Evosep One

User Guide

v. 19

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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 may result in damage to the instrument, personal injury, or loss of life.

Please familiarize all laboratory personnel with the following warning and caution symbols as they appear throughout the User Guide at the beginning of each Chapter:

Symbol	Description
	Indicates a risk of danger is present. This may refer to any type of hazard. A safety statement will coincide by this symbol.
	Warning / Electrical shock hazard
	Caution / Risk of fire
	Warning / Risk of infection
	Caution / Corrosive hazard

	Warning / Broken glass
	Warning / Toxic fumes
	Caution / Risk of impact
	Caution / Risk of entrapment
	Warning / Sharp objects

1.3 Contacting Us

Support: support@evosep.com

Sales: sales@evosep.com

1.4 Declaration of Conformity

We:

Company name	Evosep Aps
Postal address	Billedskærervej 15
Postcode	5230
City	Odense M
Country	Denmark
Telephone	+31 651063191
E-mail	jf@evosep.com

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

Apparatus model (P/N)	Evosep One (EV1000)	
Type	General Laboratory equipment	
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 3 Directive 2015/863/EU • WEEE Directive 2012/19/EU • Regulation 2023/1230/EU – Machinery
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).

Joanna Freeke, PhD
Product Manager

July 11th, 2024

2 Introduction

Evosep aims to improve the quality of life and patient care by radically innovating protein based clinical diagnostics. We 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:

1. 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 and gradient formation that cause less wear and tear
2. Increased productivity with higher duty cycle utilization owing to:
    - Fewer LC household steps
    - Minimized dwell time as gradient formation occurs at a high flow rate and close to the column
3. Increased performance with better data quality owing to:
    - Reduced cross-contamination using disposable tips
    - 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. Rather, the tips are loaded directly into Evosep One for analysis. This new, unique 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, uninterrupted analysis. Upon sequence acquisition, the autosampler places one, pre-loaded sample tip at the time in-line with the solvent system, see Figure 1.

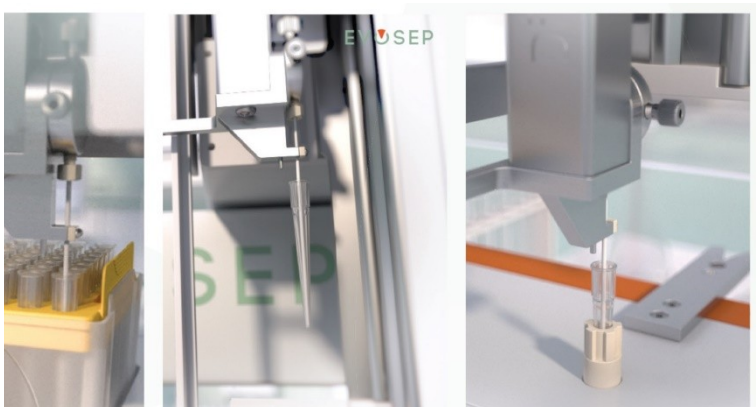


Figure 1. Upon sequence acquisition, the autosampler places one, pre-loaded sample tip at the time in-line with the solvent system.

3 Installing the Evosep One Hardware

Warning/Caution	
	**Risk of danger: exercise caution when lifting the instrument as improper lifting can lead to injuries. Wear appropriate clothing during instrument relocation.**
	**Electrical shock hazard: the Evosep One, MS, and data system hardware must have common grounding to avoid a ground loop that can cause noise and interference or produce an electrical shock.**

Important notes:

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

These instructions do not replace a required instrument installation by an Evosep service engineer.

Please store the original Evosep shipping crate and packaging safely. If needed, the Evosep One is always to be transported in the original packaging!

3.1 Lifting Instructions

Important notes:

DO NOT lift the instrument with side panels mounted! They are magnet mounted and can come off during lifting.

Only lift the instrument to place it on a table. Use a cart for moving the instrument.

The instrument weighs approximately 37 kg. For safety precautions, please use two people when moving the instrument and lift from underneath the instrument on each side.

Before lifting and/or moving the instrument, please verify that the following actions have been performed:

1. The two side panels have been removed.
2. The autosampler has been parked in lock position.
3. The instrument has been switched off.
4. The network, power, and contact closure cables have been disconnected from the backside of the instrument and set aside.
5. The transfer line has been disconnected from MS ion source.
6. The waste tubing has been removed from the waste container (not applicable for instruments with the waste container on the door).
7. The Evotip boxes have been removed from the Evosep One sample tray.

8. The instrument can now be lifted by two persons. Please lift from 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 lbs

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. It must be 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 400 mm.

3.3 Power Requirements

Line Voltage:

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

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

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

3.4 Temperature and Humidity Requirements

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

Temperature fluctuations < 1 °C/hr (2 °F/hr).

Important notes:

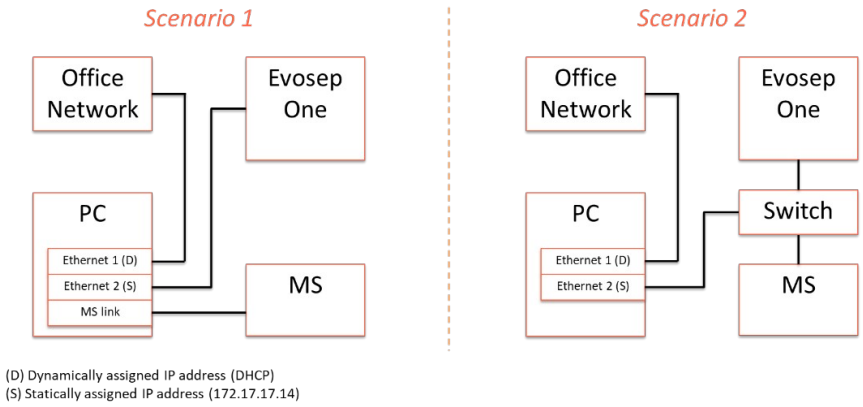
Analytical specifications require a temperature range of 22 ± 3 °C (72 ± 6 °F) with an operating humidity range of 20 – 80%, non-condensing.

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

Avoid instrument locations with high air humidity or fluctuations in temperature, such as direct sunlight, drafts, directly below air conditioning, or directly beside a mass spectrometer vent.

3.4.1 Connecting the Ethernet Communication Cable and Checking Network Adapter Settings

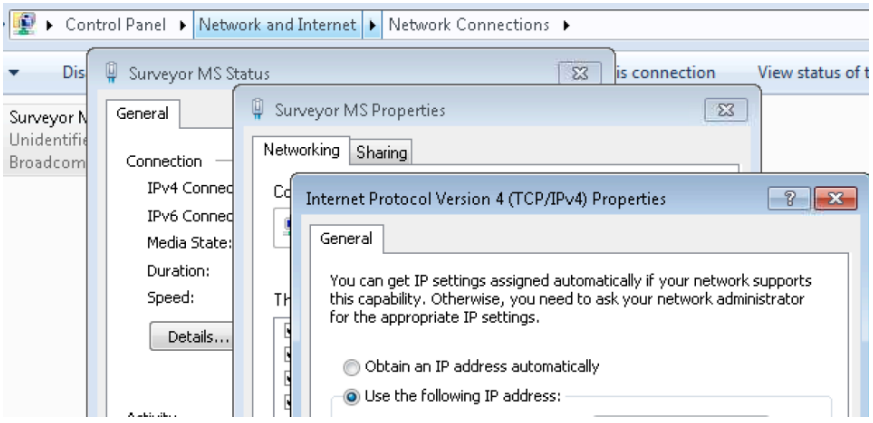
The Evosep One communicates with the MS data system through an Ethernet switch that is connected to the MS and MS data system, or directly to a dedicated network card as outlined below.



Important notes:

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

Go to the PC's network connections for the specific network adapter card and ensure that it is configured with a static IP address. If in doubt on how to set this up correctly, please contact your IT administrator.

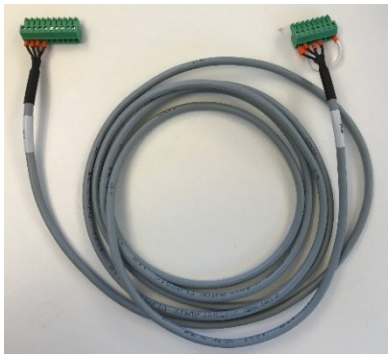


Please plug the ethernet cable into the LAN port on the pump box backside and plug the other end into the MS ethernet switch.

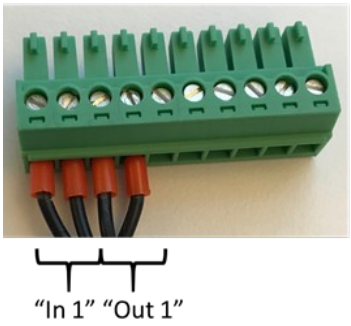
3.4.2 Connecting the Contact Closure Cable

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

Several MS-specific contact closure cables exist and can be ordered with the instrument (see example below).



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 Evosep One's pump box.

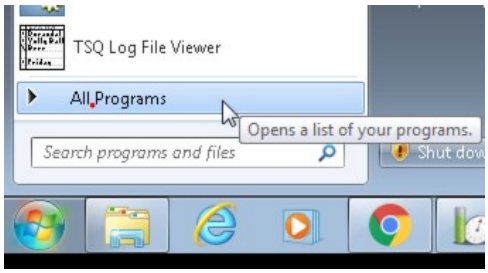
Please refer to the MS documentation on how to connect and establish contact closure for your specific mass spectrometer.

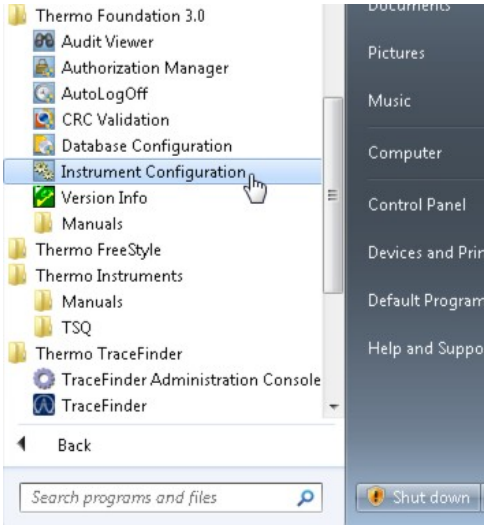
3.4.3 How to Remove Other LC Devices from MS System Configuration

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

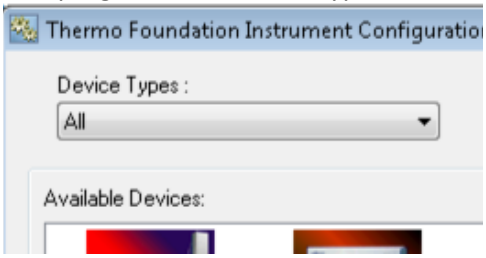
Please check for, and remove, other connected LC/Autosampler devices from the MS Instrument configuration before connecting the Evosep One. The example below is given for Xcalibur:

1. Close Xcalibur.
2. From Windows Start button, click “All Programs” and open the “Instrument Configuration” program. This can typically be found in one of the Thermo specific folders.

