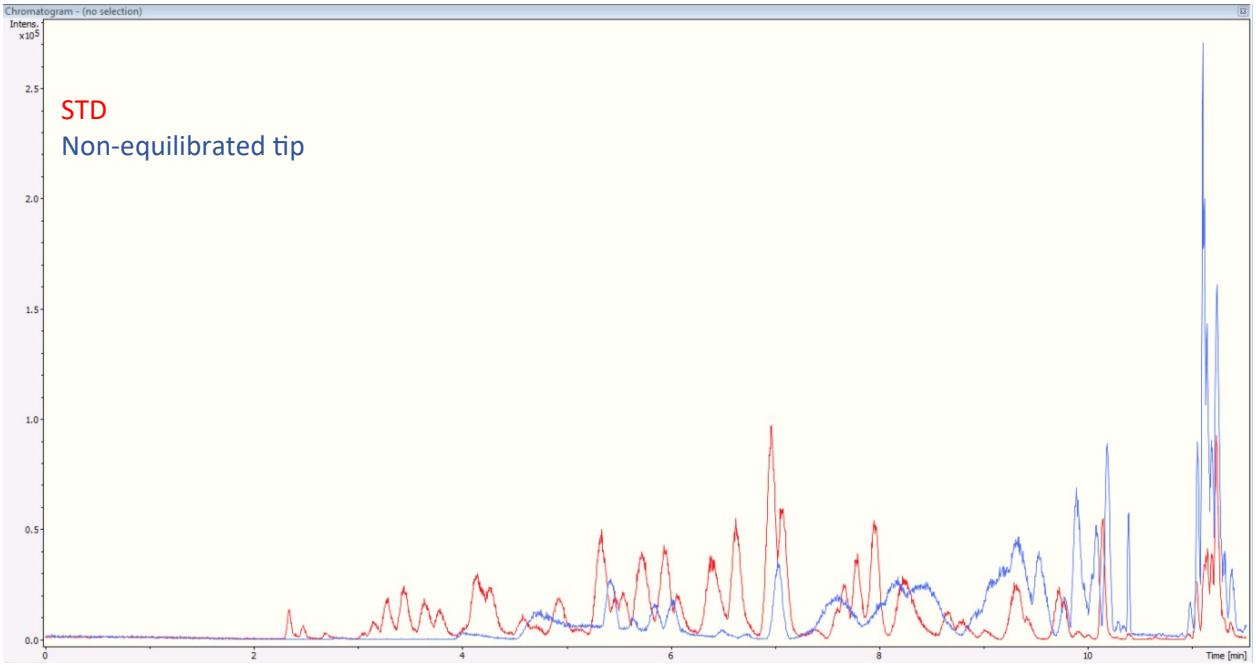


Figure 5 Tip which has not been equilibrated correctly before load of sample

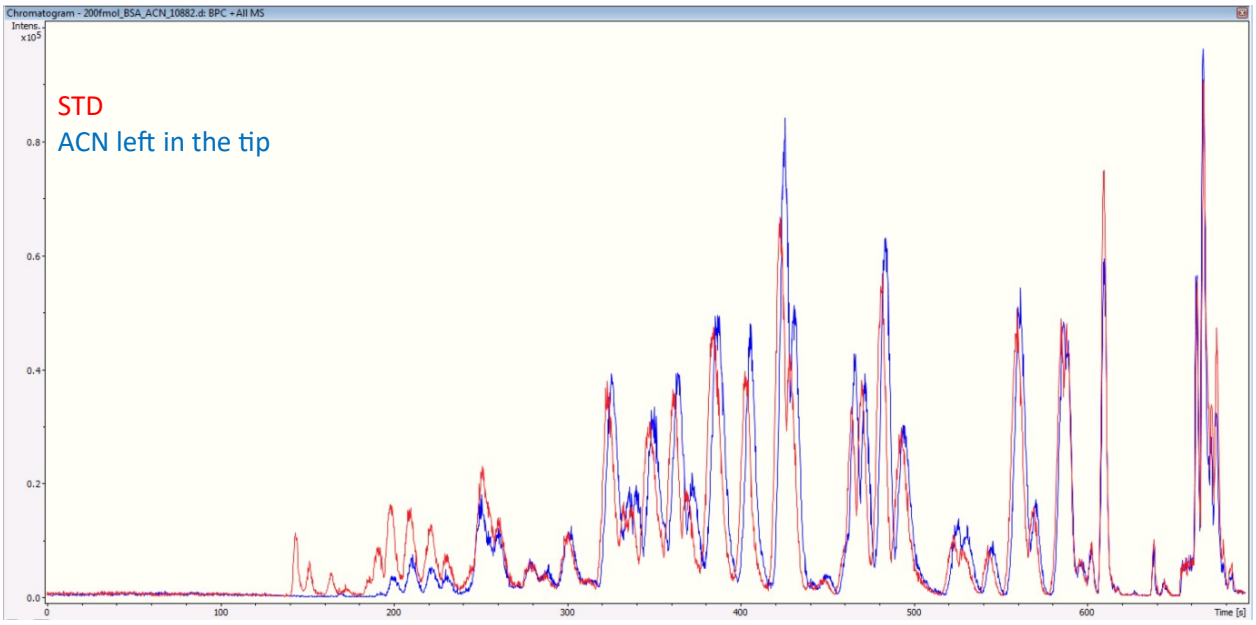
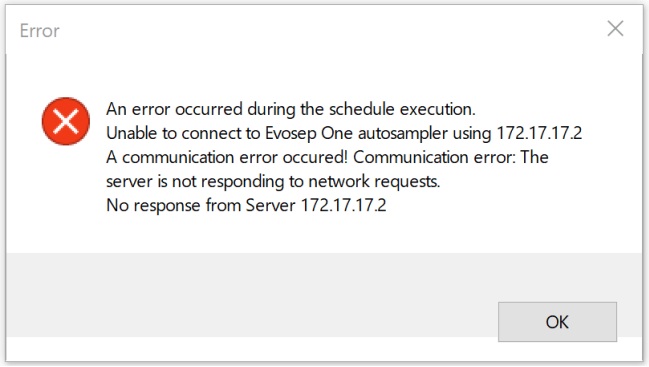


Figure 6 ACN left in the tip after the wash step

This error occurs if the ACN in the wash step is not sufficiently removed from the tip before loading the sample. This can happen if the centrifuge is not correctly adjusted for the SOP, e.g. with too low g-force or too short centrifugation time.

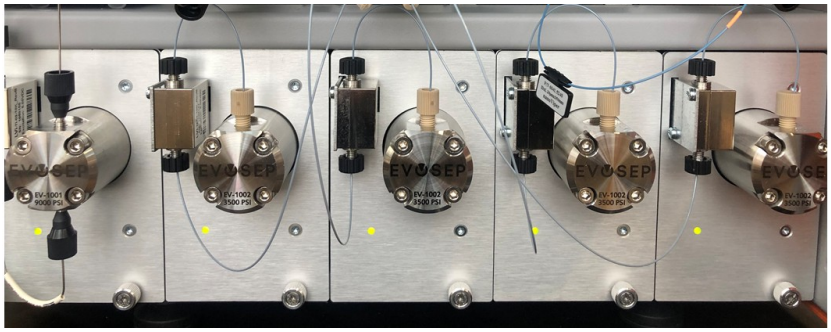
9.2 How to troubleshoot connection problems between PC and Evosep One

If you are experiencing communication problems between MS PC and the Evosep One, or getting error messages about not being able to connect to the Evosep One instrument, then please follow this guide to work through the most common issues.



9.2.1 Check that both the pump box and autosampler are powered on.

1. Pump box: Open the door on the pump box and verify that the LEDs on the five pumps are on. The LEDs could be either orange or green depending on the status of the instrument.

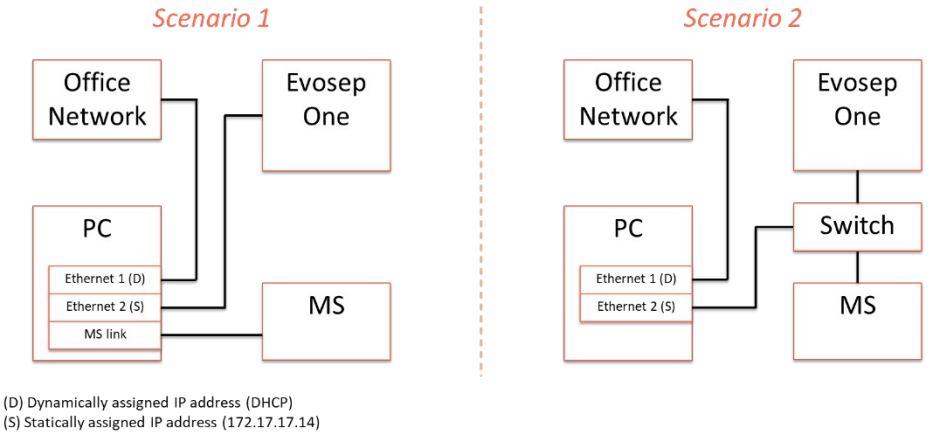


2. Autosampler: Check that the LED on the right-hand side of the X-axis is green.

If LEDs are off, please check that power cords are correctly inserted from the power outlet and up to the Evosep One, and that the power switch on the autosampler power supply is switched on.

9.2.2 Check Lan connection

In most cases the Evosep One is connected with an ethernet cable to the PC via a switch, or directly to the PC network card.



Please verify that.

1. Ethernet cables are connected correctly as in one of above examples (normally scenario 2).
2. The switch between PC and Evosep One is powered on.

9.2.3 Check network adapter set up.

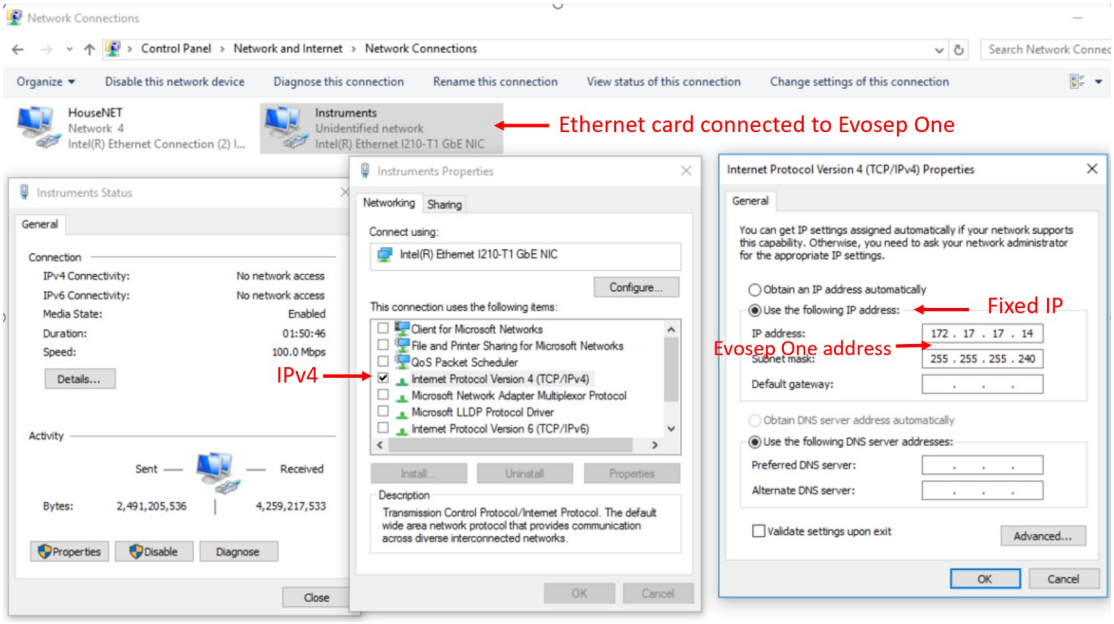
When the Evosep One plugin is installed, the ethernet card of the PC is configured to be able to communicate with the instrument. Please check following properties for the ethernet card. If more ethernet cards are installed, make sure to check the card that is connected to the Evosep One instrument.

a. The ethernet card is set up to use a fixed IP address.

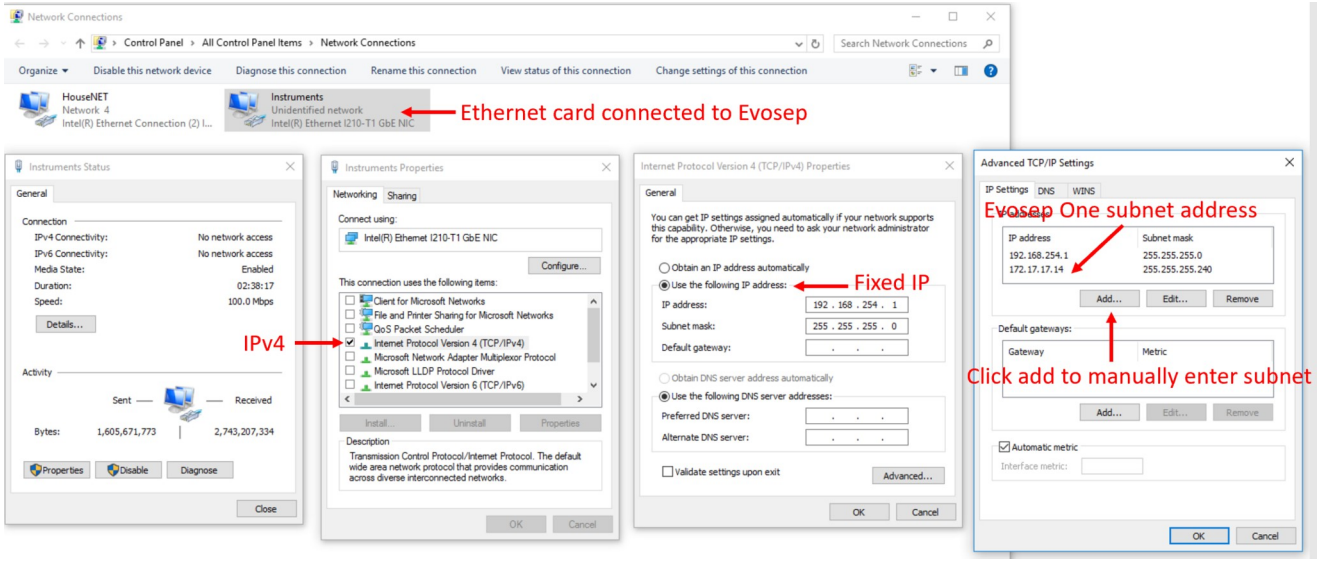
b. A subnet has been generated with IP address 172.17.17.14 and subnet mask 255.255.255.240 for the “Internet Protocol version 4(TCP/IPv4)”

1. If the ethernet adapter is not set up to use a fixed IP address, the plugin installer will not be able to add the subnet. In that case change the ethernet card to use a fixed IP address, and manually configure its settings as per below description:

   IP address 172.17.17.14 and subnet mask 255.255.255.240



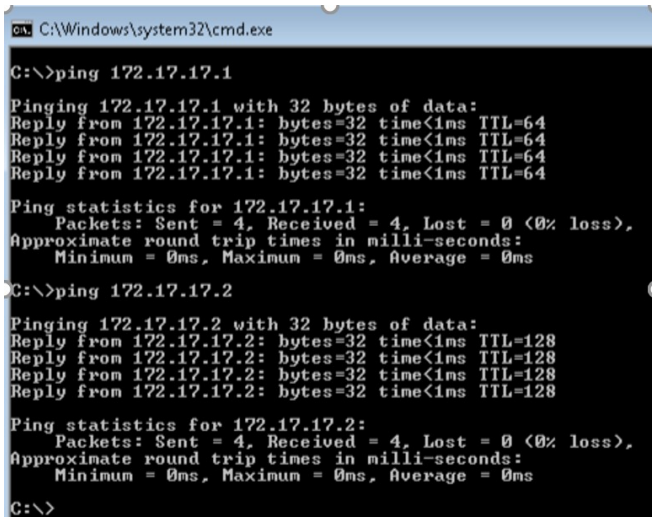
2. If the adapter is set up to use another fixed IP address, but an Evosep One subnet has not been created in the Advanced TCP/IP Settings, try to uninstall the plugin and reinstall it. Make sure the Evosep One is powered on and connected to the PC through ethernet cable. If reinstalling does not set up the subnet automatically, do it manually as per below instructions.