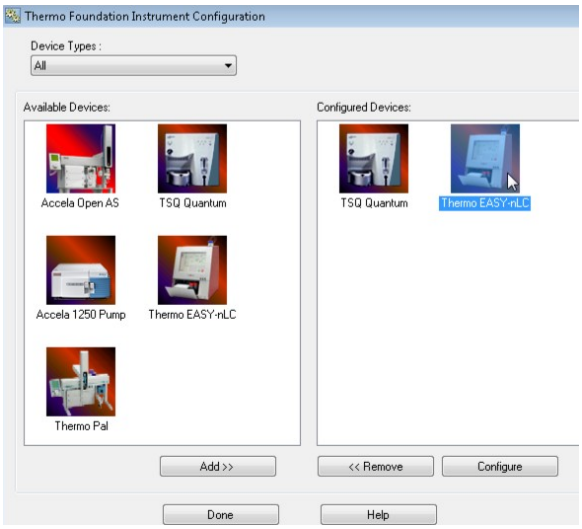




3. In the “Instrument Configuration” program, set “Device Types” to “All”.



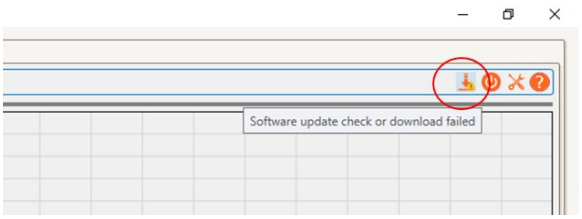
4. If any LC systems are visible in the “Configured Devices” window, select them and click “<< Remove” to remove them from the configuration (*note: do not remove the MS from the configuration*). Then click “Done” and re-open Xcalibur.



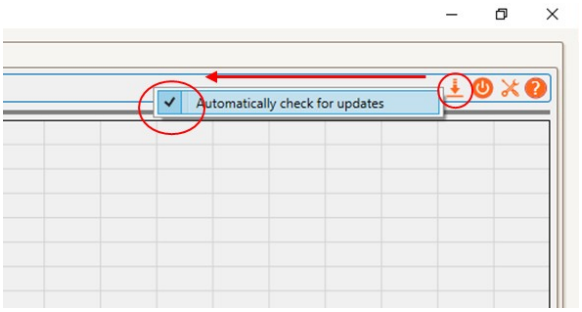
4 Installing Control Software

4.1 Automated Software Plugin Update

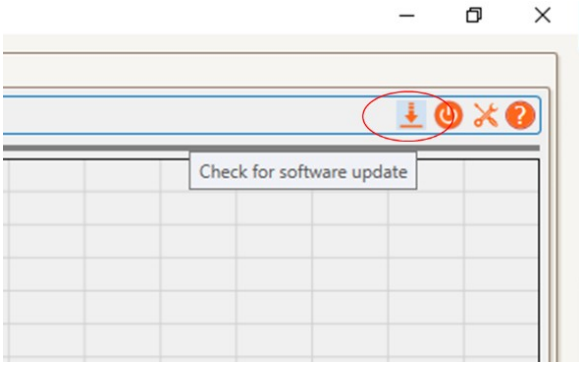
The plugin software will automatically detect if a newer version has been released and assist the user in the update process (available from plugin v1.4 for Chronos and v1.2 for HyStar). For this feature to work, the PC must have access to evosep.com. If this page is blocked, a warning will be displayed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



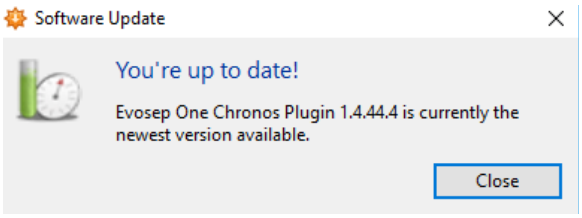
1. The software update process can be triggered in two ways:
   - Automatically during restart of Chronos/HyStar. The automated feature can be turned on/off by right clicking “Check for software update” button and checking /unchecking the box.



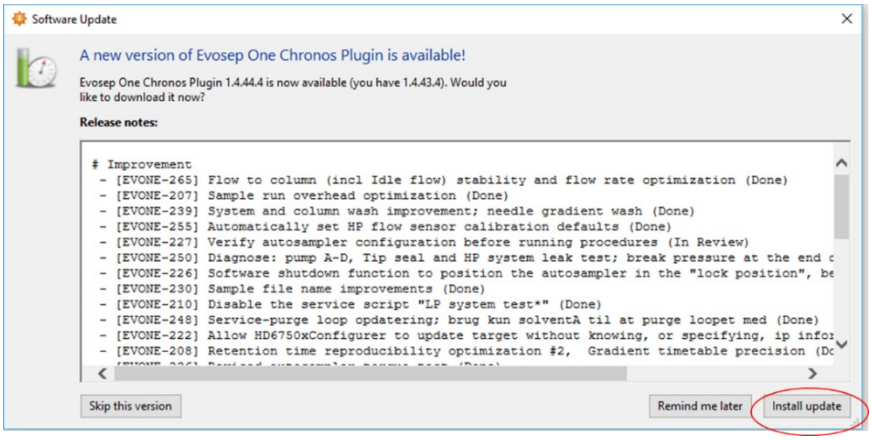
   - Manually by pressing the “Check for software update” button on the graphs page.



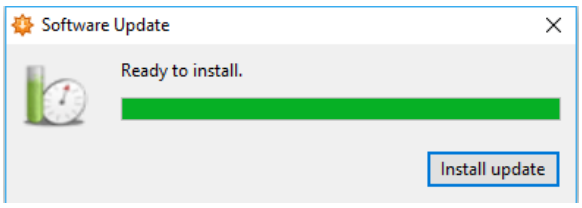
2. The software update window will open and show if the installed plugin is up to date.



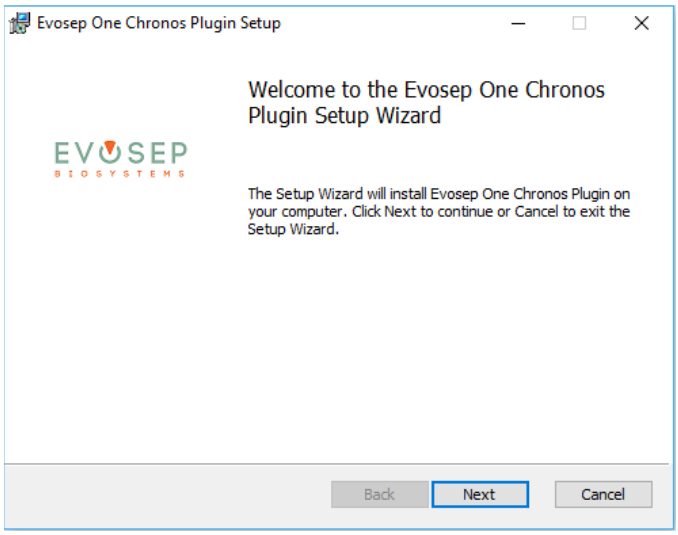
3. If not, the release note for the most recent version of the plugin will be displayed, read carefully and then Press “Install update” to proceed.



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

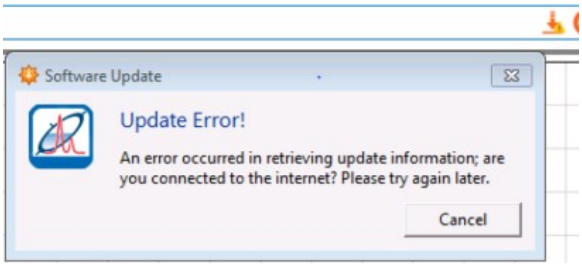


5. Press “Next” to proceed with the installation procedure.



Important note:

The automatic 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 Evosep webpage at [Evosep Support](#).



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.

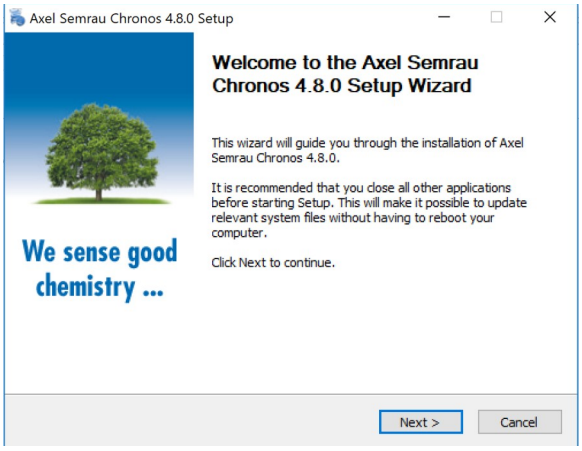
The Evosep One instrument is controlled through the “Chronos” sample acquisition software via an Evosep One plugin.

Chronos comes as a dongle-dependent version with limitations outlined below.

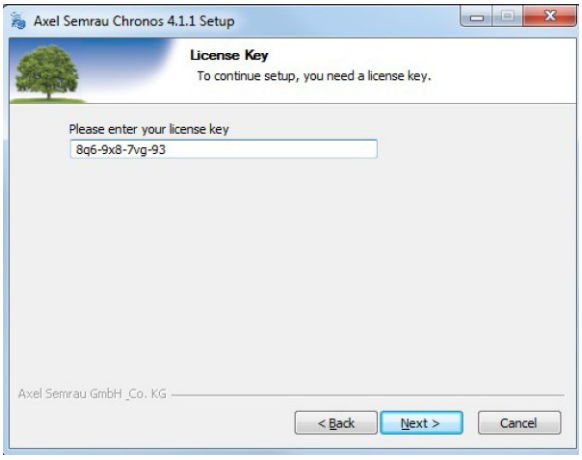
Dongle-dependent versions are full versions, without a time limit. They can be installed and used on any number of computers. To use Chronos, the USB dongle supplied with the software must be inserted and a drive letter must be assigned by Windows. No activation is necessary. If the dongle is not inserted 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 error message is displayed.

4.2.1 Installing Chronos

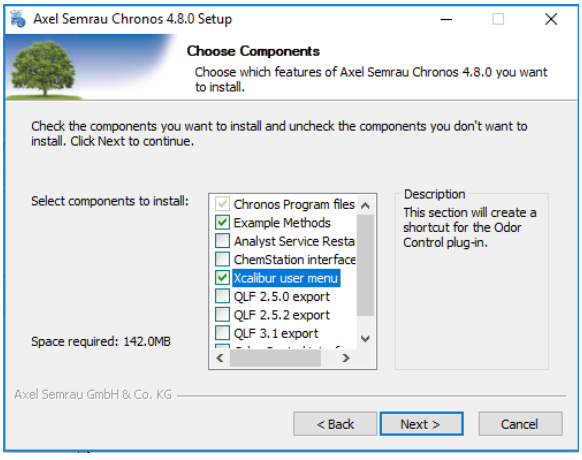
1. Insert the Chronos USB dongle into an available port on the MS data system.
2. Run the Chronos setup application file found in the root of the dongle.



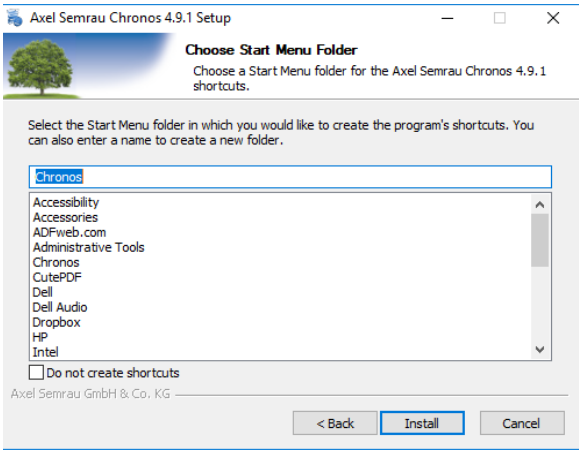
3. Click “I agree” to the software license agreement.
4. Type in the License key found in the USB dongle.



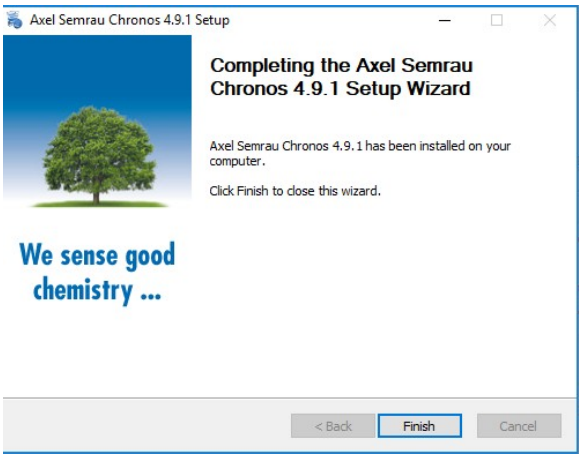
5. For installation on a Thermo MS data system, please tick the “Xcalibur user menu” checkbox. For Analyst, tick the “Analyst” checkbox.