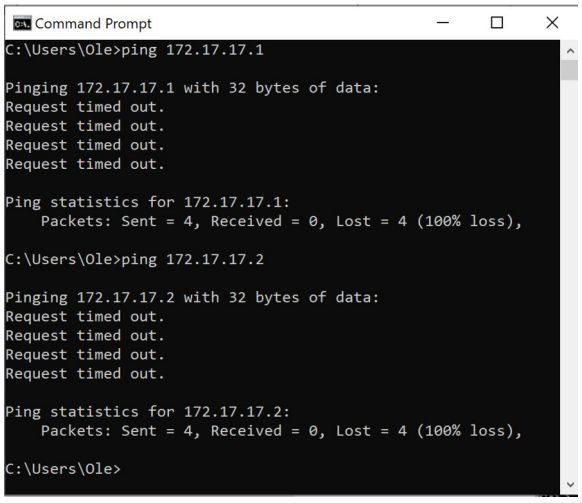
9.2.4 Ping hardware units

If there is still no connection to the instrument after all the above items have been verified, the communication to the hardware units can be tried with the “ping” command from Windows command prompt.

1. From Windows start menu, open the “command prompt” and type ping followed by the IP address of the hardware unit. E.g. “ping 172.17.17.1” (pump box) and “ping 172.17.17.2” (autosampler) as in below example:



2. If the connection can be established there will be a reply as in above example.
3. If no connection can be established the request will time out as in below example



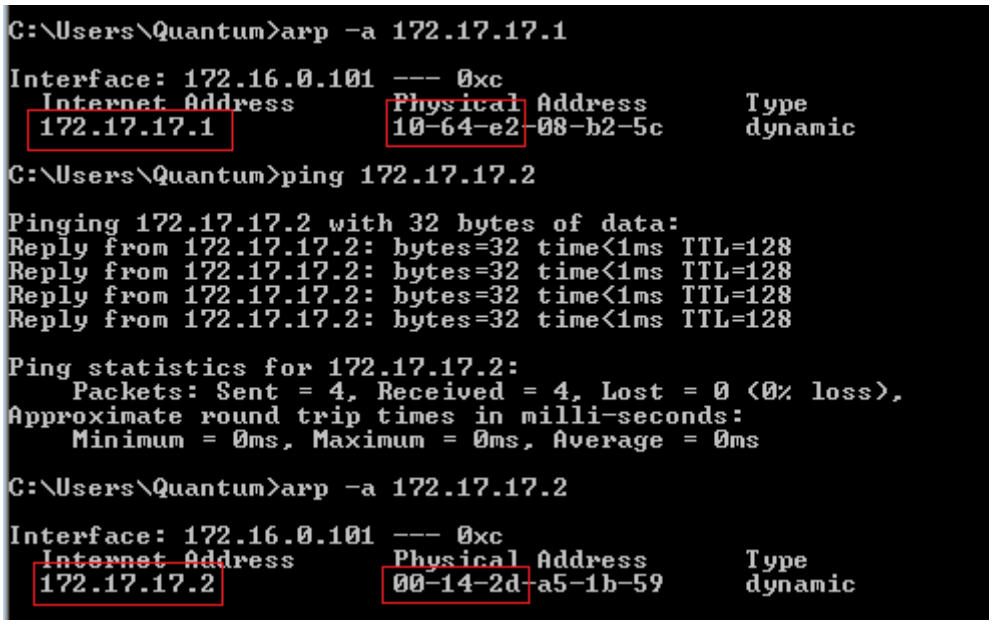
4. To verify that the Ping reply is coming from the Evosep One hardware and not another piece of hardware with the same IP address, run following commands

   arp -a 172.17.17.1

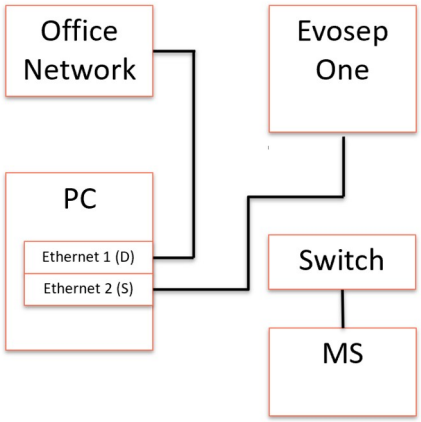
   And check that the physical address has prefix 10-64-e2

   arp -a 172.17.17.2

   And check that the physical address has prefix 00-14-2d



5. If there is no reply on either of the hardware units or the ping reply is not coming from the Evosep One, then please try and bypass the switch by connecting the Evosep One directly to the PC as in below diagram and then retry pinging the units.



6. If pinging without the switch is not giving a positive reply, then proceed with removing the ethernet cable from the autosampler and then disconnect the ethernet cable from the pump box and plug it into the ethernet connector on the autosampler (this will bypass the built-in gateway of the Evosep One) and then redo the ping to IP address 172.17.17.2 (autosampler)
7. If still no reply, please repeat step 4 with a known working ethernet cable.
8. If that is not working either, please reconnect all cables as they were and restart the PC and try to reconnect with the Evosep One software
9. If restarting the PC does not work, then power cycle the pump box and autosampler and try to connect again.
10. If still no success, then please contact your local support or support@evosep.com, preferable with TeamViewer access credentials, so we can log on to the PC and work out the problem.

9.3 Error messages

If something unforeseen happens on the instrument an error message will typically be shown as a pop-up window and or in the run log.

Below is a list of the most common error messages. To find more information, locate the number in front of the error message in the table and look for more info on the error message further down in this chapter.

Please note that IP addresses stated in the table are the default. For a non-default installation, IP addresses may differ but possible cause and action will be the same:

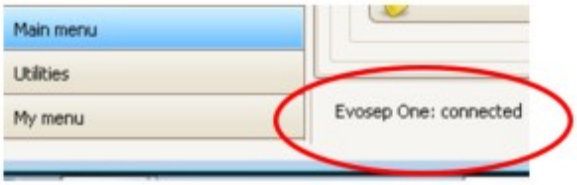
Error messages
1) Instrument at 172.17.17.1:2 gateway target device failed to respond
2) Pumpa at 172.17.17.1:7 gateway target device failed to respond, or Pumpb at 172.17.17.1:6 gateway target device failed to respond, or Pumpc at 172.17.17.1:5 gateway target device failed to respond, or Pumpd at 172.17.17.1:4 gateway target device failed to respond, or Pumphp at 172.17.17.1:3 gateway target device failed to respond, or all of the above
3) An error occurred the schedule execution A communication error occurred! Communication error: The server is not responding to network requests. No response from Server 172.17.17.2 or 172.17.17.1
4) [pumpa 172.17.17.1:7 (6/5/4/3)] An existing connection was forcibly closed by the remote host
5) Pump(hp,a,b,c,d) A connection attempt failed because the connected party did not properly respond after a period of time, or established connection failed because connected host has failed to respond (172.17.17.1:502)

6) [pump(hp,a,b,c,d)] Flowmeter not detected, or [pump(hp,a,b,c,d)] Loadcell not detected
7) An error occurred during the schedule execution. Most probable an instrument or tray/agitator/injector was not defined in the setting or was forgotten to be set in a method
8) The software does not support the device hardware/firmware - please contact Evosep support! or The device firmware must be updated to be used with this software - if this does not happen automatically next time the software is connected to the hardware, please contact Evosep support! or This software does not support the device firmware - please upgrade the Evosep One instrument software package to obtain compatibility with the connected hardware!
9) An error occurred during the schedule execution. There is already a listener on IP endpoint 127.0.0.1:64001. This could happen if there is another application already listening on this endpoint…
10) Tip expected but not present
11) Autosampler – Unable to access autosampler. Please check that it's powered on, the ethernet cable connected and that it's not locked by the handheld terminal
12) Pumphp – Pressure overload
13) Pump(a,b,c,d) – Pressure overload
14) MoveToObject(Rack 1,1,True,True,False) (or TipCheck, Inject, Wash etc.)
15) MoveTorqueMode(3,10 mm,400 mA,5 mm/s..
16) MoveValveDrive(Valve Drive 1, xxxdeg, xx rad/s)
17) Xxx samples per day (x.x min) – Pump HP does not contain sufficient solvent to perform the analysis. Please check the solvent bottle levels and run the Prepare – Pump preparation - Degas.
18) Preparation – Low pressure pump(s) did not meet preparation criteria, and or Preparation – High pressure pump did not meet preparation criteria
19) High pressure on pump A and/or B detected. Please wipe the needle tip and perform a Diagnose - restriction test.
20) No Evotip was present during the analysis and the sample was aborted. Please check if the Evotip position in the autosampler matches the sample list.
21) [Pump(hp,a,b,c,d)] fan detection fault
22) [Pump(hp,a,b,c,d)] drive not responding

23) Couldn't verify method script authencity
24) Flow sensor offset
25) Measured difference in flow between solvent A and B is x /min, please check that the solvents are correct and run Tip seal and HP system tests

9.4 Error messages regarding communication issues with Chronos and Evosep plugin

Please note that the status of the Evosep One is "not connected" until a schedule is started. When a schedule is started, the data system will connect to the instrument and the instrument status will change to "connected"



Error message:

1) **Instrument at 172.17.17.1:2 gateway target device failed to respond**

Possible cause:

Not possible to establish connection to the backplane. Reason for this could be that the backplane is defect

Action:

Look through the grills on the back when the instrument is powered on. The small LED on the left-hand side of the backplane should be blinking or steady-on for a correctly functioning backplane.

Error message:

2) **Pumpa at 172.17.17.1:7 gateway target device failed to respond**
   or
   Pumpb at 172.17.17.1:6 gateway target device failed to respond

or
Pumpc at 172.17.17.1:5 gateway target device failed to respond
or
Pumpd at 172.17.17.1:4 gateway target device failed to respond
or
Pumphp at 172.17.17.1:3 gateway target device failed to respond
or
all of above

Possible cause:

- Not possible to establish connection to one/several or all the devices connected to the backplane (pump hp and pump a-d).
- This could be caused by a device not fully inserted into the backplane connector, a faulty connector etc.

Action:

- Ensure that all pump cassettes are fully inserted and that the finger tight front screw is tightened.
- Verify that the pump LED on the front of the pump cassette is on.

Error message:

3) **An error occurred the schedule execution.**
A communication error occurred! Communication error: The server is not responding to network requests.
No response from Server 172.17.17.2 or 172.17.17.1

Possible cause:

- The error occurs when the data system cannot connect to the instrument, either because the instrument is not switched on, the ethernet cable is not connected or the data system network configuration is not set up correctly.

Action:

- See Chapter 9.2 How to troubleshoot connection problems between PC and Evosep One

Error message:

4) **[pumpa 172.17.17.1:7 (6/5/4/3)] An existing connection was forcibly closed by the remote host**

Possible cause:

- The error message "…An existing connection forcibly closed by the remote host" for any of the hardware devices is typically caused by the Evosep One being power cycled with Chronos previously connected to the system.
- The error message will appear when trying to connect to the system.

Action:

- If the Evosep One is being power cycled or moved to another data system, Chronos should always be restarted before connecting.

Error message:

5) **Pump(hp,a,b,c,d) A connection attempt failed because the connected party did not properly respond after a period of time, or established connection failed because connected host has failed to respond (172.17.17.1:502)**

Possible cause:

- Typically indicates loss of communication when the instrument is connected. E.g. if the ethernet cable is disconnected.

Action:

- Check ethernet cable and that all devices/pumps are fully inserted.
- Power cycle the Evosep One and restart Chronos and connect to the system.

Error message:

6) **[pump(hp,a,b,c,d)] Flowmeter fault**
Or
[pump(hp,a,b,c,d)] Loadcell fault

Possible cause:

- Indicates wrong/no signal from either the flow sensor (flow meter) or the pressure sensor (load cell).
- Could be caused by a broken sensor or cable.

Action:

- Power off the instrument and check that the relevant sensor cable is connected correctly.
- Power back on the instrument and reconnect to instrument.
- For flow sensor try to swap cables on the flow sensor to see if error message still is on the same sensor (see note below)

Please note:
Instrument only checks for connection to the pressure and flow sensor during power on, if there is no connection, an error message will be shown **when** connecting.
When troubleshooting make sure to power off instrument when switching cables/sensor

Error message:

7) **An error occurred during the schedule execution. Most probable an instrument or tray/agitator/injector was not defined in the setting or was forgotten to be set in a method**

Possible cause:

- Method stopped by user when the autosampler is active.
- Method stopped by system due to other error state (e.g. reaching max pressure).