6. Click “Install” to start the installation.

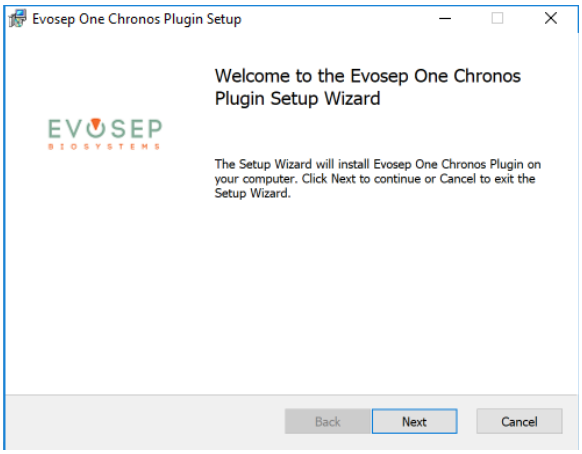


7. Click “Finish”.

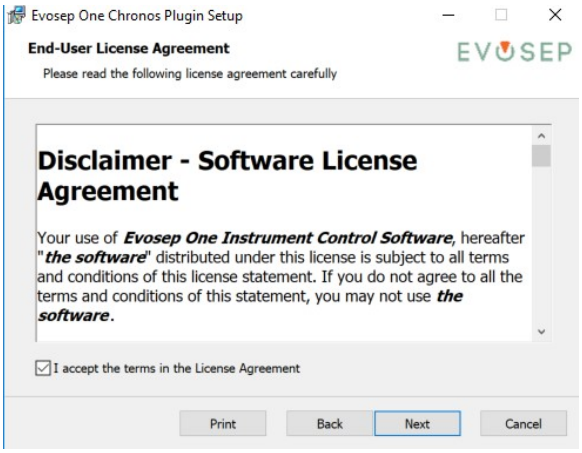


4.2.2 Evosep Chronos Plugin Installation

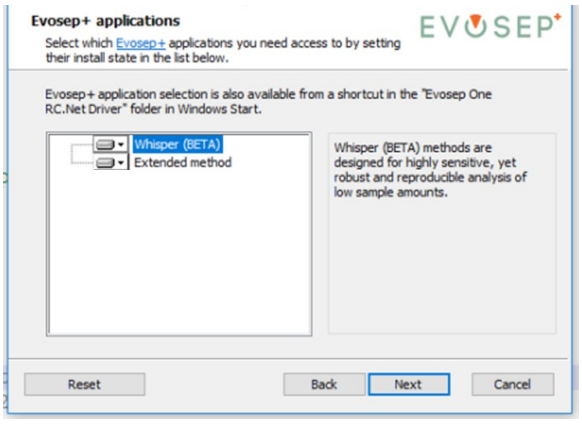
1. Please ensure that Chronos is NOT running before starting the installer.
2. Connect the Evosep One instrument to the computer, or through a network switch, with an ethernet cable.
3. Ensure both the pump box and the autosampler of the instrument are switched on before starting the plugin installation.
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”.



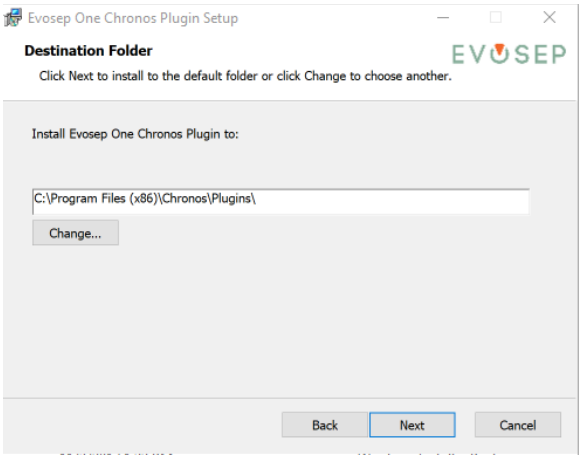
8. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.



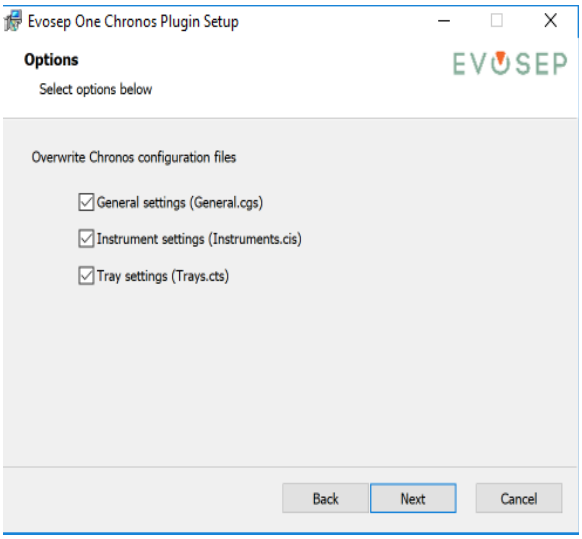
9. Please read the information in the “Prerequisites” window carefully, then click “Next”.



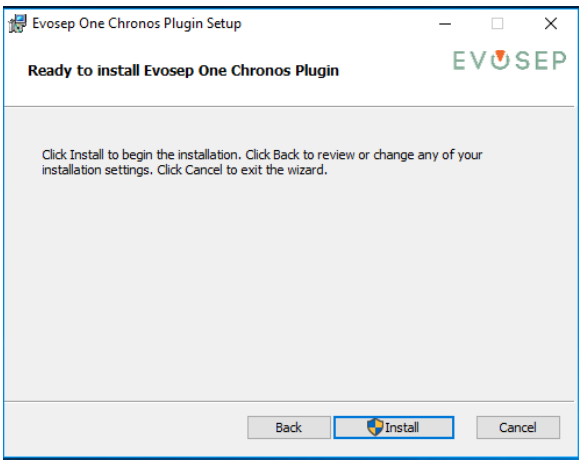
10. Choose which Evosep+ applications you wish to install in addition to the standard methods.
11. Click “Next” to install the plugin in the suggested folder.



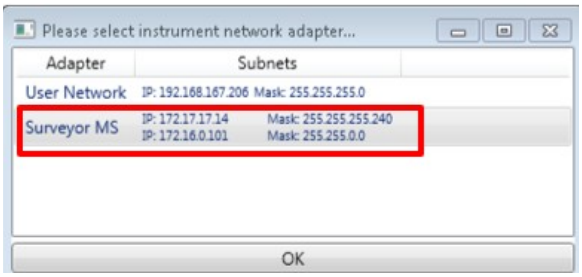
12. Verify that all three checkboxes are ticked to overwrite the Chronos configuration files with the Evosep One configuration files. Click “Next”.



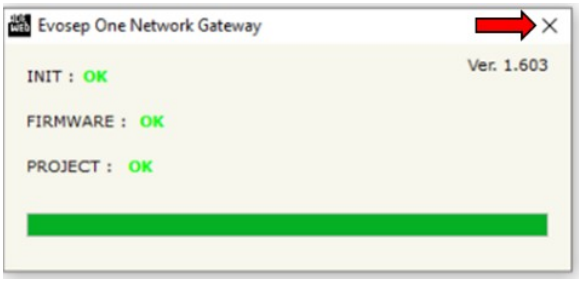
13. Click “Install” to begin the installation. Click “Yes” to any popups during the installation.



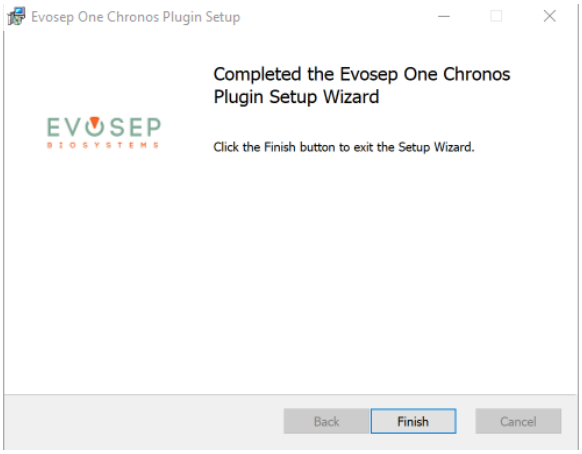
14. If asked to select instrument network adapter, always choose the MS network adapter and then click “OK”.



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

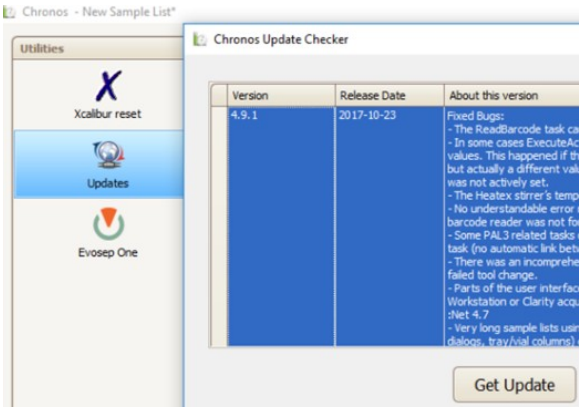


16. When the installation is complete, click “Finish”, to exit the installer.

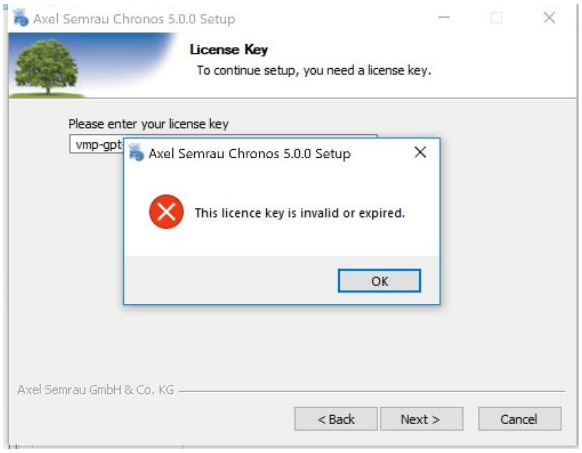


When updating Chronos, please note the following:

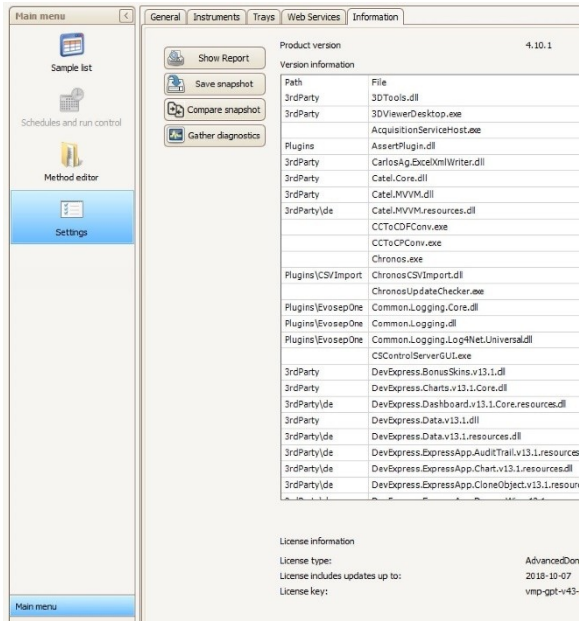
- The newest version of Chronos can be installed by opening Chronos and clicking on Updates under Utilities.
    - Prior to updating, please check for compatibility issues in the release notes for your version of the Evosep One plugin.



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



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



When updating the Evosep One plugin, please note the following:

- 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 same version number plugin, please uninstall the Evosep One plugin using the Windows program uninstaller feature.

Important 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.

4.2.3 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

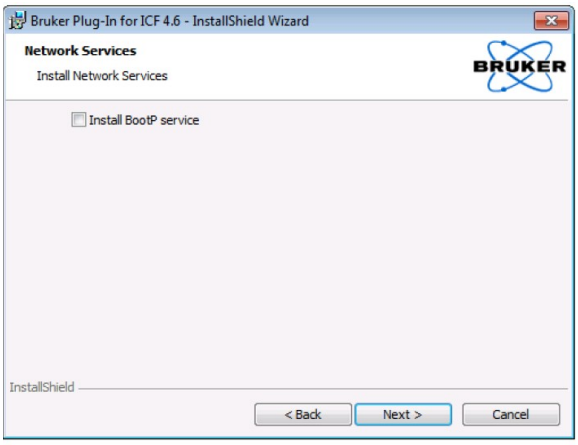
4.3.1 Installing ICF for Bruker Compass HyStar

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

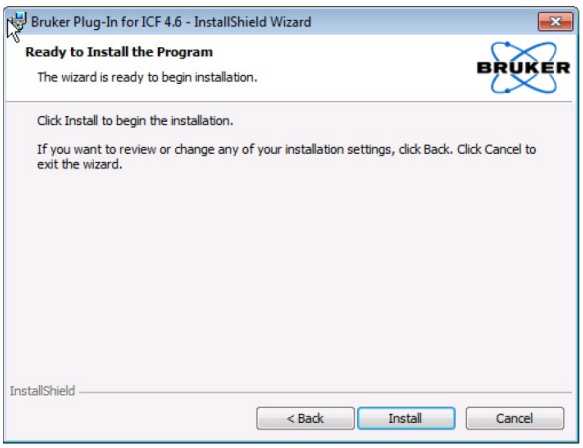
1. Insert media containing the ICF plugin for HyStar.
2. Navigate to the ICF plugin X.X for HyStar Y.Y folder for either Windows 7 or Windows 10.
3. Run the “CD Start” application file and click “Install” to install the Plugin.

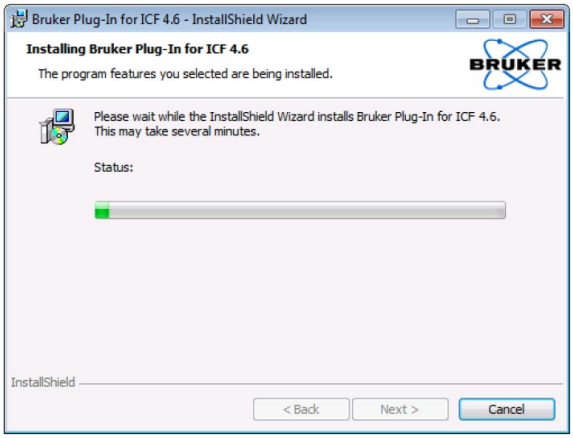
4. Follow the various pop-up windows with information regarding the installation.
5. Accept the terms in the License Agreement and click “Next”.

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

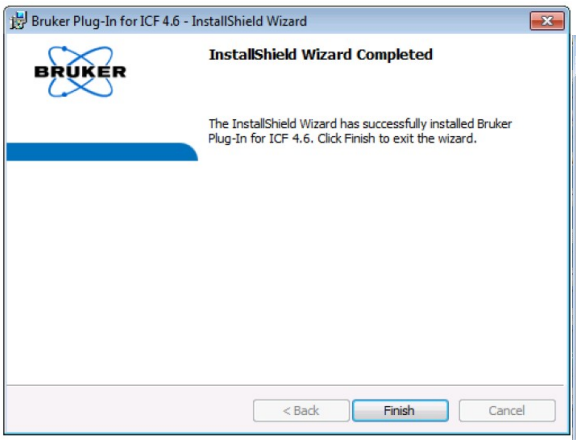


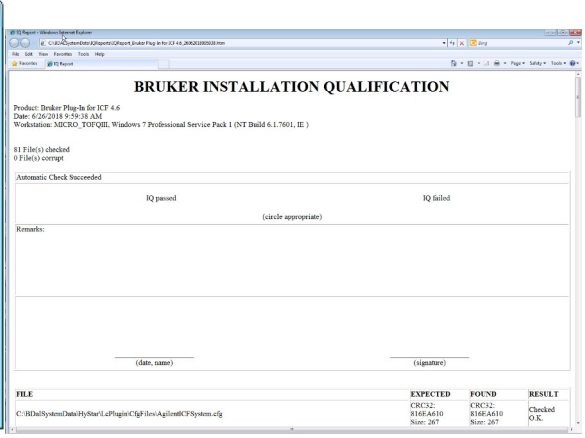
7. Now click “Install” to install the program.



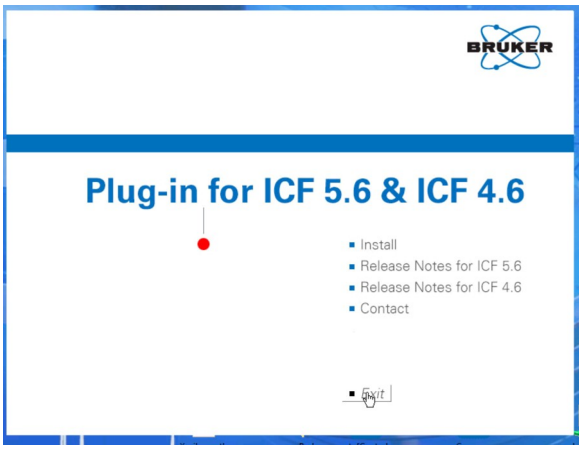


8. Click “Finish”. In the “Bruker Installation Qualification” pop-up window, verify that all parts of the installation have been “Checked O.K.”.





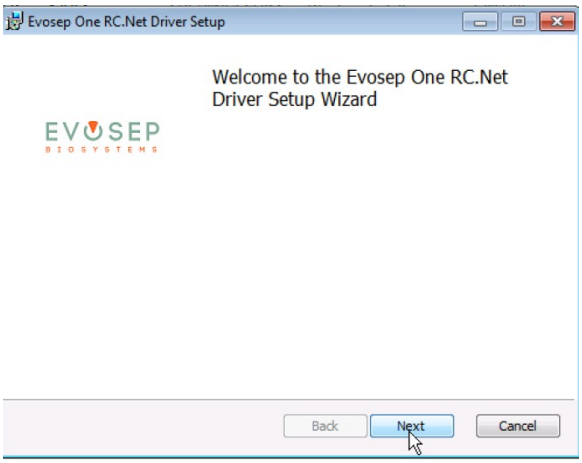
9. Click “Exit” to close the CD start menu.



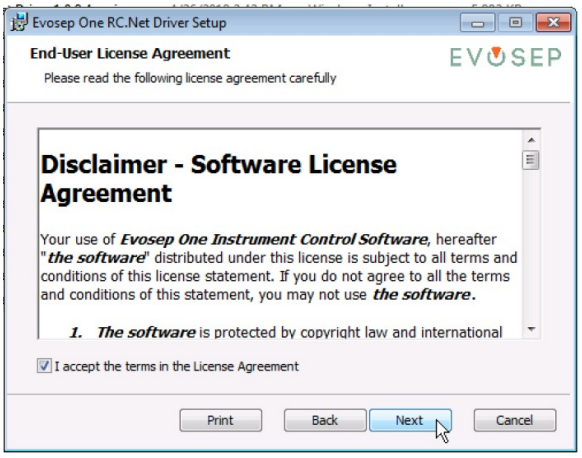
4.3.2 Installing the Evosep One RC.Net Driver 2.x.x.x.msi

Make sure the ICF plugin for HyStar is already installed.

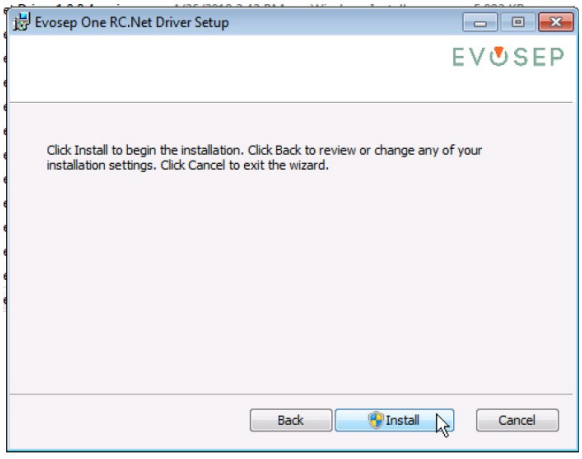
1. Connect the Evosep One instrument to the computer, or through a network switch, via ethernet cable.
2. Insert the media containing the Evosep One RC.Net driver.
3. Run the Evosep One RC.Net Driver 2.x.x.x Windows installer package file.
4. Click “Next”.



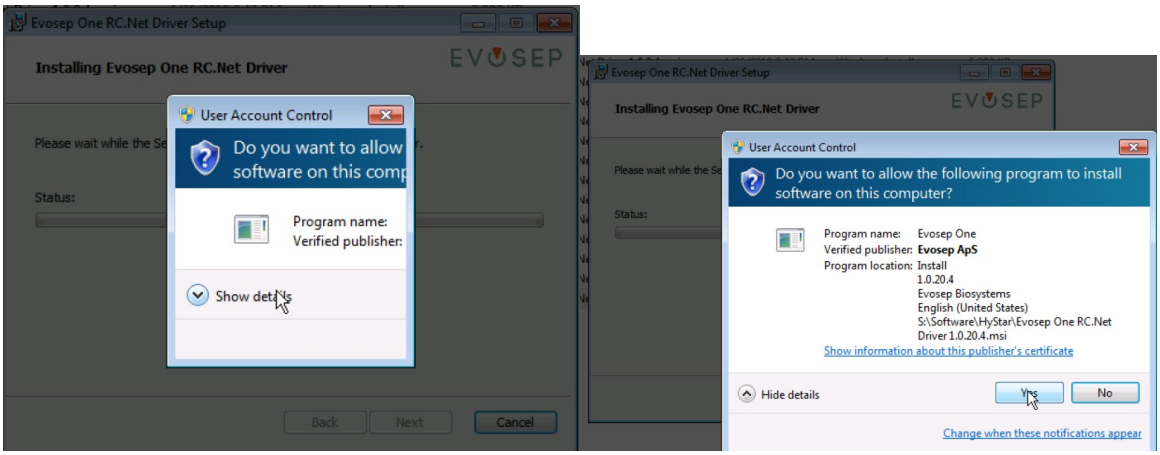
5. Tick the “I accept the terms in the License Agreement” checkbox and click “Next”.



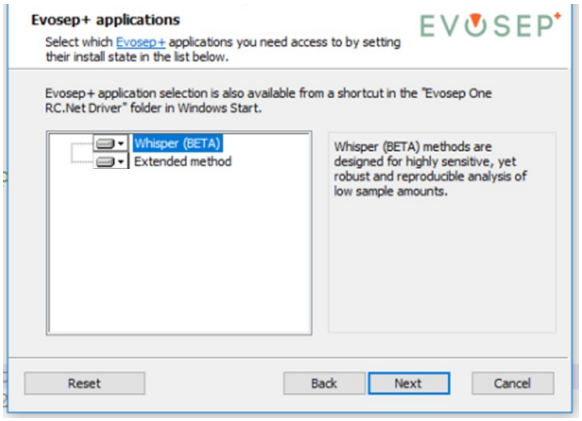
6. Please read the information in “Prerequisites” window carefully, then click “Next”.
7. Click “Install” to begin the installation. Click “Yes” to any pop-up windows during the installation.