Action:

- If method is stopped on purpose by the user, click ok and ignore message.
- If method is stopped unexpectedly click ok to ignore message and see error message causing the method to stop unexpectedly.

Error Message:

8) **The software does not support the device hardware/firmware - please contact Evosep support!**
or
The device firmware must be updated to be used with this software - if this does not happen automatically next time the software is connected to the hardware, please contact Evosep support!
or
This software does not support the device firmware - please upgrade the Evosep One instrument software package to obtain compatibility with the connected hardware!

Possible Cause

- Mismatch between firmware version of Evosep One and the Evosep One software plugin installed on the PC. Please note that this only refers to the Evosep One software and not Chronos.

Action

- Update the Evosep One software on the PC to the latest version and connect to the Evosep One from that PC.

  Before updating the SW please read the release note for more information on new features, changes etc. in the software

Error Message:

9) **An error occurred during the schedule execution. There is already a listener on IP endpoint 127.0.0.1:64001. This could happen if there is another application already listening on this endpoint or if……..**

Possible cause:

- This IP address is used for configuring the connection to the autosampler while connecting to the instrument. The error message will be shown if the address is not available. This can occur if Chronos and or the Evosep plugin has not been closed correctly.

Action:

- Close Chronos and Chronos processes (using the Windows Task Manager) and try to connect again. If this does not work, please restart the computer.

Error Message:

10) **Autosampler – Unable to access autosampler. Please check that it's powered on, the ethernet cable connected and that it's not locked by the handheld terminal**

Possible cause:

- During maintenance autosampler has been controlled with the terminal and the action has not been completed.

Action

- With Terminal check and finish pending action e.g. change tool
- Check that autosampler is powered on and all cables connected

9.5 Error messages regarding hardware

Consult below list of error messages for hardware issues during method acquisition. Error messages will typically be shown as popup windows and in the run log window.

Error Message:

11) Tip expected but not present

Possible cause:

- No tip detected on needle. Typically caused by executing a sample run without having an Evotip in the correct position.

Action:

- Verify that a tray has been placed in correct position and that an Evotip is present.
- For sample acquisition any position can be chosen in the sample list. For Diagnostic runs EvoSlot 1, pos 1 is hard coded.
- Check that the needle is not broken.

Error Message:

12) Pumphp – Pressure overload

Possible cause:

- Pump HP (high-pressure pump) has reached maximum pressure during a method run, typically caused by a blocked emitter or column, or using a column not suitable for the chosen method

Action:

- Verify with Table 1: Evosep One Methods in chapter 7 that the column being used is compatible with the chosen method.
- Run a blank tip with the same method with and without the emitter connected - a well-functioning emitter will only give a few bars added backpressure to the setup.
- Replace column with a new one and verify that backpressure drops to an acceptable level
- Run the Diagnose-Restriction test to verify that the instrument back pressure without column connect is ok

Error Message:

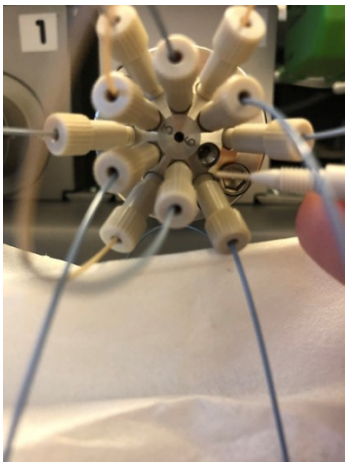
13) Pump(a,b,c,d) – Pressure overload

Possible cause:

- One or more of the low-pressure pumps has reached maximum pressure during method run. Typically caused by a blocked restrictor tubing or a blocked needle.

Action:

- Run the Diagnostic – Restriction test:
- If the backpressure is too high on both pump A and B replace the needle and re-run the test.
- If only one of the 4 low pressure pumps pressure is too high, disconnect the restrictor tubing for the failing pump from the 12-port valve (restrictor tubing's are labelled with a red marker). To identify the correct tubing follow the tubing from the flow sensor down to valve 12, See below photos (flow sensors are positioned from left to right A,B,C,D) With the tubing disconnected from the 12 port valve run the Prepare – Pump preparation - Degas script to flush out the valve port and then reconnect the restrictor tubing and re-do the restriction test. If this does not solve the problem a new restrictor tubing should be installed.



Error Message:

14) MoveToObject(Rack 1,1,True,True,False) (or TipCheck, Inject, Wash etc.)

Possible cause:

- Movement for robot X, Y or Z axis could not be completed because of a collision or error. Example given is movement to rack but it could also be Tipcheck, Inject, Wash etc.

Action:

- Verify that nothing is physically stopping the movement of the robot arm. It could be another instrument or a wall that the instrument is standing too close to. Be aware that the Y-axis needs some room to move, also on the backside of the instrument.
- Make sure that the A,B transfer line going to the needle tee has free movement etc.

Error Message:

15) MoveTorqueMode(3,10 mm,400 mA,5 mm/s..

Possible cause:

- Movement for robot Z axis (up down) into Inject port or other position, could not be completed

Action:

- Verify that nothing is physically stopping Z-axis movement (up and down), it could be the AB transfer tubing or a tip in a wrong position
- Verify that the needle is securely tightened to the needle Tee

Error Message:

16) MoveValveDrive(Valve Drive 1, xxxdeg, xx rad/s)

Possible cause:

- Valve could not be switched into position. This can happen if there is too much friction to move valve or if valve drive is disconnected

Action:

- Verify in error message what valve drive has a problem (Valve Drive 1 = Valve 6, Valve Drive 2 = Valve 12, Valve Drive 3 = Valve loop
- Verify that p-bus cable from robot X-axis to valve drive and between valves drives are fully connected into connector on x-axis and valve drives
- Power cycle instrument and retry

Error Message:

17) **Xxx samples per day (x.x min) – Pump HP does not contain sufficient solvent to perform the analysis. Please check the solvent bottle levels and run the Prepare – Pump preparation - Degas program.**

Possible cause:

- If pump HP uses too much solvent (20ul) to build up pressure during the column equilibration the analysis is stopped. This is done to avoid the risk of the pump emptying completely during the gradient.

Action:

- Check solvent levels in solvent bottles, run Preparation degas. If the problem persists run the leak test for pump HP found under Diagnose

Error Message:

18) **Preparation – Low pressure pump(s) did not meet preparation criteria**
And or
Preparation – High pressure pump did not meet preparation criteria

Possible cause:

- During Degas and Solvent exchange, solvent volume to build up pressure (LP 50bar, HP 200bar) is measured to verify that pump can build up pressure. If volumes needed to build pressure is too high the instrument will stop with above error message. The most likely cause is that the solvent bottles are empty or the tubing in the bottles are not submerged.

Action:

- Please perform following steps:
- Check solvent levels in bottle A and B and that solvent lines are submerged
- Perform a visual inspection for obvious damage, kinks or leaks on the flow lines between the pump(s) listed in the error message and Valve 6 and 12

Error Message:

19) **High pressure on pump A and/or B detected. Please wipe the needle tip and perform a Diagnose - restriction test.**

Possible cause:

- If Pump A/B sample loading pressure is above 50 bar this error messaged will be triggered.

Action

- Please perform a Diagnose – restriction test to verify that instrument flow paths are not blocked

Error Message:

20) No Evotip was present during the analysis and the sample was aborted. Please check if the Evotip position in the autosampler matches the sample list.

Possible cause

- If pump A/B sample loading pressure is above 50 bar and a tip is not present on the needle this error message will be triggered
- Can happen if a Tip is not present on the needle when going into the injection port or if no tip is detected on the needle after injection when the needle is moved to tip eject.

Action

- Please check if the Evotip position in the autosampler matches the sample list. E.g. check that the same Evotip position was chosen twice or that a wrong Evotray slot has been chosen
- Check that no tip is sitting in the Tip inject port
- Check needle for damage
- Re-run with blank Evotip and visually inspect the Evotip pick, inject, eject etc.

Error Message:

21) [pump(hp,a,b,c,d)] fan detection fault

Possible cause

- Pump fan faulty or not connected

Action

- Power cycle instrument and verify that error is still present and reported for the same pump
- Contact support@evosep.com , and inform about error message.

Error Message:

22) [pump(hp,a,b,c,d)] drive not responding

Possible cause

- Faulty pump PCB (Printed Circuit Board)

Action

- Power cycle instrument and verify that error is still present and reported for the same pump
- Contact support@evosep.com , and inform about error message.

Error Message:

23) **Couldn't verify method script authencity**

Possible cause

- The method script has been edited and is no longer in its original form.

Action

- Reinstalling the Evosep plugin will overwrite the changes and return the instrument methods to their original state.

Error message:

24) **Flow sensor offset**

Possible cause:

- Within all sample runs the system detects the A/B/C/D flow sensor values when there is no flow on the system. If these flow rates deviate more than +- 100 nl/min from 0 nl/min, the system shows a warning, since it will affect the sample run retention times.

Action:

- Check that the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile)
- Check that the system is level
- If both above is correct, then perform a prepare/solvent exchange and run a new sample
- If the warning continues to appear, please perform a Calibrate/Flow sensor ABCD

Error message:

25) **Measured difference in flow between solvent A and B is x /min, please check that the solvents are correct and run Tip seal and HP system tests**

Possible cause:

- To find the loop volume, the system relies on being able to distinguish if solvent A or solvent B is present in the transfer line. It does so by measuring the actual flow rate at 40 bars. When solvent A is present a lower flow rate will be measured compared to when solvent B is present in the transfer line, due to the difference in viscosity of the 2 solvents. If this difference is less than 100 nl/min, the system cannot distinguish the solvent change, and hence not calculate the exact loop volume
- This can happen if the solvents of the system are wrong or if a leak is present

Action:

- Check that the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile)
- If both above is correct, then perform a prepare/solvent exchange
- Run a Diagnose/Tip seal and Diagnose/HP system test to check for leaks

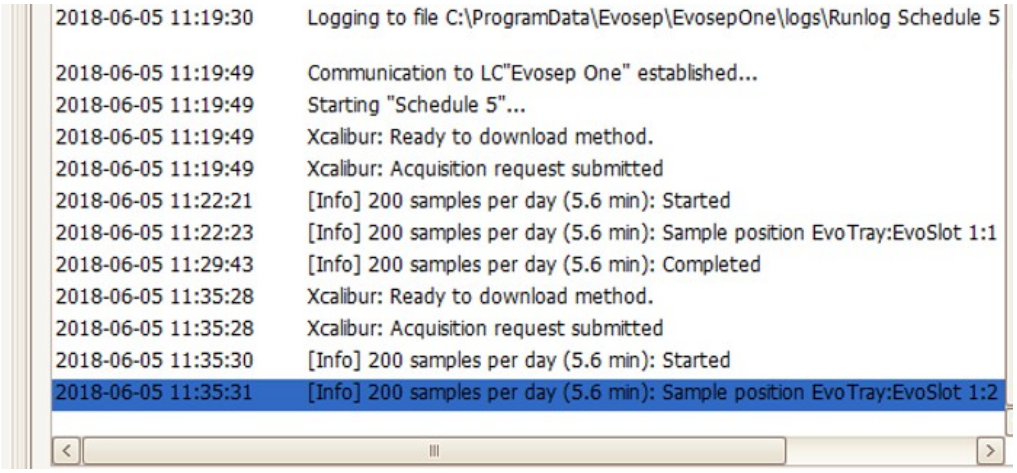
9.6 Schedule / Sample not starting / Contact closure problems

9.6.1 Troubleshooting tips for Xcalibur set-up.

How it works:

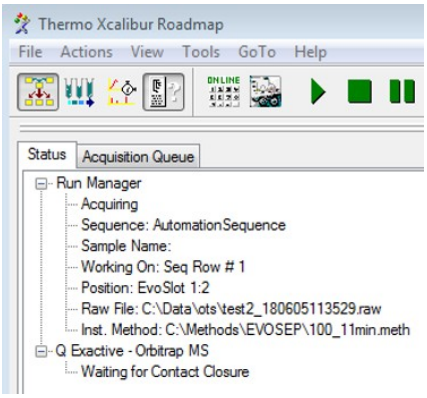
The Evosep One is set up to wait for the MS to be ready before starting a sample run, this feature ensures that the Evosep One does not run any samples if the MS is not ready to acquire data.

The run log for a typical schedule will look like this:



When Chronos has verified that Xcalibur is in the “Ready to download method” state, the acquisition request is submitted, and the sample started.

When the acquisition request is submitted the MS will change status from “ready to download” to “Waiting for contact closure” and when the contact closure signal is sent from the Evosep One at the start of the gradient the status will change to “Running”.



Typical issue 1:

1. After a schedule is started it takes several minutes before the first sample acquisition is started.
    a. Info: One or more pumps not referenced, referencing now
    b. Info: One or more pumps low on solvent, refilling now

Possible cause:

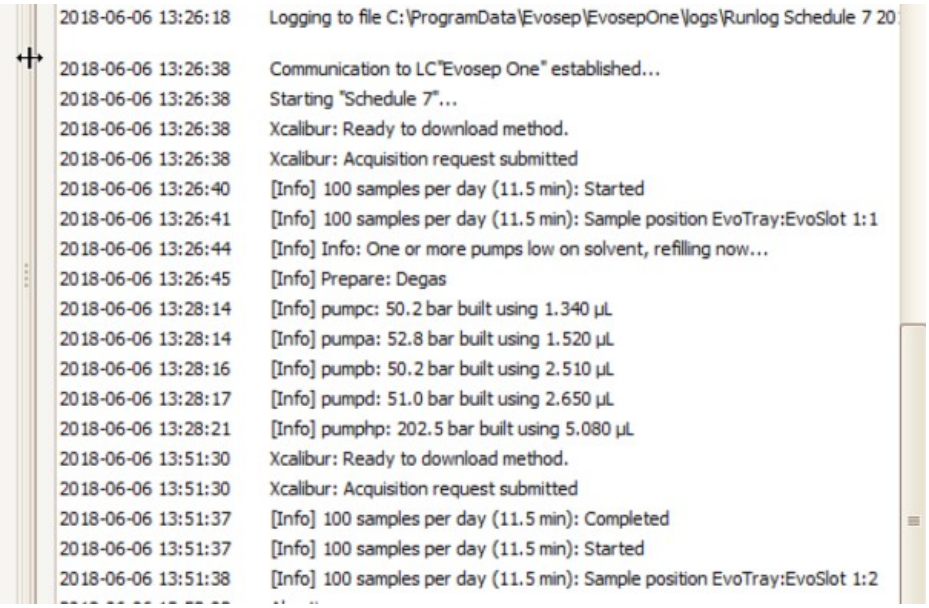
- If the instrument has been switched off or the low-pressure pumps has been stopped with not enough solvent to complete a sample run the instrument will automatically initiate a reference or refill followed by Prepare – Pump preparation - Degas and Align solvent before starting the first sample in the schedule. This is shown in the Run log with following message:

    Info: One or more pumps not referenced, referencing now

    Or

    Info: One or more pumps low on solvent, refilling now

When the “degas” and “align solvents” activities are competed, the instrument will start the sample.