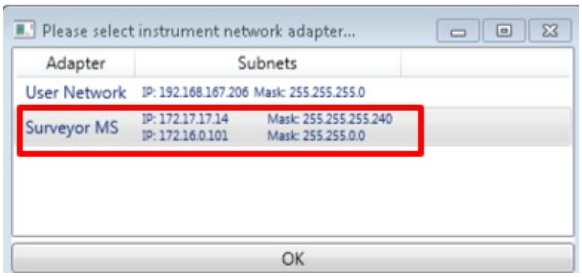
8. Click show details (if window not fully visible) then click “Yes” to allow the program to install the software.



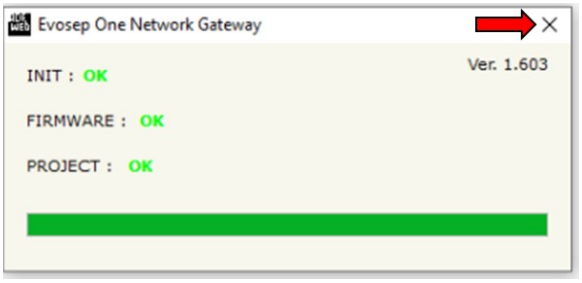
9. Choose which Evosep+ application you wish to install in addition to the standard methods.



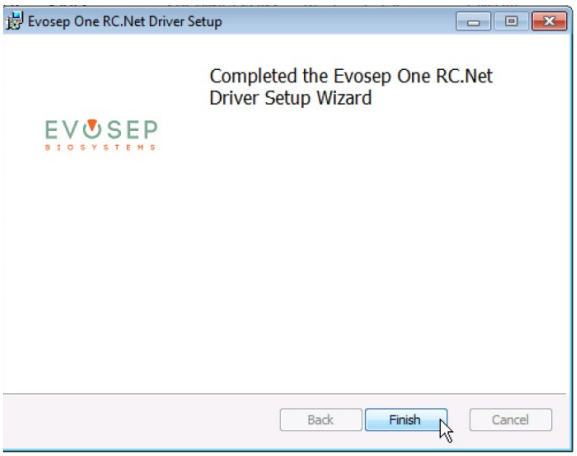
10. If asked to select an instrument network adapter, always choose the MS/LC network adapter and click “OK”.



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

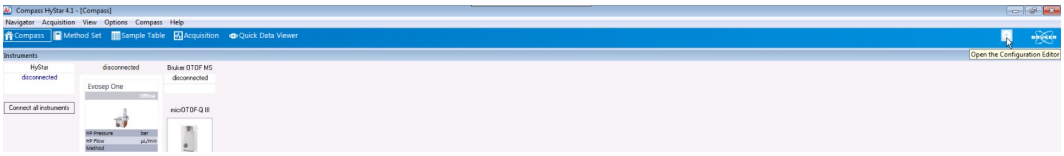


12. When the install is complete, click “Finish” to exit the installer.

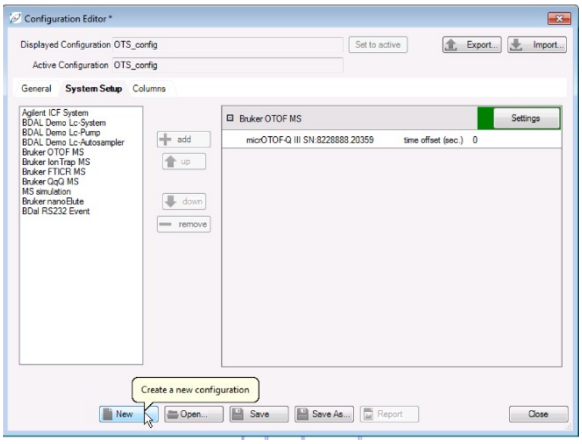


4.3.3 Create HyStar IFC Configuration for Evosep One

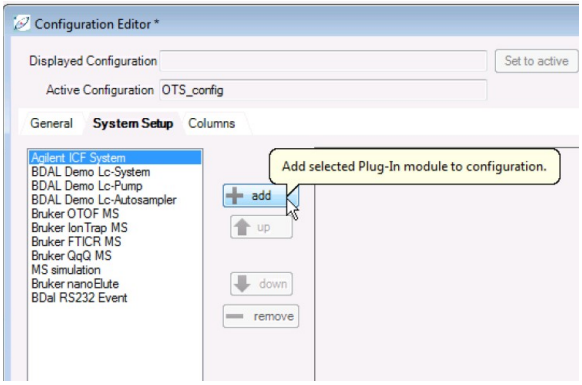
1. Open Compass HyStar and click on the gearwheel icon to open the Configuration Editor.



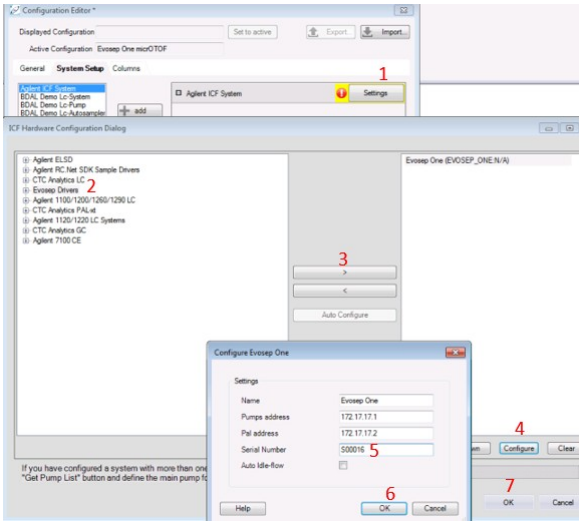
2. In the “Configuration Editor” window, click “New” to create a new configuration.



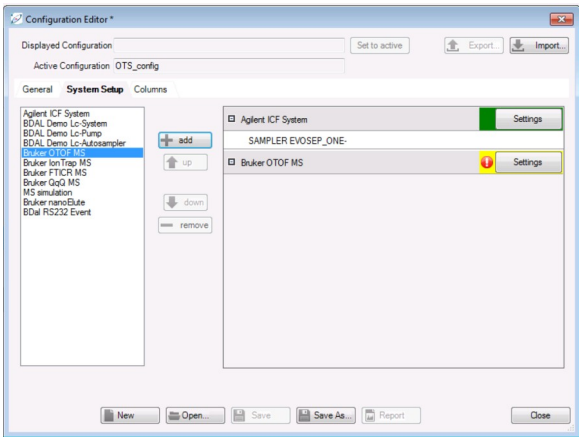
3. Mark “Agilent ICF System” and click “add”.



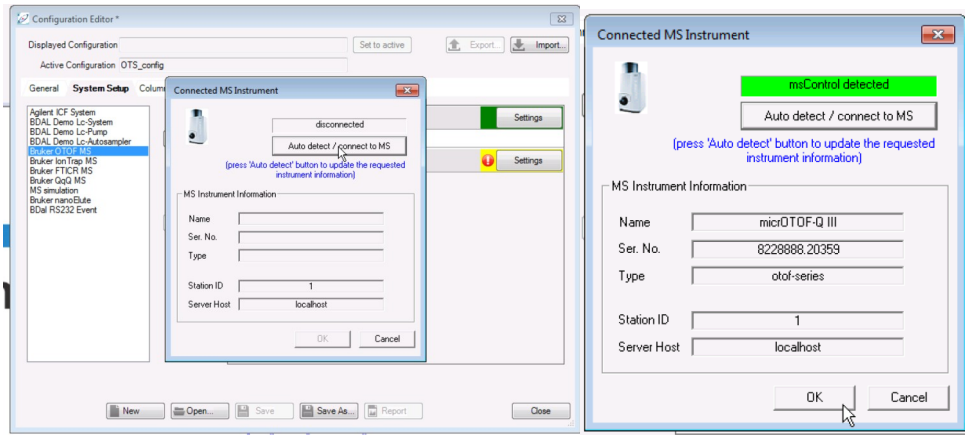
4. Click “Settings”, “Evosep Drivers”, “>”, “Configure”, enter the Evosep One Serial Number and check “Auto Idle-flow” if needed, “OK” in the “Configure Evosep One” window, and “OK” in the “ICF Hardware Configuration Dialog” window.



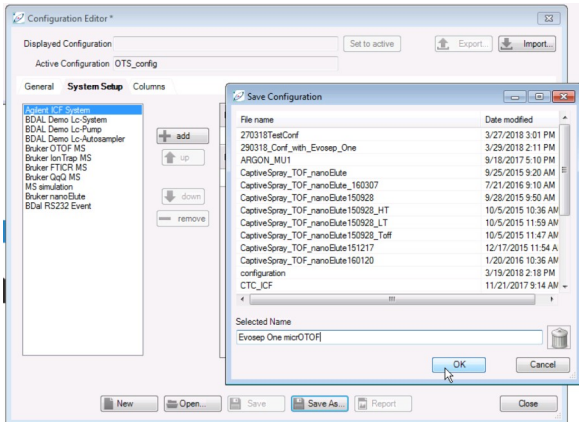
5. In the “Configuration Editor”, select the MS model being used and click “add”. Click “Settings” for the newly added MS.



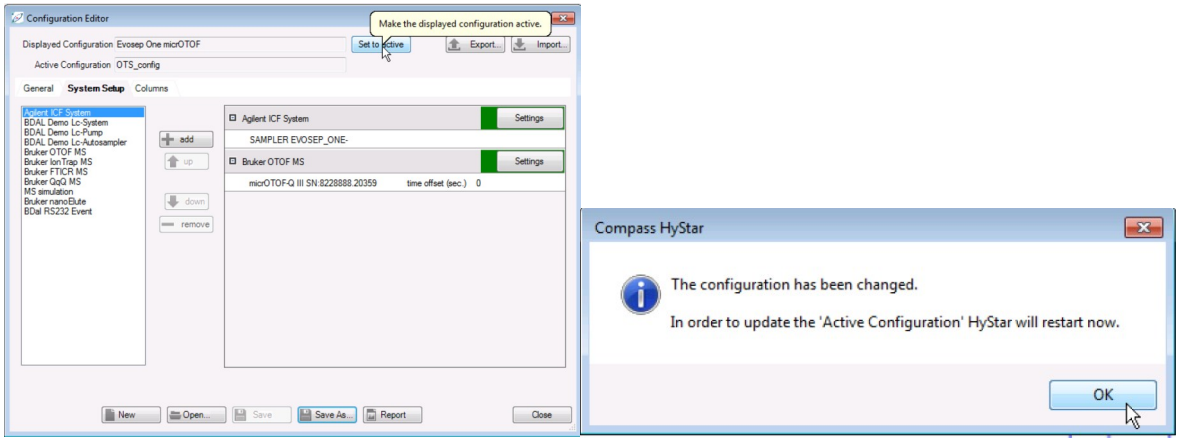
6. Click “Auto detect/connect to MS”. Verify that the MS is detected and click “OK”.



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



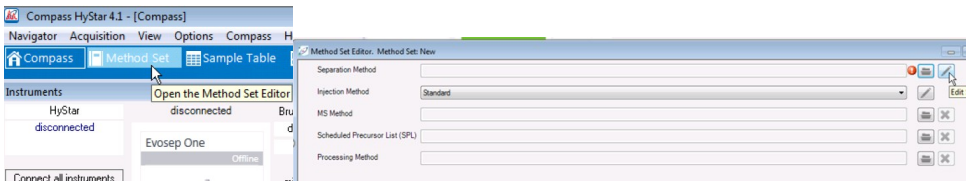
8. Click “Set to active” to use the Evosep One configuration. Click “Close” and “OK” to restart HyStar.



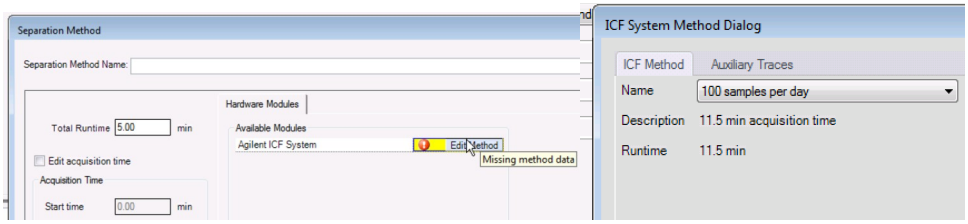
9. If upgrading from an earlier ICF plugin, carefully check all the HyStar hardware profiles (using ICF). Recreate if needed.

4.3.4 Create Evosep Separation Methods

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

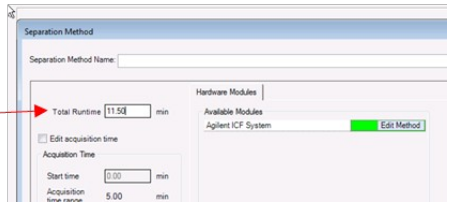


2. Click “Edit Method” and choose one of the predefined Evosep methods, e.g. “100 samples per day” and click “OK”. Please note the Runtime for the chosen method name in the ICF System Method dialog.

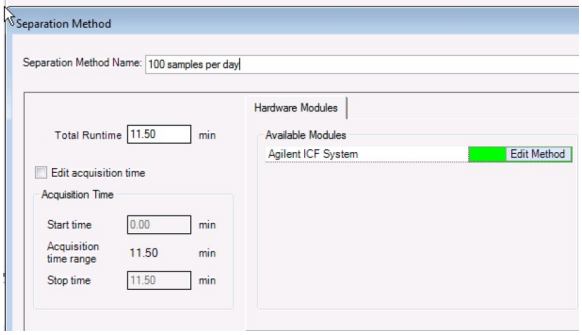


3. Now, if not done automatically, set the "Total Runtime" for the chosen method. The example below is given for the 100 samples per day method.

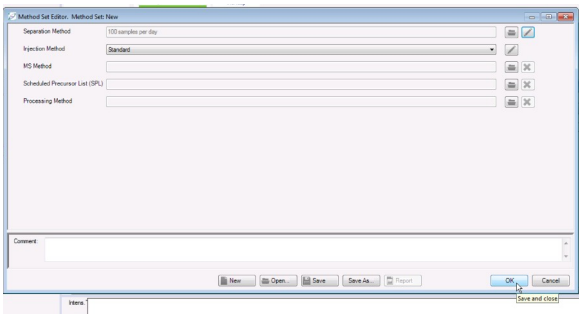
Throughput	Gradient Length
Samples/day	Minutes
300	3.2
200	5.6
100	11.5
60	21
30	44



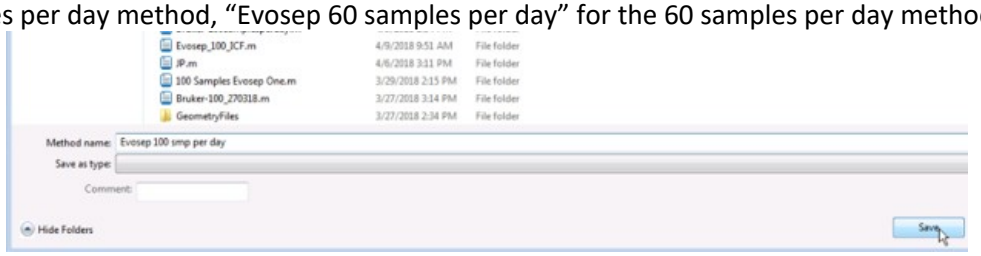
4. Name the Separation Method the same name as chosen in the ICF System Method Dialog, e.g. "100 samples per day". Click "OK".



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.

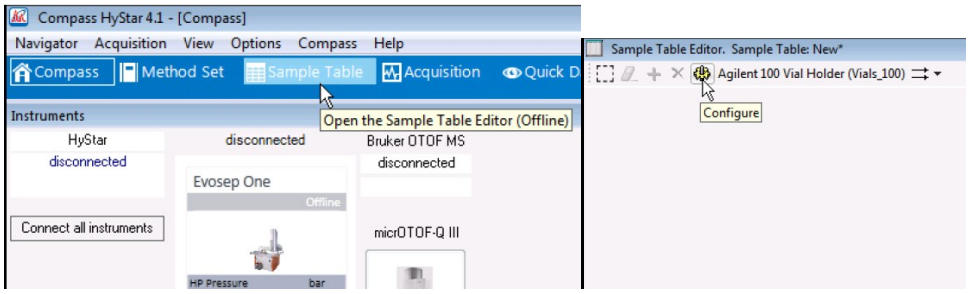


7. Repeat Steps 1 – 6 to create separation methods for the remaining methods below.

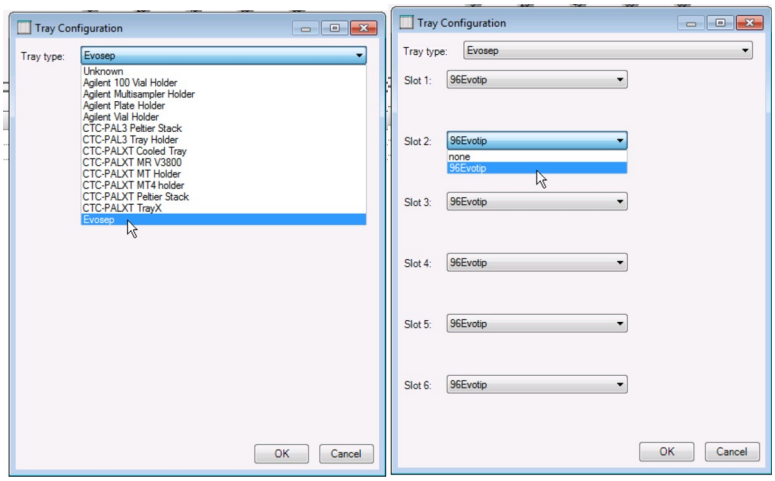
- 30 samples per day
- 60 samples per day
- 100 samples per day
- 200 samples per day
- 300 samples per day

4.3.5 Create Evosep One Tray Type and Sample Table

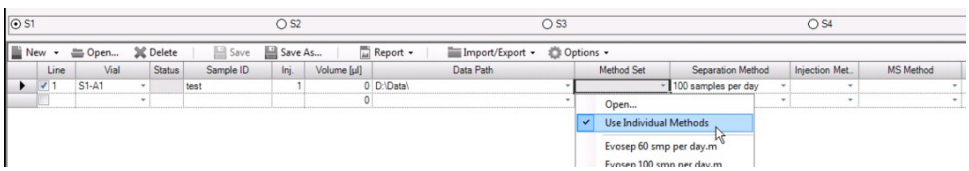
1. When HyStar has restarted, create the Evosep One tray type by clicking “Sample Table” followed by the small gearwheel icon in the “Sample Table Editor” window.



2. Choose “Evosep” as the “Tray type”. Select “96Evotip” for “Slot 1” – “Slot 6”. Click “OK”.



3. In the sample table, enter the following parameters in Line 1:
   - Vial: S1-A1
   - Sample ID: test
   - Method Set: Click the small arrow and select “Use individual Methods”
   - Separation Method: 100 samples per day



4. Click “Save As…” with the name “Evosep One Sample table”. Click “OK” to save the sample table.

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

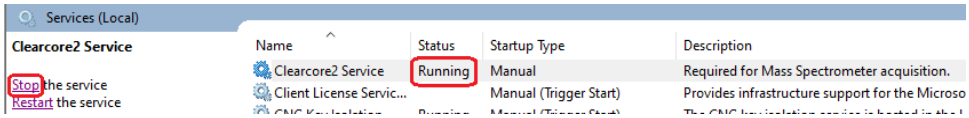
4.3.6 Flow to Column – 20% Solvent B Enables MS calibration

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

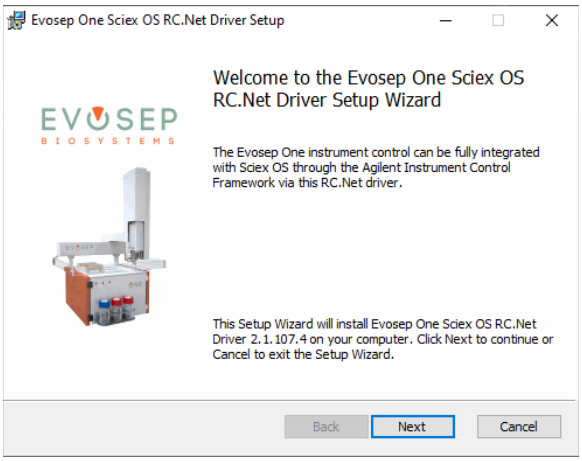
4.4.1 Installation Prerequisites

1. Ensure SCIEX OS 2.0, or newer, is installed.
2. Close SCIEX OS, if running.
3. Open Windows Services app and Stop the Clearcore2 Service, if running.

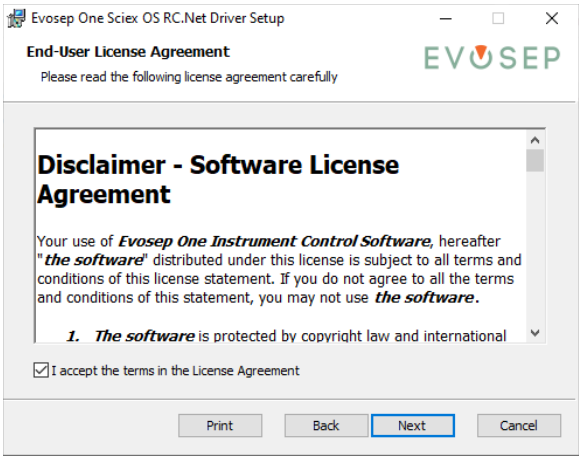


4.4.2 Installation Procedure

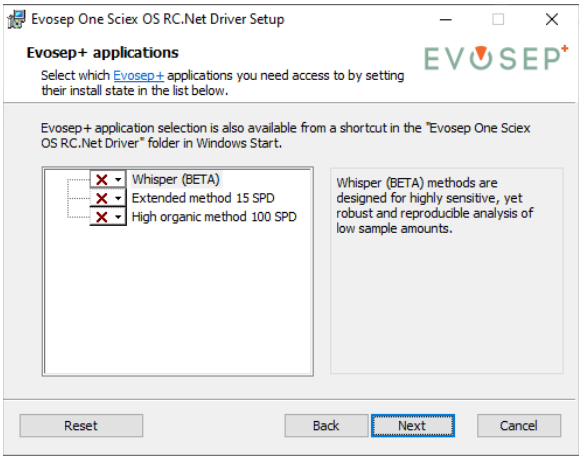
1. Connect the Evosep One instrument to the computer, or through a network switch, via ethernet cable and make sure that the instrument is switched on.
2. Run the Evosep One SCIEX OS RC.Net Driver 2.x.x.x Windows installer.
3. Click “Next”.



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



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

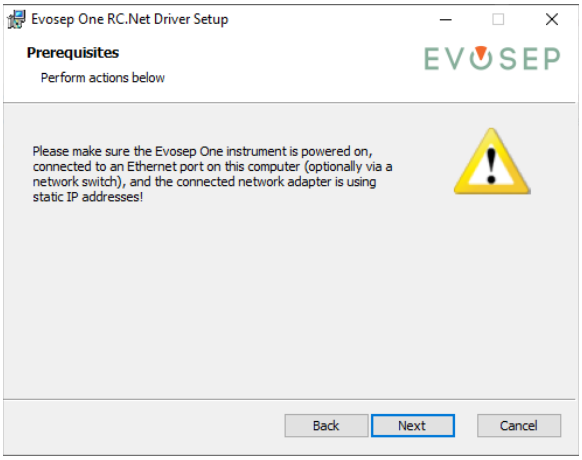


Important notes:

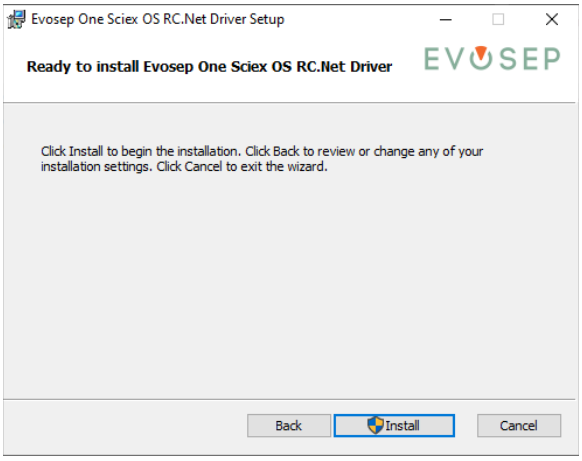
The +applications are not installed during a standard installation as Evosep prefers that the customer actively chooses the +applications they need.