Action:

- None, after successful degas and align solvents the instrument will start the sample

Typical issue 2:

- Evosep One does not continue the schedule after first sample, MS does not change status from Waiting for contact closure to Running.

Possible cause:

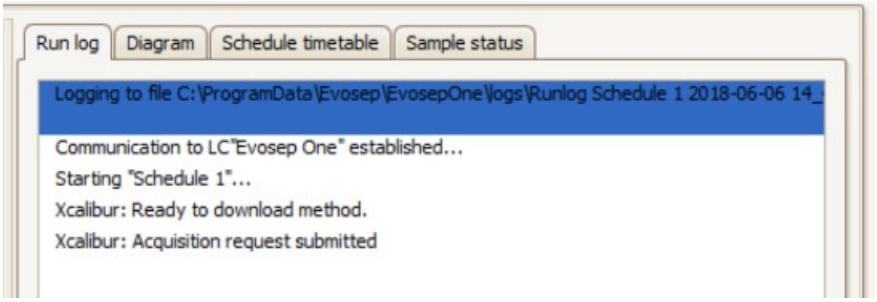
- Contact closure cable not connected, faulty or not configured correctly
- When a schedule is started the instrument will check if MS is ready and then start the first sample, however if the contact closure cable is not connected the MS will not be started and will be staying in waiting for contact closure mode, and therefore not ready to receive the next sample from the Evosep One.
- Other LC device present in MS system configuration

Action:

- Verify that contact closure cable is connected, configured correctly and not faulty.
- Please see Connecting the contact closure chapter in this manual and info regarding setting up contact closure in the MS manual
- Other LC/autosampler device present in MS Instrument Configuration, see chapter ”How to remove other LC devices from MS system configuration”

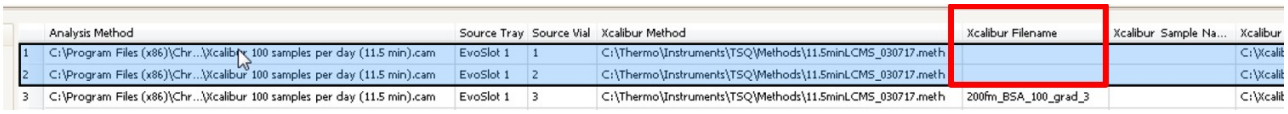
Typical issue 3:

- Schedule does not start even though the Run log shows Acquisition request submitted



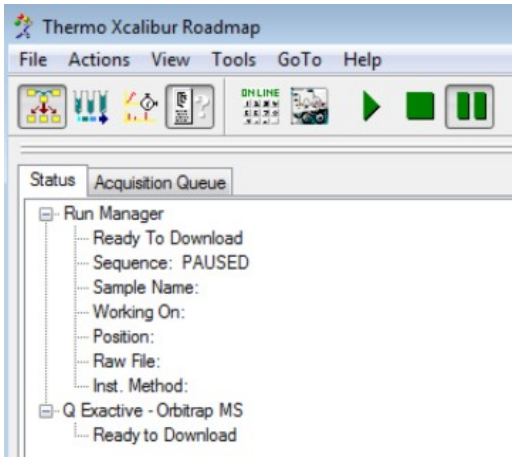
Possible cause:

- Most likely the MS is not ready.
- Other LC device present in MS system configuration
- Chronos Sample list not complete e.g. Xcalibur Filename has not been filled in.



Action:

- Verify in status view for MS that Sequence is not set to PAUSED and that MS status is “Ready to Download”
- Other LC/autosampler device present in MS Instrument Configuration, see chapter ”How to remove other LC devices from MS system configuration”
- Check that Chronos Sample list is filled in correctly



Typical issue 4:

- Evosep One is still in the middle of a sample but the MS has stopped the acquisition
- .. or the MS is still acquiring sample 1 while Evosep One has finalized sample 1 and does not proceed to sample to the next sample in the sample list

Possible cause:

- Most likely the MS method is not set to the same length as the Evosep method

Action:

- Verify that the correct MS method length is the same as the Evosep method

9.7 Hardware troubleshooting

The Evosep One software is preconfigured with several diagnostic programs, that enable the user to do basic system leakage and restriction tests. The programs run automated procedures to pinpoint failing hardware parts and in turn provide information on how to fix the most common errors. The section below describes the diagnostic programs and provide some additional background information on the error causes and repairs.

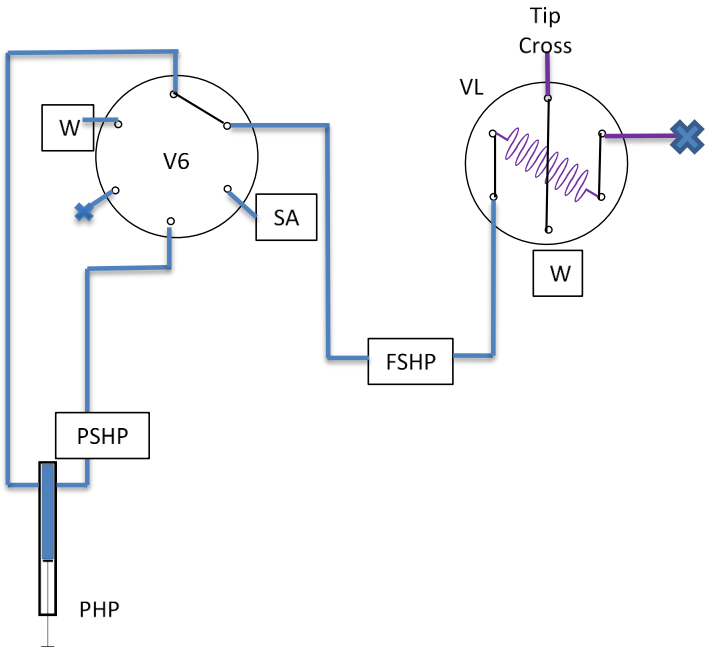
9.7.1 Leak in the HP system

If there are indications of a leak in the HP system, run the script diagnostics/HP system. The script will test the systems leak tightness at 500 bar and provide feedback on the position of any diagnosed leaks.

When asked to blind the transferline please use the stainless steel cap P/N EV1062

For all leaks indicating a leak in the valve, inspect the rotor seal and stator for scratches. If the rotor seal or valve stator is scratched exchange the scratched part.

	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☐	☐	☐	☐	☑



The flow is too high, > 0.10 µl/min, and is observed on the HP flow sensor (FSHP) when running the HP leak script.

The high flow is observed both when the valve loop is in fill loop and in elute position.

- This indicates a leak in either the valve or in a tubing connecting to or from the valve.
- Tighten the connecting tubings and re-run the script.
- If the script fails, blind the line FSHP to valve loop with the cap and re-run the script.
- If the script passed, it indicates that the leak is in the valve. Exchange the rotor seal and re-run the script.
- If the script fails, call for assistance.

The flow is too high, 0.10 µl/min, and is observed on the HP flow sensor (FSHP) when the valve is in the elute position.

No flow observed when the valve is in the fill loop position.

- This indicates a leaking loop.
- Tighten the loop and re-run the script.
- If the script fails again, exchange the rotor seal and re-run the script.
- If the script fails again, call for assistance.

The flow is too high and is observed on the pump speed, >0.50 µl/min, and no flow is observed on the flow sensor. A leak measured as pump speed is a sum of a leak before and after the FSHP. If both are failing fix the leak after the flow sensor first.

- This indicates a leak before the FSHP. It could be in the pump, in the valve or in the connecting lines.
- Check all lines and re-run the script.
- If the script fails, please run the Pump HP script.

	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☑	☐	☐	☐	☐

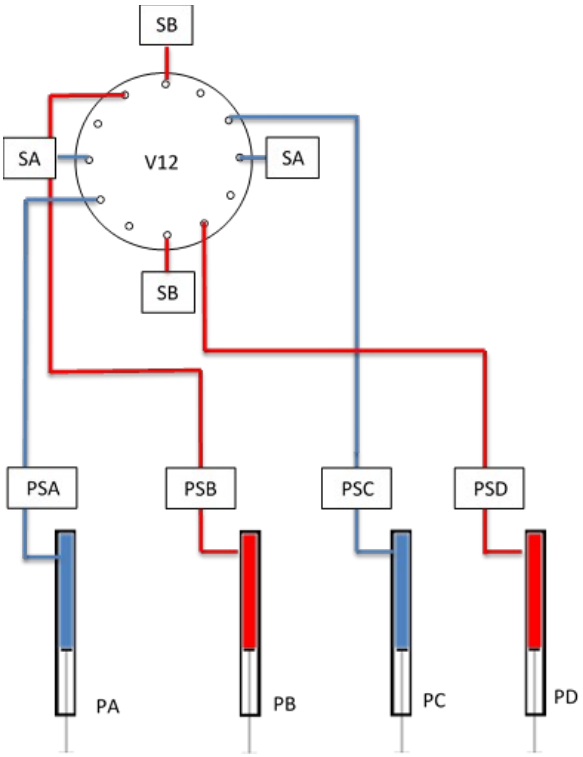
- If the script fails, exchange the rotor seal, and re-run the script.
- If the script fails, call for assistance.

9.7.2 Leak in the LP system pump to V12 area

If there are indications of a leak in the LP system, run the leak script Pump A-D.

For all leaks indicating a leak in the valve, inspect the rotor seal and stator for scratches. If the rotor seal or valve stator is scratched, exchange the scratched part.

	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☐	☑	☐	☐	☐



A leak is observed as a flow that is too high on pump speed for PSA, PSB, PSC or PSD, pump speed >1.0 µl/min.

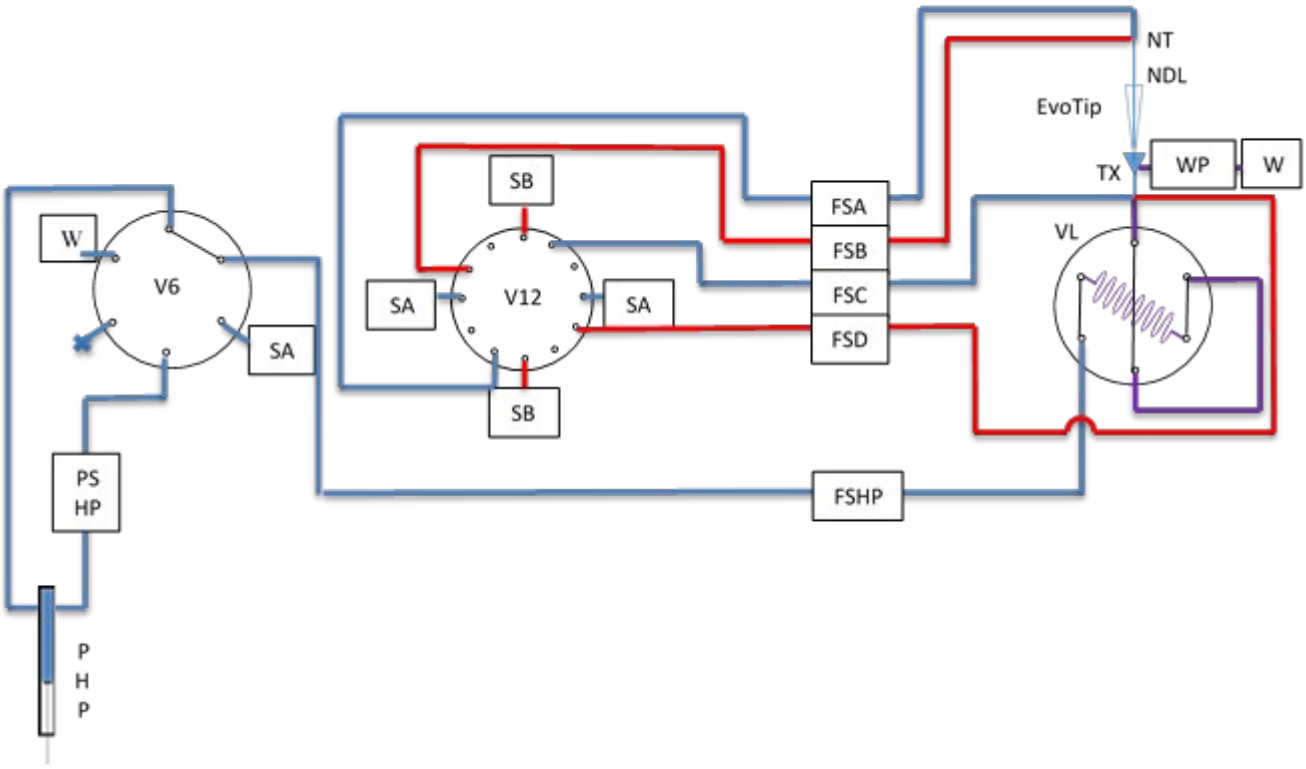
- Tighten the connections in the subsystem having a leak and re-run the script.
- If the script fails, block the line going from the pump pressure sensor to the V12 at the V12 end with the 1/32” cap, and re-run the script.
- If the script passes, exchange the rotor seal and re-run the script.
- If the script fails, call for assistance.

9.7.3 Leak in the LP system, Tip seal area

If there are indications of a leak in the Tip seal area, run the leak script Tip seal.