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

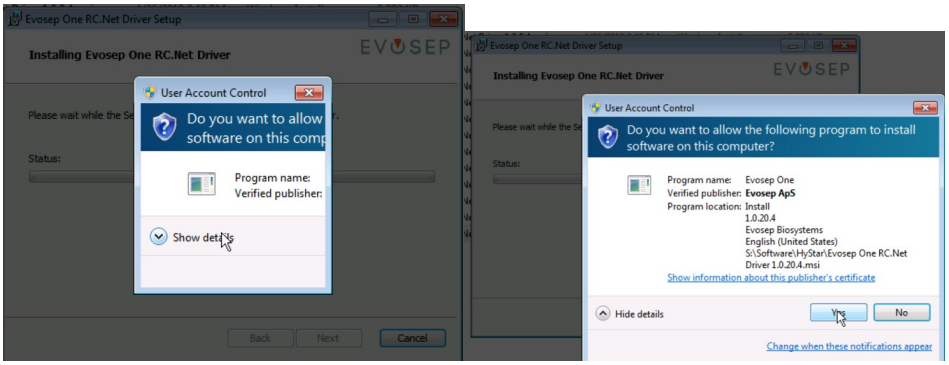
6. Please read the information in the “Prerequisites” window carefully, then click “Next”.



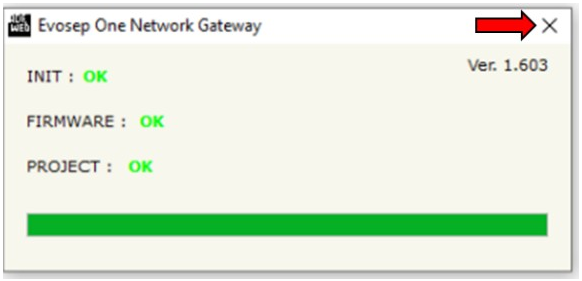
7. Click “Install” to begin the installation. Click “Yes” to any pop-up windows during the install.



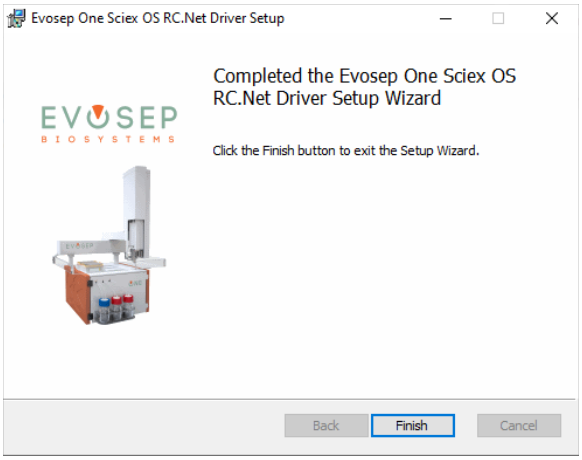
8. Click show details (if window not fully visible) then click “Yes” to allow the program to install the software.



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

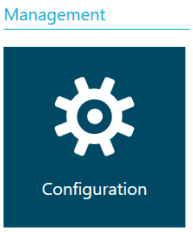


10. Click “Finish” to close the window when the installation is complete.



4.4.3 Create SCIEX OS Hardware Configuration for Evosep One

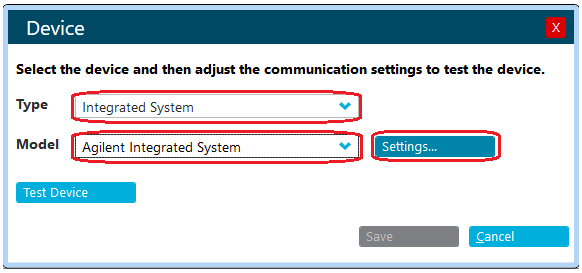
1. Start SCIEX OS – this will automatically start the Clearcore2 Service.
2. Select “Configuration”.



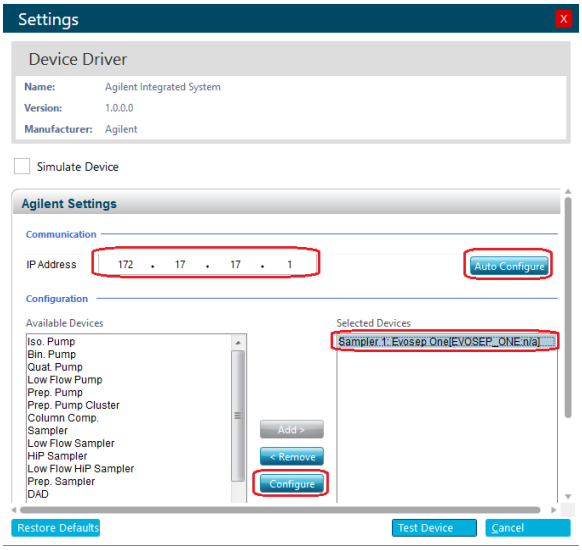
3. If the current configuration is active, click the “Deactivate” button on the toolbar.

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

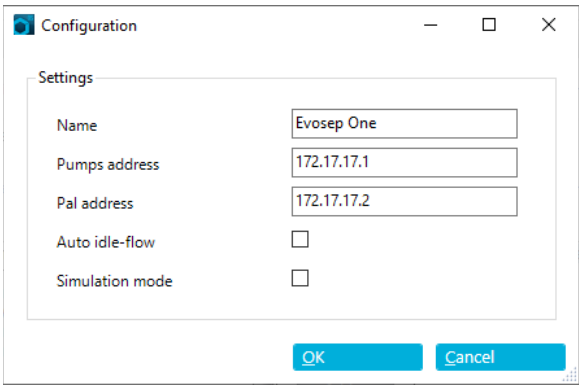
5. On the “Device” dialog, select “Integrated System” and “Agilent Integrated System”. Click “Settings…”.



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



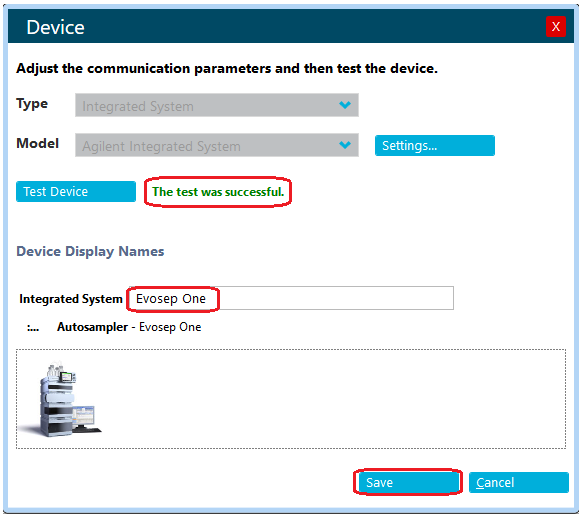
7. After a moment, the Evosep One instrument appears in the “Selected Devices” list. Select it and click “Configure”.
8. On the “Configuration” dialog, set the following instrument parameters:



- Name: Used for display, leave at default.
- Pumps address: Communication setting, leave at default.
- Pal address: Communication setting, leave at default.
- Auto idle-flow: Start idle-flow after a few minutes of inactivity.
- 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.

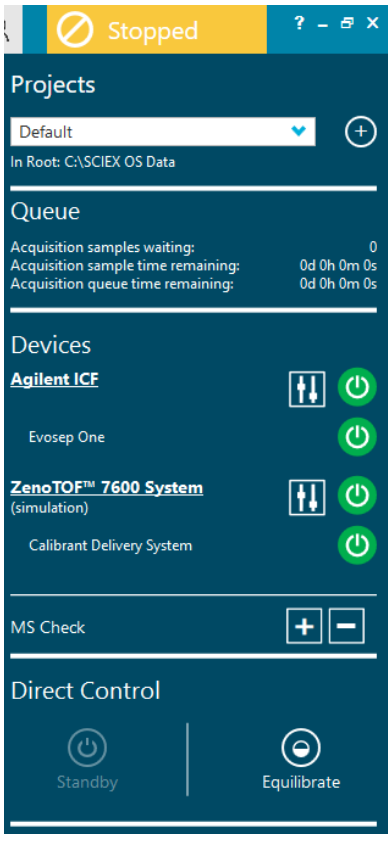
9. When satisfied with the configuration, click “OK” to save and close the dialog.
10. On the “Settings” dialog, click “Test Device”.

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



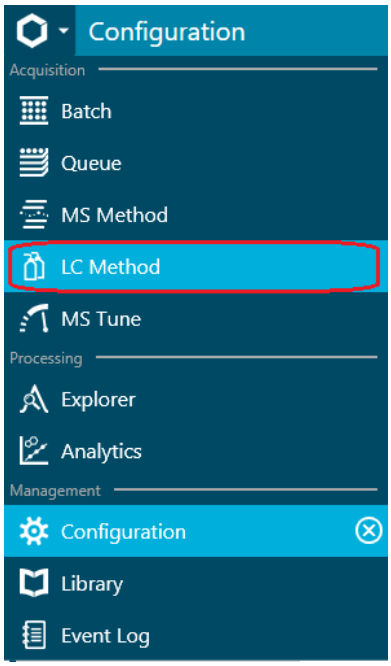
12. Click “Save”.
13. Click “Activate Devices”.

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



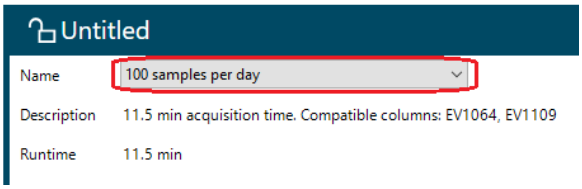
4.4.4 Create SCIEX OS LC Methods for Evosep One

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

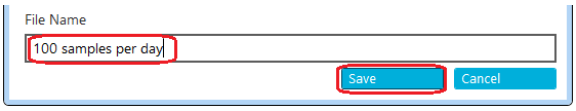


2. Click “New”.

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. Repeat Steps 3 – 4 to create a LC method for each of the Evosep One methods you want to use in your project.
   - Note that the method acquisition runtime, which you need when creating the corresponding MS method, is MS dependent and not described here.
   - The Evosep One standard methods have the following gradient lengths:

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

Important note:

The duration of the "System and column wash" method is column dependent (approximately 10 min) and there is no need to collect MS data during the wash; therefore, the MS acquisition time should be set to 1 minute.

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

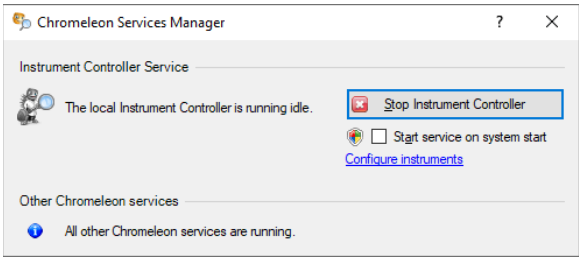
For the operating system and environment in general, follow the requirements for the version of Chromeleon or SII for Xcalibur, which the driver will be running under. Currently, Windows 10 64-bit US English is supported.