For all leaks indicating a leak in the valve, inspect the rotor seal and stator for scratches. If the rotor seal or valve stator is scratched exchange the scratched part.

	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☐	☐	☐	☑	☐



A leak is observed with a flow that is too high on FSHP, flow > 0.10 µl/min and negative flow on FSA, FSB, FSC or FSD.

- Tighten the line connecting the FS to the V12 and re-run the script.
- If the script fails, place the endcap on the line connecting to the V12 and re-run the script.
- If the script passes, the leak is most likely in the V12.
- Exchange the rotor and re-run the script.
- If it fails with negative flow on the flow sensor, call for assistance.

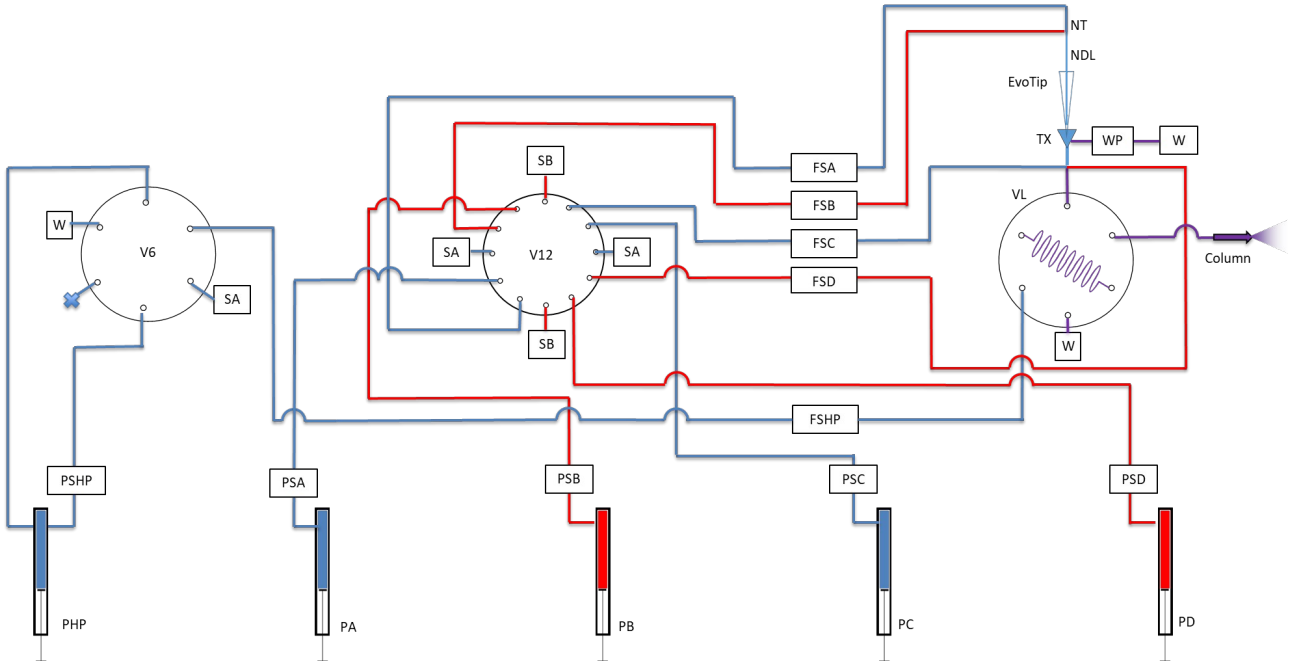
A leak is observed with a flow that is too high on FSHP, flow > 0.10 µl/min, and the script for the HP system pass.

- Check all connections in the Tip seal region, the lines going to the FSA, FSB, FSC and FSD, the line going to VL, the needle connection and re-run the script.
- If it fails, call for assistance.

9.7.4 High restriction in the system

If there are indications of high restriction in the system, run the script Restriction.

	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☐	☐	☑	☐	☐



The restriction test is divided in 3 parts:

1. Low pressure system
    a. A pressure above 70/50/70/50 bar for pump A/B/C/D respectively is a sign of a partially blocked subsystem
    b. If both pump A and pump B are restricted
        i. This indicates a blocked needle.
            1. Please try to rinse the tip of the ceramic needle with Kimwipe or similar, soaked in methanol.
            2. Exchange the needle.
        ii. Re-run the script and if the restriction still is too high, call for assistance.
    c. If either pump A/B/C/D are restricted
        i. This indicates a blocked line from V12 to the respective flow sensor.

1. Disconnect the tubing on valve V12, flush the valve port with ethanol, to remove residual material and reconnect the tubing.
2. Exchange the tubing.

ii. Re-run the script. If the restriction is still too high, call for assistance.

2. High pressure system
    a. A pump HP pressure above 150 bar indicates a partially blocked high-pressure subsystem
        i. Remove the transfer line and re-run the script.
        ii. If the pressure drops to less than 70 bar, the transfer line is blocked or partly blocked. Install a new transfer line and re-run the script to confirm the blocked transfer line.
        iii. If pressure is still >70 bar, remove the line FSHP to VL and re-run the script.
        iv. If the pressure drops to less than 40 bar, the line FSHP to VL is most likely blocked or partly blocked. Install a new line and re-run the script to confirm the blocked line.
        v. If the pressure is still >40 bar, replace the line going from the V6 to FSHP and re-run the script.
        vi. If the pressure remains >40 bar call for assistance.

3. Tip interface and loop
    a. A pressure above 50 bar for either pump A/B/C/D indicates a partial restriction of the tip interface or the loop
        i. Disconnect the loop on the loop valve, flush the valve ports with ethanol to remove residual material and reconnect the loop.
        ii. Exchange the loop. Re-run the script. If the restriction is still too high, call for assistance.

10 Routine Maintenance

To maintain the Evosep One instrument, please follow the procedures described in this chapter.

Most of the instrument components can be accessed by removing the left- and right-side panel, opening the front door, removing the tray plate, and setting the autosampler into exchange position with the terminal.

10.1 Recommended maintenance schedule

10.1.1 Daily Maintenance

- Visually inspect solvent level in bottle A and B and refill if necessary.
- Visually inspect solvent level in waste bottle and empty if necessary.
- Visually inspect tip disposal container and empty if necessary.

10.1.2 Weekly Maintenance

- Empty, rinse and refill solvent bottle A and B
- Empty waste bottle
- Remove empty/not in use Evotip boxes from tray

10.1.3 Prepare instrument for storage

If the instrument is not going to be used for 1-2 weeks, please perform following tasks. To prevent bacterial growth, it is recommended to switch to organic solvents.

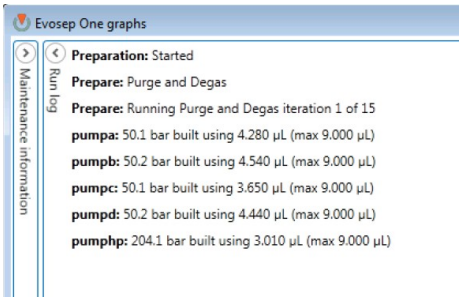
To minimize risk of instrument contamination always wear gloves when handling the tubing going to the solvent bottles

- Place both A and B line in solvent B bottle as illustrated above
- Run the Prepare – Pump preparation -Solvent Exchange
- Park autosampler in lock position
- Switch off instrument

When starting up after storage

As Acetonitrile is a much better solvent for degassing the pumps, start out with a few “Solvent exchange” cycles with acetonitrile on both channel A and B, to ensure there is no air trapped inside of the pumps

- Empty, rinse and refill solvent bottle A and B
- Place both A and B line in Solvent B bottle as illustrated above
- Empty waste bottle
- Run 3-4 cycles of the Solvent exchange script and ensure that values in the run log for
    - **Pump HP is less than 6 ul**
    - **Pump A-D is less than 7 ul**



- **Move the A tubing back into bottle A.**

- 

- Check that the solvent lines are inserted in the right bottles.
- Run the Prepare – Pump preparation – Solvent Exchange all 15 cycles.

11 Replacing spare and wear parts

11.1 Recommended wear part list

Evosep recommends to have following wear parts available to quickly fix minor issues with the instrument:

- EV1008 – valve 12 rotor
- EV1011 – valve 6 rotor
- EV1014 – valve loop rotor
- EV1103 – valve 12 to flow sensor LP (EV1023 for systems with <S00088)
- EV1018 – needle
- EV1058 – HP piston seal
- EV1060 – LP piston seal
- EV1034 – Transferline

Please find pictures of all parts in our web shop

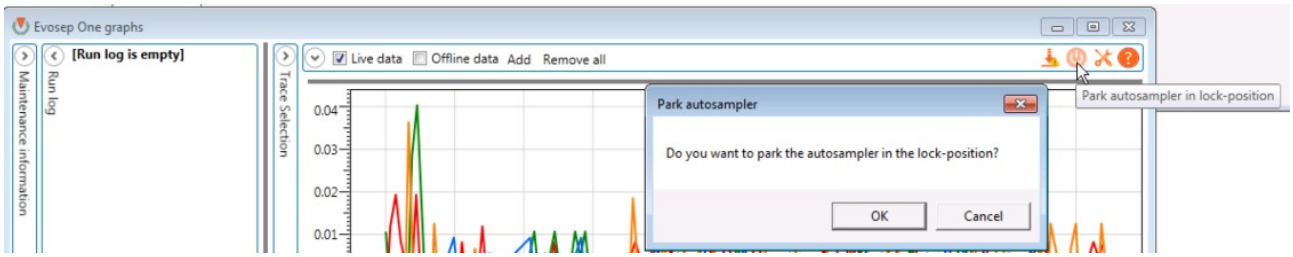
Visit the Evosep webshop: Spare parts and consumables for Evosep One

Please follow below instructions on replacing spare and wear parts on the system

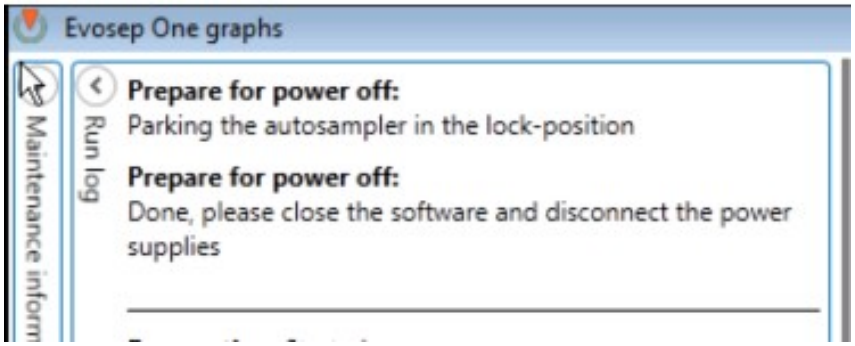
11.2 Power off the instrument

1. Stop any running procedures including idle flow.
2. Go to the Graph viewer window.

3. Click the orange off-icon, then click OK in the pop-up window to park the autosampler in the lock-position.



4. Autosampler Z-axis will now be parked in lock position and when completed, a message will be shown in the run log. Thereafter the software can be closed, and the power supplies disconnected.



If some reason it is not necessary to switch off the instrument after moving the Autosampler Z-axis to the lock position a new procedure can be started.

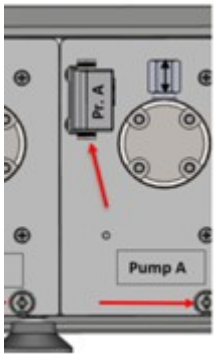
11.3 Replacing the HP Pump cassette

1. Remove the left side panel
2. Power off the instrument
3. Disconnect Viper tubing from top port on HP pressure sensor
4. Disconnect Viper tubing from top port on HP pump

5. From the left side, push out the cable binder holding the waste tubing
6. Loosen the knurled nut in the lower right corner of the pump cassette
7. Gently pull out the pump cassette from the instrument by pulling the knurled nut, be careful not to kink or break the surrounding tubing
8. Insert pump cassette in reverse order
9. Run Preparation – Pump preparation – Solvent exchange to ensure the new pump is fully purged and degassed
10. Run the Diagnose Pump HP to ensure that no leaks are present after the replacement of the pump cassette

11.4 Replacing the LP Pump cassette

1. Power off the instrument
2. Disconnect the peek tubing from the bottom port on the LP pressure sensor
3. Loosen the knurled nut in the lower right corner of the pump cassette



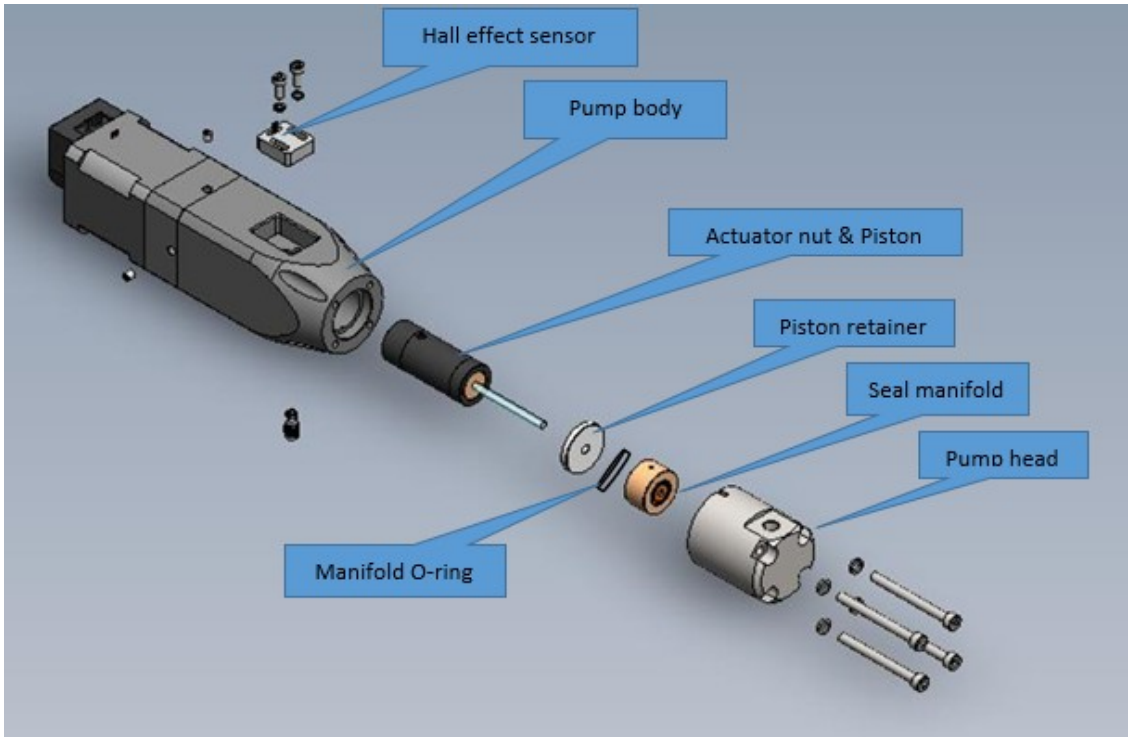
4. Gently pull out the pump cassette from the instrument by pulling the knurled nut, be careful not to kink or break the surrounding tubing
5. Insert pump cassette in opposite order

6. Run Preparation Solvent exchange to ensure that the new pump is fully purged and degassed
7. Run the Diagnose Pump A-D to ensure that no leaks are present after the replacement of the pump cassette

11.5 Replacing HP/LP pressure sensor

1. Remove pump cassette from instrument as described in the subsection “replacing the HP/LP pump cassette”
2. Disconnect the tubing from the top port on the LP pressure sensor (for the HP pressure sensor this is already done when removing the cassette)
3. Using a T10, remove the 2 screws that holds the pressure sensor
4. Gently slide the pressure sensor away from the cassette and disconnect the pressure sensor cable
5. Install pressure sensor in opposite order making sure the pressure sensor cable connects securely into the pressure sensor

11.6 Replacing pump piston seals including seal manifold



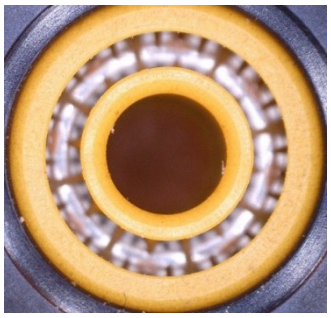
1. Run service script to fill pumps, which will fully retract piston within the pump house
2. Disconnect tubing from pump head

3. Use a 3mm hex key to loosen and remove the 4 pump head screws, then loosen the screws diagonally to evenly loosen the pump head
4. Carefully and in a straight line from the pump block, slide the pump head away from the pump

> ***WARNING:*** *Sapphire pistons are very shock sensitive. Use extreme caution, and do NOT shock or side-load the piston in any way!*

5. Remove the seal manifold with its two piston seals and O-ring, by sliding it off the piston.

Seal Manifold wetting procedure



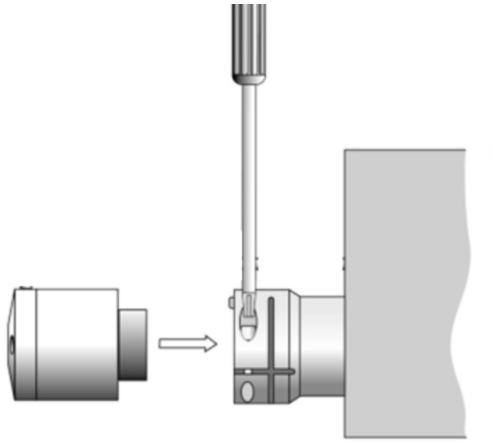
typical high-pressure seal spring cavity

Upon setup or when servicing the seals, wetting seal & pump head piston bore with acetonitrile will help reduce air bubbles and will allow faster flow stabilization. Use an adjustable pipette with a plastic tip to avoid damaging the sealing surfaces. Flush the spring cavity of the pressure seal 3-5 times with acetonitrile. Fill the pump head bore with acetonitrile and carefully assemble as described below:

6. To install: First insert the seal manifold in the pump head with the O-ring pointing outwards and make sure to align the manifold drain slots vertically
7. Now take the pump head with the manifold and slide the manifold and pump head onto the piston
8. Tighten the 4 pump head screws finger-tight and then tighten them securely, diagonally.
9. Reconnect tubing to the pump head
10. Run the degas script to remove air from the pump

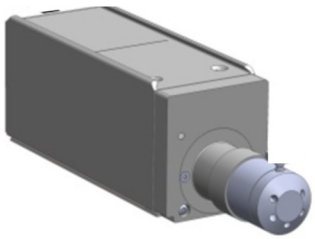
11. Run leak test for pump to ensure that the new seal is sealing correctly

11.7 Replacing a valve stack (Field Service)

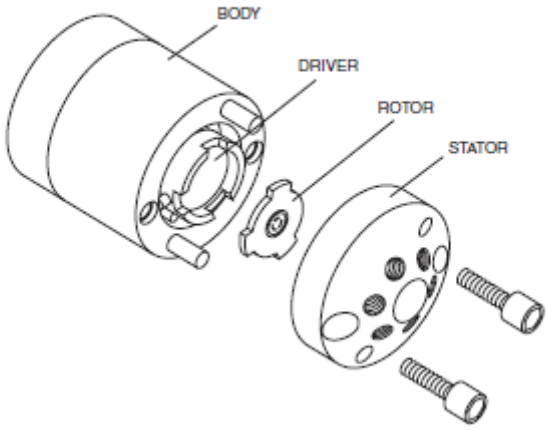


1. Disconnect all tubing from the stator port
2. For replacement of Valve 6, run the service script called “Set valve 6 pos 2-3”
3. Unscrew the Torx screw at the valve adapter, which holds the valve to the valve drive
4. Install the replacement valve and reassemble the above steps in the reverse order.

11.8 Replacing valve drive actuator (Field Service)



11.9 Replacing a valve rotor and or valve stator



1. Use a 9/64" hex driver to remove the socket head screws which secure the stator on the valve. Start by alternating between the two screws, loosening them in quarter-turn (90°) increments until all load is removed
2. Slide the Stator off the two guiding rods, and be careful not to stress any tubing connected to the stator
3. Visually inspect the stator surface within the inner ring
    a. If stator surface is not 100% clean, remove residues with a lint free tissue soaked in appropriate solvent
    b. If Stator surface cannot be cleaned or is scratched, it should be replaced.
4. With your fingers or a small tool, gently pry the rotor away from the driver
5. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The tab pattern is asymmetrical to prevent improper placement.
6. Slide the stator back onto guiding rods with port one pointing upwards, while doing this be careful that rotor sits securely in valve drive and that no tubing is caught between stator and valve driver
7. Insert the two socket head screws and tighten them gently until they start to get snug. Then alternate between the two screws, tightening them in quarter-turn (90°) increments until the stator is flush against the valve body. Do not overtighten the screws – they simply hold the assembly together and do not affect the sealing
8. If a new stator has been installed, please refer to tubing diagram for connecting all tubing to the correct stator ports

11.10 Tubing and fittings

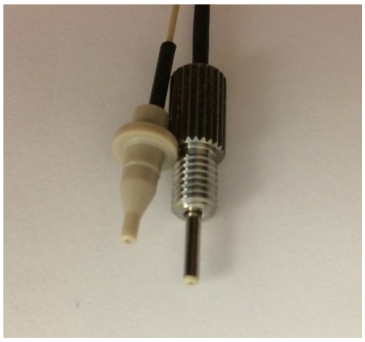
the following paragraph contains a description of the various kinds of tubing and fittings used on the Evosep One system. It is essential to the instrument performance to use official Evosep parts with correct dimensions and lengths. At the end of the section a tubing diagram and a table which indicates tubing positions in valve ports can be found:

Viper and nanoViper tubing

Viper and nanoViper tubing are finger tight fitting systems, which requires only very small torques to seal. Therefore, it is essential to follow the below guidelines to avoid damage by over-tightening:

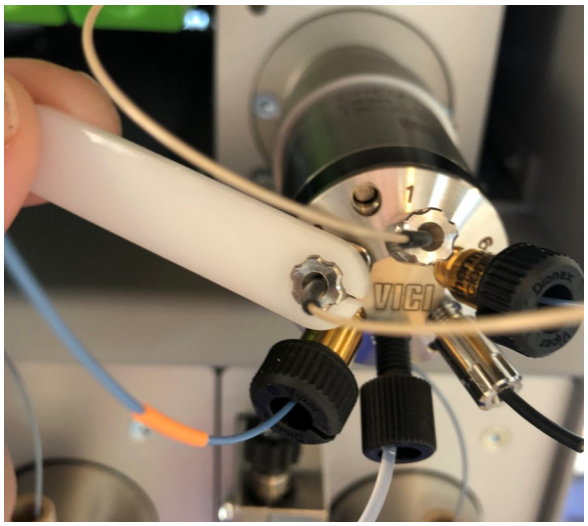
1. Insert Viper or nanoViper into the receiving port
2. Tighten the screw until you feel resistance
3. Now turn the screw a maximum of 45 degrees (1/8 of a full turn)
4. Verify that the connection is leak free, usually the fitting systems is tight after 45 degrees.
5. If the fitting system is leaking turn the screw up to an additional 45 degrees. But do not turn the screw beyond an angle of 90 degrees from where the initial resistance was felt.

NanoConnect tubing



Tubing for 5/16-24 Coned ports on Tip cross, 10-32 coned port on Needle Tee, Valve Loop, and flat bottom receiving ports on low pressure flow sensors, Loop

1. Insert the NanoConnect fitting into the receiving port and finger-tighten the nut securely
2. For male NanoConnect fittings use the Nanoconnect torque wrench



Peek

Peek tubing is used for low pressure connections on the system with various fittings.

- All of them are finger-tightened and no tools should be used for tightening

Tubing for flat-bottom-ports comes with a pre-swaged super flangeless ferrule or a one-piece-assembly, either for 1/32”, 1/16” or 1/8” outer diameter tubing

1. Check that the tubing is level or sticking slightly out of ferrule
2. Insert into receiving port and finger-tighten the nut securely

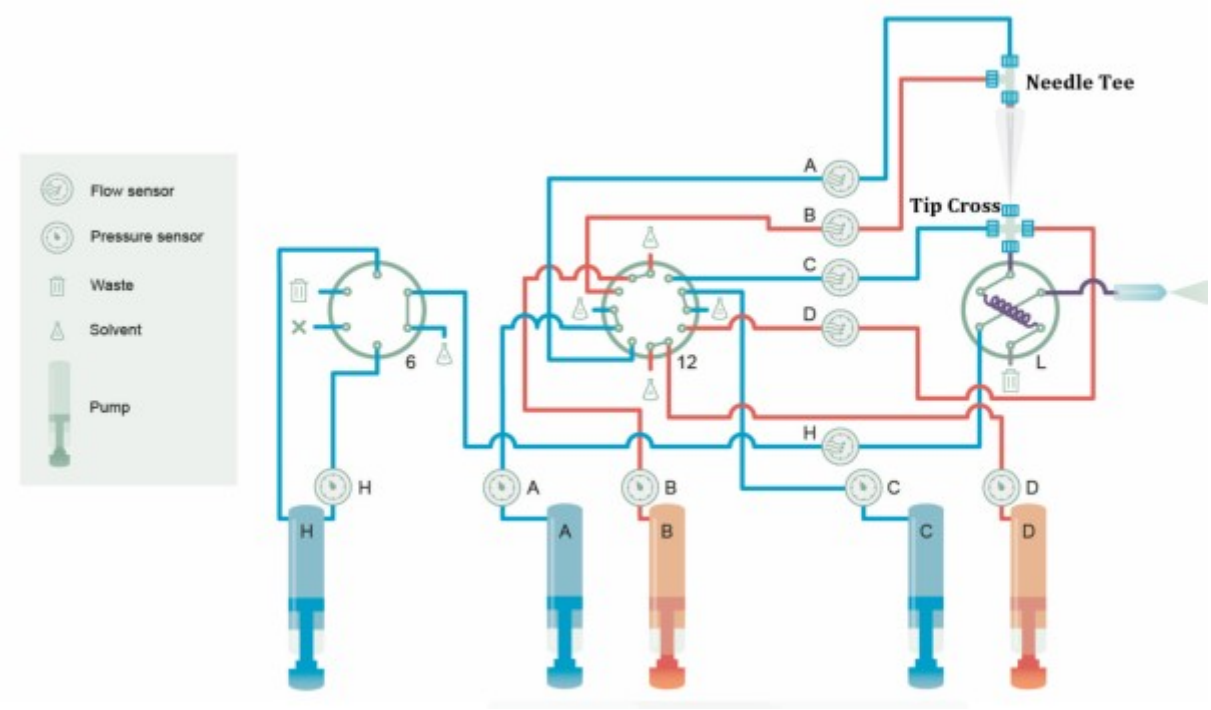
Tubing for 1/32” coned ports found on Valve 12 comes with a One piece no twist peek nut

1. Slide the peek nut onto the tubing
2. Insert the nut and tubing into the receiving port
3. Finger-tighten the nut securely while making sure that the tubing is bottomed out in the port
4. When tightened gently pull the tubing to verify it is seated correctly

Tubing for Viper compatible ports on LP pressure sensors, comes with a 10-32 coned fitting for 1/32” OD tubing.

1. Check that tubing is sticking slightly out of fitting
2. Insert fitting and tubing into pressure sensor port, push tubing against port bottom and then tighten the fitting using the tightening tool

Tubing diagram and ports valve port positions



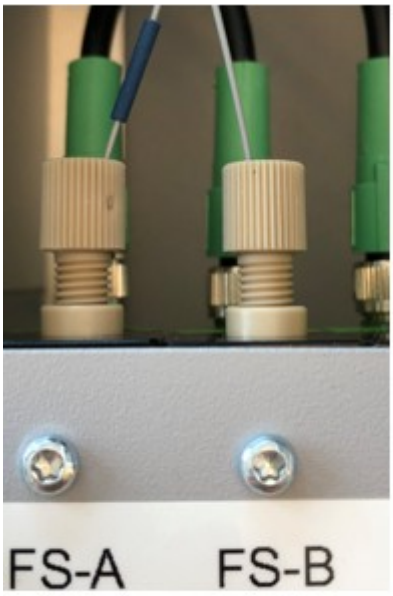
Valve Drive 1 Valve 6 tubing position		Valve Drive 2 Valve 12 tubing position		Valve Drive 3 Valve Loop tubing position	
P1	Pump HP	P1	Solvent B Tee	P1	TipCross

P2	Waste	P2	Pressure sensor B	P2	Loop
P3	Blank	P3	Flow sensor B	P3	Flow sensor HP
P4	Pressure sensor HP	P4	Solvent A Cross	P4	Waste
P5	Solvent A Cross	P5	Pressure sensor A	P5	Loop
P6	Flow sensor HP	P6	Flow sensor A	P6	Transferline
		P7	Solvent Tee		
		P8	Pressure sensor D		
		P9	Flow sensor D		
		P10	Solvent C Cross		
		P11	Pressure sensor C		
		P12	Flow sensor C		

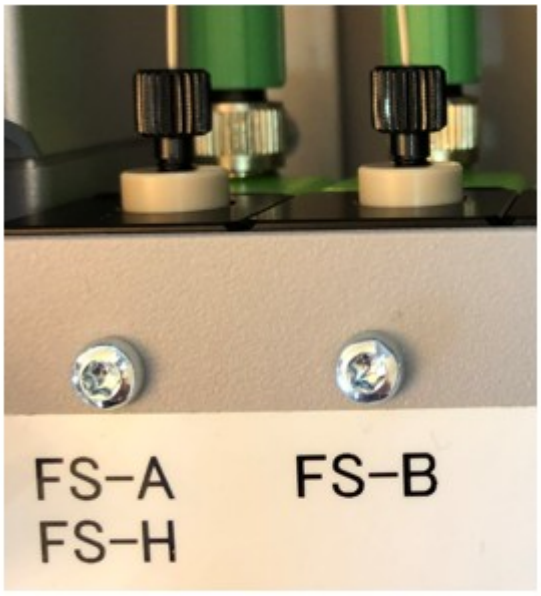
11.11 Replacing tubing A, B flow sensor to Needle Tee tubing

Please note that this tubing comes in 2 versions depending on the low-pressure flow sensor port configuration.

For more information on ordering the correct part please go to the evosep.com support page or contact support@evosep.com

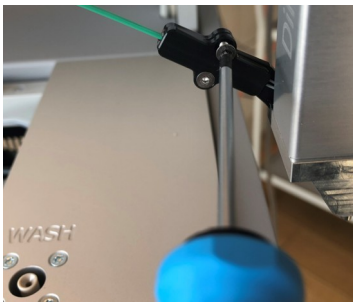


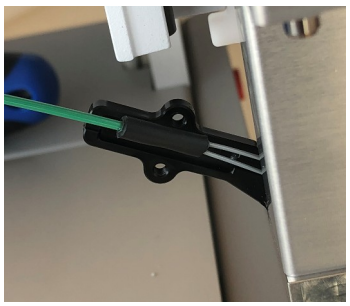
Flat bottom version



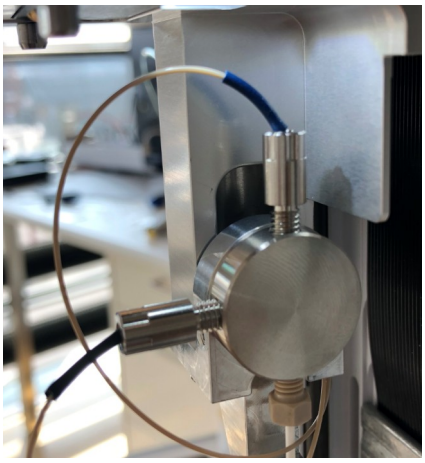
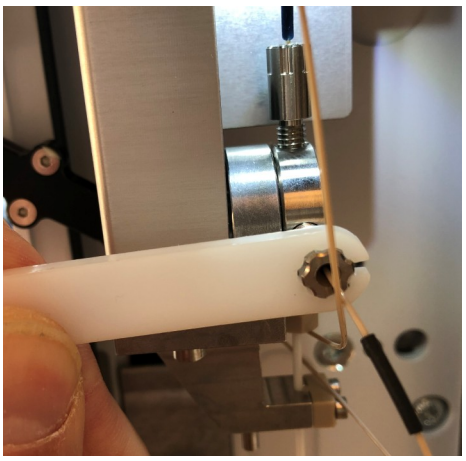
6-40 coned version

1. Remove sample tray from instrument
2. From the Pal Terminal select “RobotArmLeft”
3. Press “Options” and select “Change Syringe”
4. Press “Move” to move the needle to the exchange position
5. With a T6 remove the two Torx screws from the black tubing holder and remove the cover

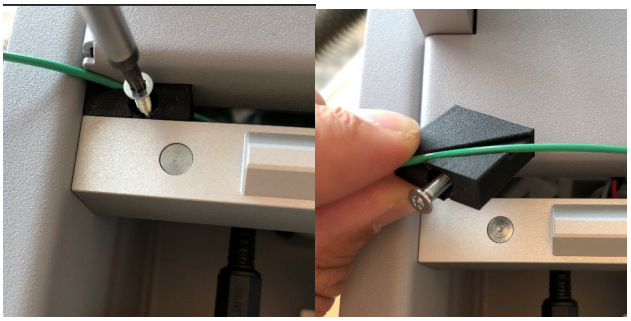




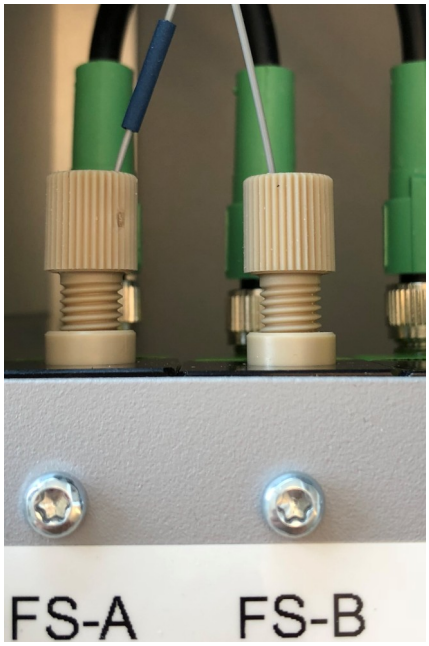
6. Now disconnect tubing A and B from the stainless steel Tee using the small torque wrench, please note that tube A has a blue label and sitting vertical and tubing B has a black label and is orientated horizontal

7. With a Torx 10 loosen the screw from the tubing holder in the back-left corner of the cabinet and gently remove the holder.

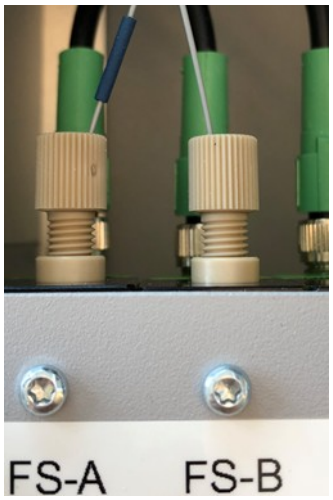


8. Disconnect the tubing from exit side of flow sensor A and B, please note that tubing A has a blue label

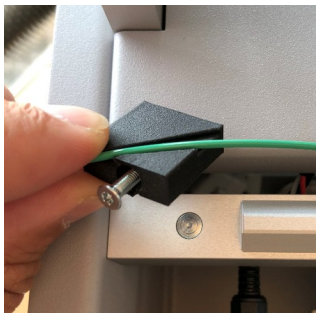


9. To remove tubing completely, slide the two metal fittings on tubing A and B down through the small opening where the tubing holder was sitting.

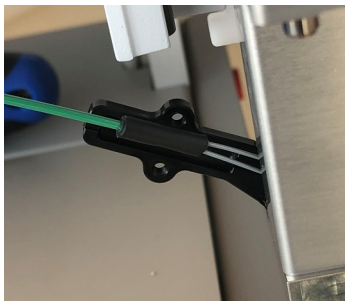
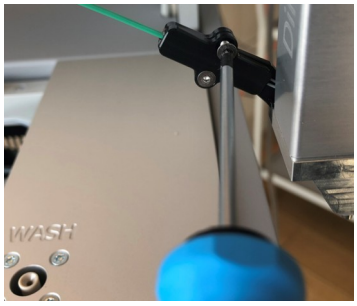
10. **To connect new tubing**,
11. From the Pal Terminal select “RobotArmLeft”
12. Press “Options” and select “Change Syringe”
13. Press “Move” to move the needle to the exchange position
14. Push the tubing with the two metal fittings up through the small opening, do this from underneath and up.
15. Route the other end of the tubing with the peek fittings underneath the tubing coming from the drain pump and connect the two peek fittings to flow sensor A and B. Please note that the tubing with the blue sleeve should be connected to flow sensor A



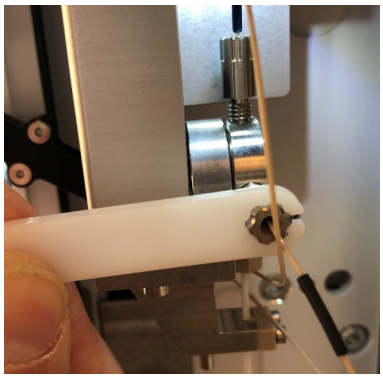
16. Put the green tubing in the groove of the tubing holder. Slide it into the small opening between the back top-cover and the metal frame. With a Torx 10 tighten the screw in the tubing holder all the way in, make sure:
    a. that the holder and screw is flush with the metal frame
    b. that as much as possible length of the tubing is going out of the instrument

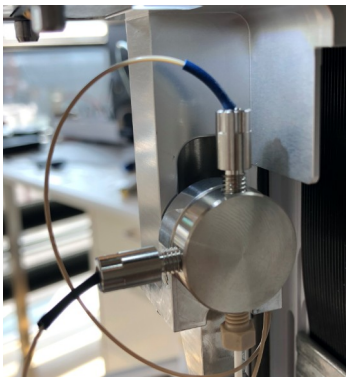



17. Put the black sleeve on the green tubing into the small groove on the black tubing holder and route the tubing on the backside of the Tee holder, put the black cover back on the tubing holder using a T6 screwdriver.

18. Now connect tubing A and B from the stainless steel Tee and tighten with the torque wrench please note that tube A has a blue label and sitting vertical and tubing B has a black label and is orientated horizontal

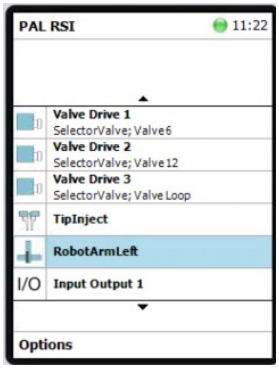


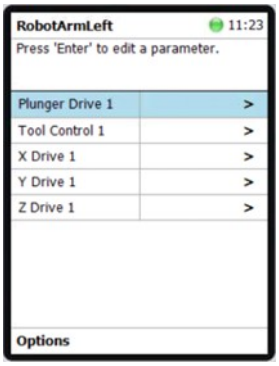


Important note: Do not use any other tools than the nanoconnect torque wrench for the nanoconnect fittings.

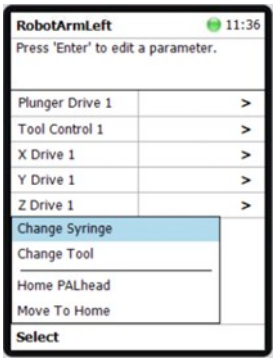
11.12 Replacing the needle

1. From the Pal Terminal select “RobotArmLeft”

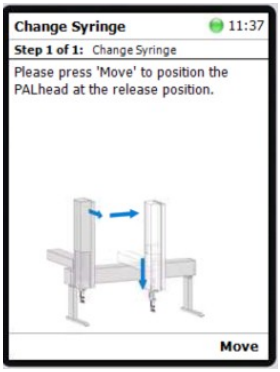




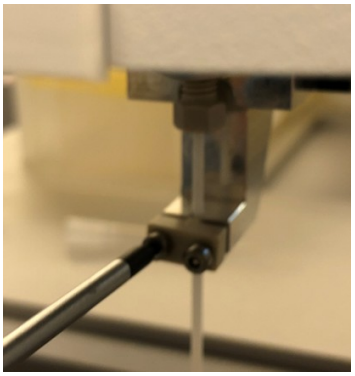
2. Press “Options” and select “Change Syringe”



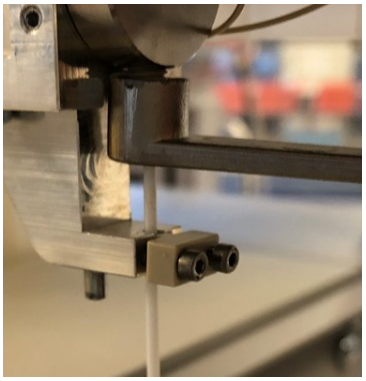
3. Press “Move” to move the needle to the exchange position



4. Loosen the 2 small T6 screws on the needle PEEK clamp



5. With one hand hold the needle while with the other unscrew the peek nut holding the needle in the needle tee




6. With the peek nut fully unscrewed remove the needle and peek nut
7. When inserting the new needle work in opposite order by pushing the non-tapered end of the needle (EV1018) through the needle clamp and then through the small PEEK nut and into the bottom port of the needle tee
8. Make sure that the needle is seated completely in the bottom of the port, then finger-tighten the PEEK fitting and use the ¼" socket wrench to tighten the PEEK nut maximum a ½ turn more. After that, tighten the needle clamp again
9. When completed press next on terminal to move "RobotArmLeft" to home position

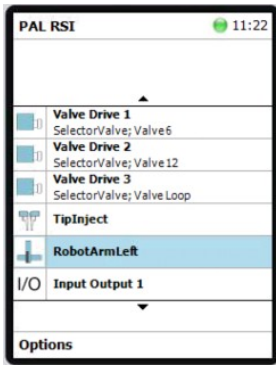
10. Press back to go to the “PAL RSI” main terminal page

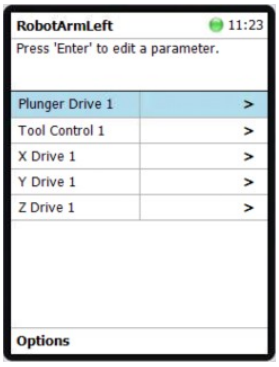
11.13 Replacing the needle tee

1. Remove the needle as described in “replacing the needle”
2. Disconnect the A and B NanoConnect tubing from the needle tee, be careful not to kink or stress the tubing
3. Loosen the lock screw from the needle tee holder and remove the needle tee
4. Install needle tee in opposite order

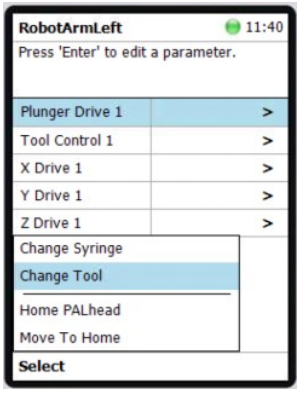
11.14 Replacing the tool

1. From the Pal Terminal select “RobotArmLeft”

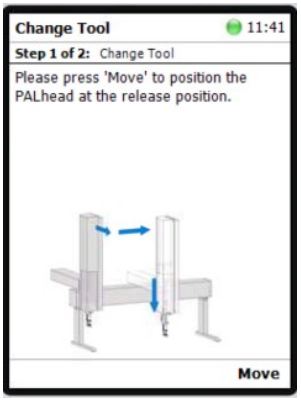




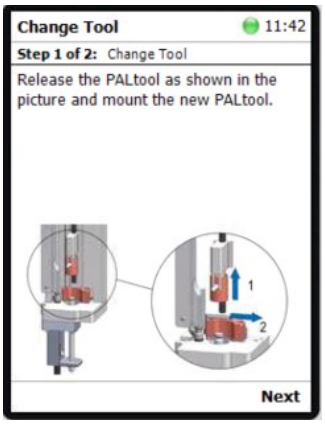
2. Press A to select “Options” and select “Change Tool”



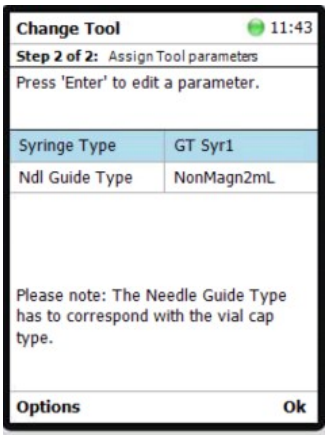
3. Press “Move” to move the tool to the exchange position



4. Remove needle
5. Disconnect the A and B nanoConnect tubing from the needle tee
6. Unscrew the two screws holding the tubing plate and remove tubing
7. Release tool as illustrated on Terminal window



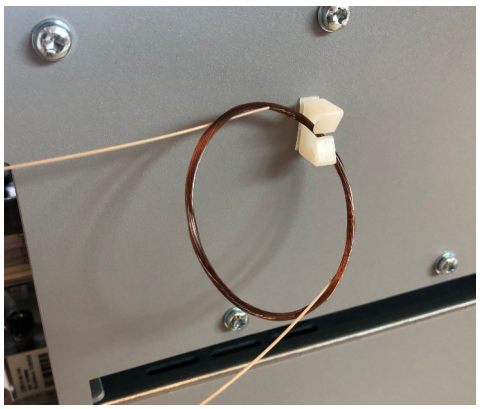
8. Install new tool and connect tubing and needle
9. Press next on terminal
10. Verify that syringe type = GT Syr1 and Ndl Guide =NonMagn2mL



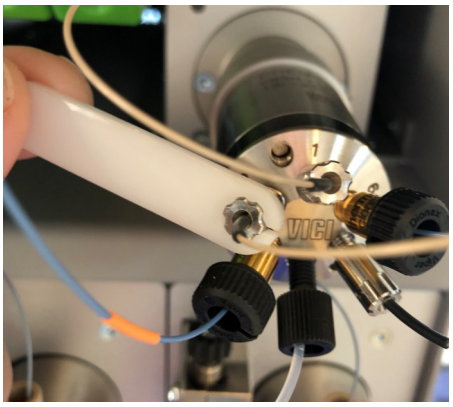
11. Press ok to move robot arm to home position

11.15 Replacing the loop

1. Remove left-hand side panel
2. Carefully without damaging the storage loop remove it from the holder



3. Using the nanoconnect torque wrench loosen and disconnect the two loop fittings from loop valve port 2,5



4. Install new loop in reverse order, using the small torque wrench to ensure correct tightening of the loop fittings
5. After installation run the Service script “Loop flush” to flush the new loop with solvent
6. Run the Diagnose script “HP system” leak test to verify that there are no leaks around the loop
7. Run the Calibrate - Loop volume script to measure the volume of the loop, see note

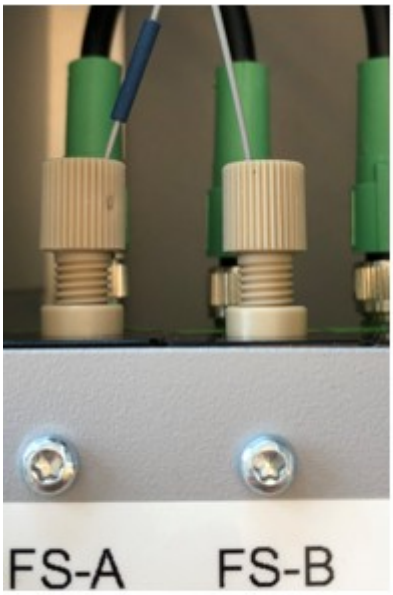
Note:

- The Loop volume calibration script measures the exact volume of the sample loop. The calculated volume is used in the sample runs to ensure higher analyte retention time accuracy. If the loop is replaced, the calibrate/loop volume script must be executed.

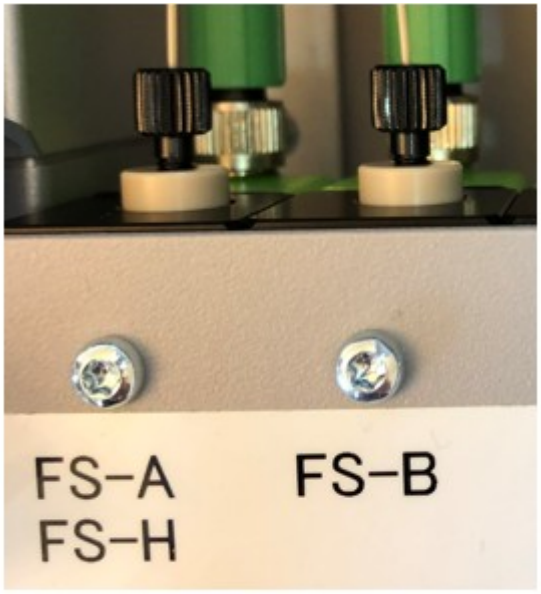
11.16 Replacing a flow sensor

Please note that the low-pressure flow sensor (FS-A-D) comes in two versions using two different fittings

For more information on ordering the correct part please go to the evosep.com support page or contact support@evosep.com



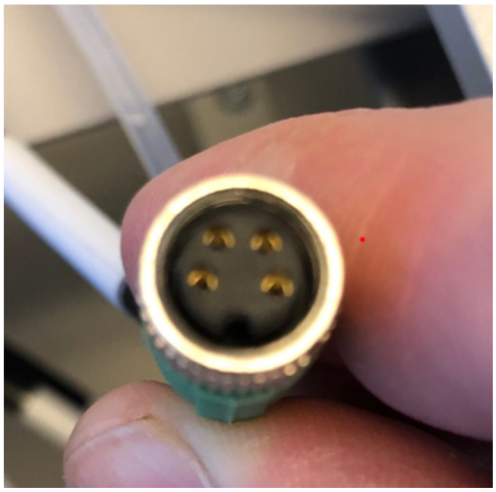
Flat bottom version



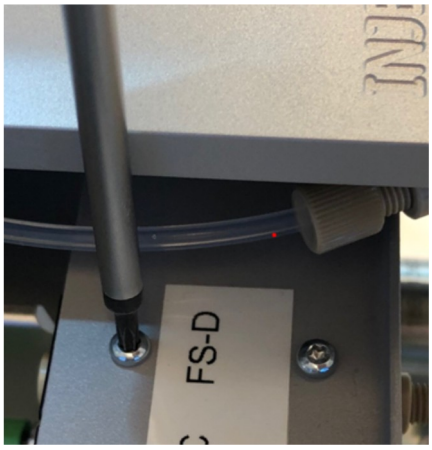
6-40 coned version

Example shown for low pressure flow sensor, but procedure is the same for Flow sensor HP

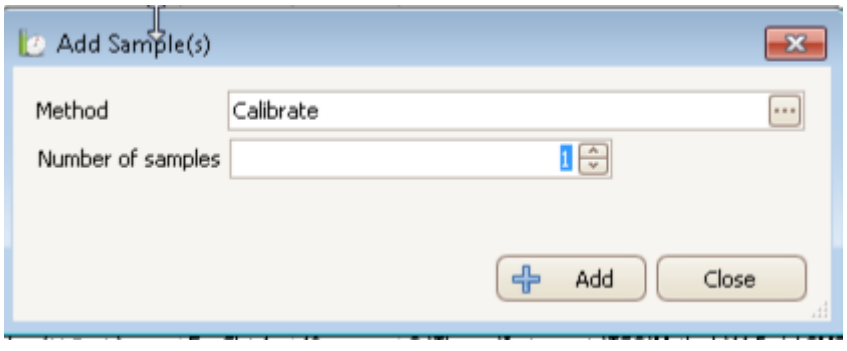
1. Power of the instrument and ensure that needle drops into lock position
2. Remove sample tray
3. Disconnect the tubing on entry and exit side of flow sensor (nanoViper connections on the flow sensor HP), then unscrew the small metal cap on the flow sensor cable and remove the flow sensor cable. (For re-connecting please note the small cut out in the connector)



4. With a Torx 10 screwdriver remove the two flow sensor screws and carefully remove the flow sensor from the instrument. For the HP flow sensor the screws are located horizontal on the front side of the instrument behind the door.



5. Install new sensor in reverse order making sure to orientate the cable correctly
6. The two tubing connections on the low-pressure flow sensors are finger-tighten fittings but need to be tighten securely to create a good seal. For the nanoViper connections on the flow sensor they should not be overtightened.
7. When new sensor has been connected and instrument switched back on, please run Prepare – Pump preparation – Degas until acceptable values are reached
8. Schedule and run the appropriate calibration method Flow sensor ABCD or Flow sensor HP to calibrate the new sensor



	Analysis Method	Flow sensor ABCD	Flow sensor HP	Loop volume*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Calibrate.cam	☐	☐	☐

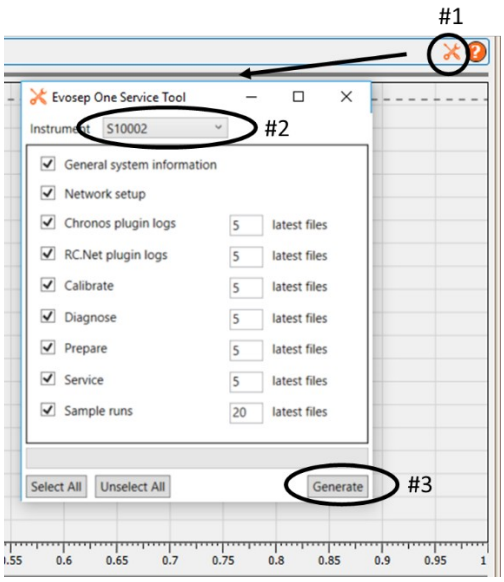
9. If a low-pressure sensor (FS-A-D) has been replaced schedule and run the Tip seal test to verify that the tubing connections around the flow sensor is leak free. If the high-pressure sensor (FS-HP) has been replaced schedule and run the Diagnose HP system leak test.

12 Support, service and warranty

12.1 How to request technical support

To obtain technical support please contact Evosep support at support@evosep.com. Your email must contain the following information:

- Instrument serial number
- Problem description
- What has been done to solve the problem
- For an already open case, please supply Case number
- Instrument logfiles. Please use the service tool to collect and compress instrument logfiles
    1. From the Evosep graph page, press the “tool” icon in the upper right corner.
    2. Select the instrument serial number of interest.
    3. Press “Generate” to extract and compress the logfiles.

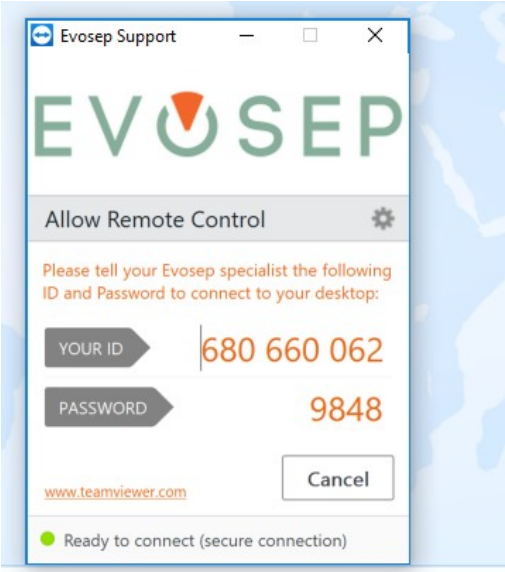


- For remote support please supply TeamViewer ID and Password as described below.

An Evosep support specialist will get back to you with a case number for tracking the problem plus further questions and recommended tests to determine the probable cause and find a solution to the problem.

12.2 Remote support via TeamViewer

Evosep uses TeamViewer to establish secure remote desktop access to the data system controlling the Evosep One. To allow remote control please go to https://get.teamviewer.com/evosep and accept to run the executable file. This will generate an ID and Password. Please supply this info to support@evosep.com, when requesting technical support.



12.3 How to arrange for a service visit

If a problem cannot be solved by technical support, please request a service visit.

Prior to the arrival of the Evosep service engineer possible replacement parts will be shipped to the instrument location. Smaller wear parts the service engineer will carry on site.

Please note that Evosep will charge for parts, travel and labor if the problem/instrument is not covered by warranty or service agreement

12.4 Product warranty

The product warranty remains in effect for a period of 12 months from the date of installation or 15 months from delivery whatever comes first. Any warranty requests must be filed within the warranty period.

The warranty covers defects or failures of the Evosep One system and its major hardware parts occurring as a result of normal use or manufacturing defect.

The warranty does not cover defect or failures resulting from damage caused by accidents, neglect, misuse or abuse.

Instrument wear parts in most cases all parts which are in contact with solvents and or sample are not covered by the warranty period. E.g. tubing, fittings, rotor, stators, seals, needle etc.