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 state that validation was done with Xcalibur 4.5 and 4.5 SP1.

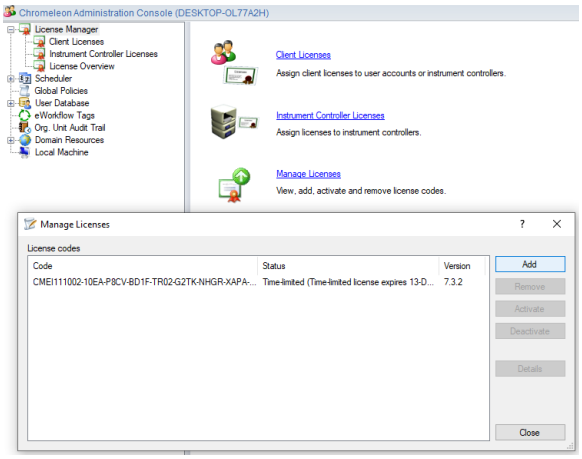
4.5.3 Prerequisites

1. Make sure that either:
   - Thermo Chromeleon 7.3.1 or newer is installed, or
   - Thermo SII for Xcalibur 1.7 or newer is installed
2. Close Chromeleon and/or Xcalibur, if running.
3. Open “Chromeleon Services Manager” and click “Stop Instrument Controller”, if running.

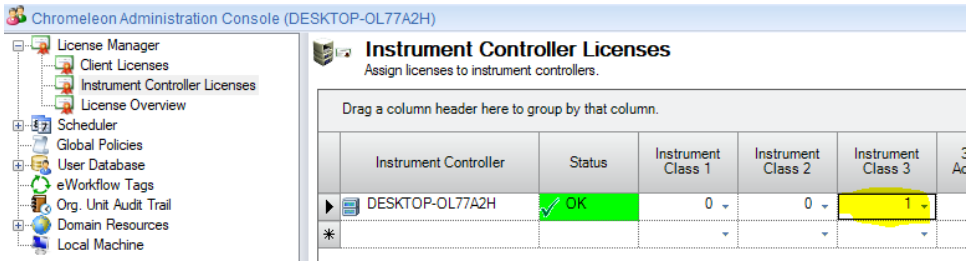


4.5.4 Chromeleon/SII for Xcalibur License

1. License can be added through the Chromeleon Administration Console.
2. Click “Manage Licenses” and “Add” the license key:

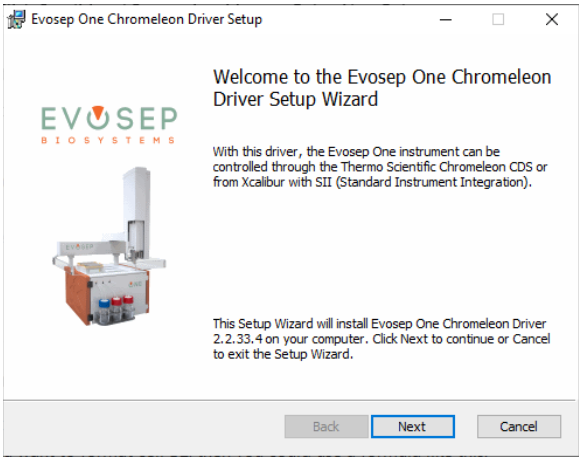


3. Under “Instrument Controller Licenses”, make sure that at least one Class 3 instrument is allowed.

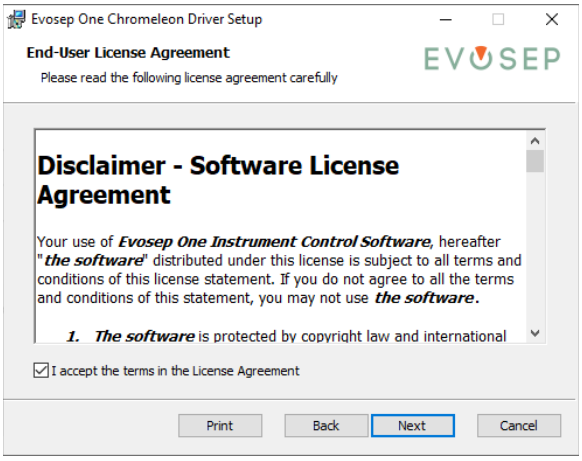


4.5.5 Installation Procedure

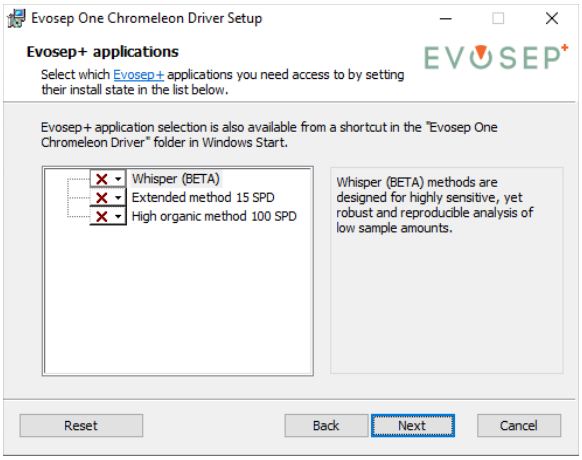
1. Connect the Evosep One instrument to the computer, or through a network switch, via ethernet cable, and make sure that the instrument is switched on.
2. Run the Evosep One Chromeleon Driver 2.x.x.x Windows installer.
3. Click “Next”.



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



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

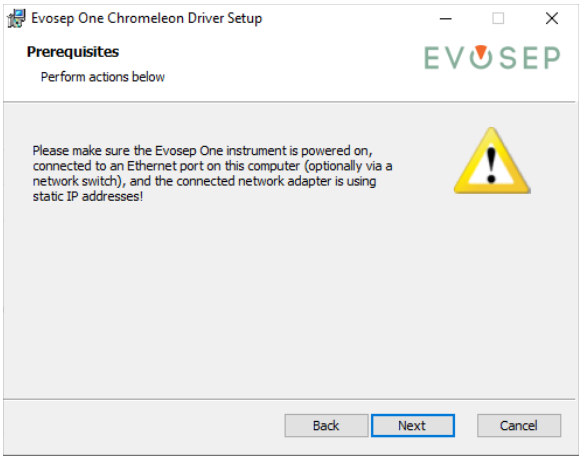


Important notes:

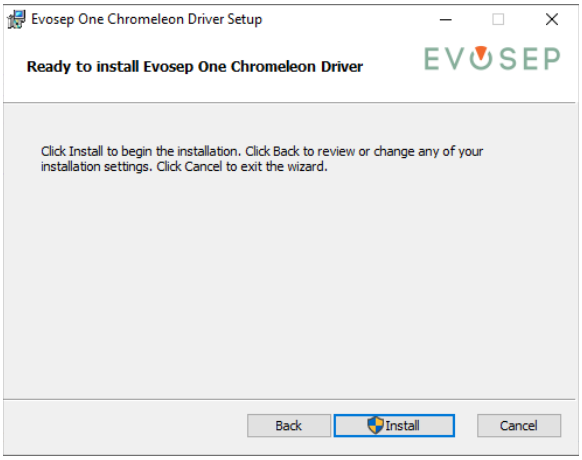
The +applications are not installed during a standard installation as Evosep prefers that the customer actively chooses the +applications they need.

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

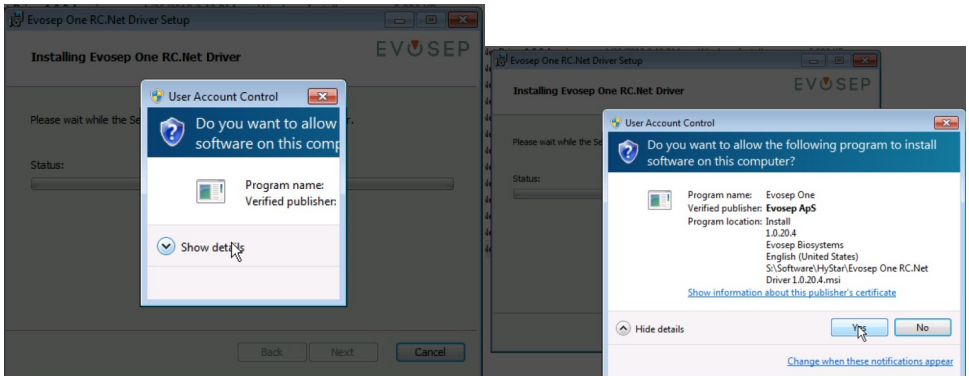
6. Please read the information in the “Prerequisites” window carefully, then click “Next”.



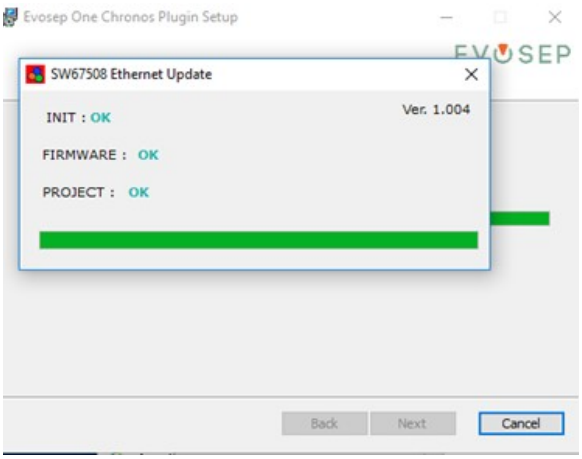
7. Click “Install” to begin the installation. Click “Yes” to any pop-up windows during the installation.



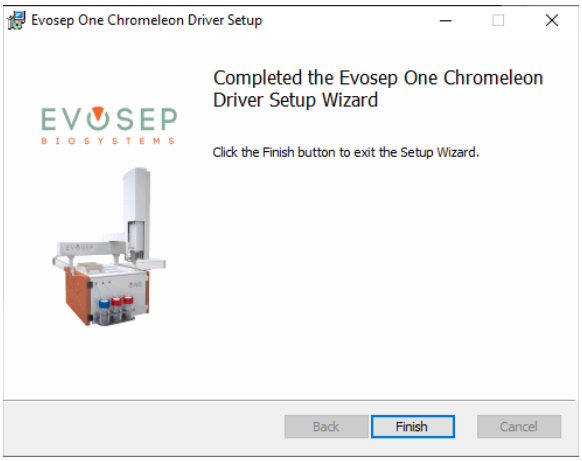
8. Click “Show details” (if window not fully visible) then click “Yes” to allow the program to install the software.



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



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

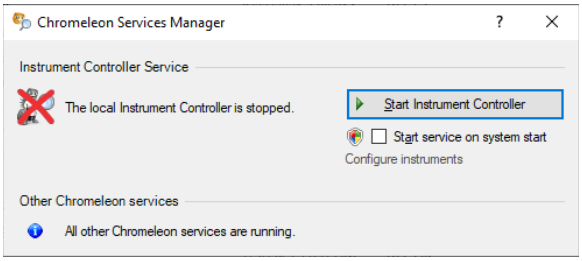


4.5.6 Checking Driver Installation

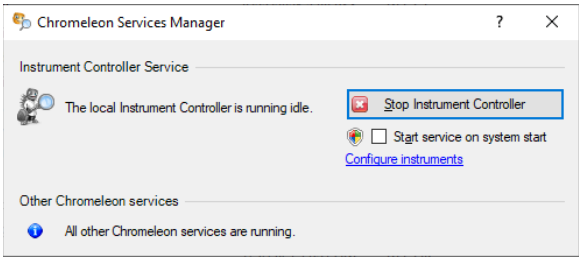
The Evosep One driver integrates into Chromeleon's Installation Qualification by registering its own inventory file. This means you can simply run "Station IQ" from the "Thermo Chromeleon 7" Start menu folder to verify the installation.

4.5.7 Create Chromeleon Hardware Configuration for Evosep One

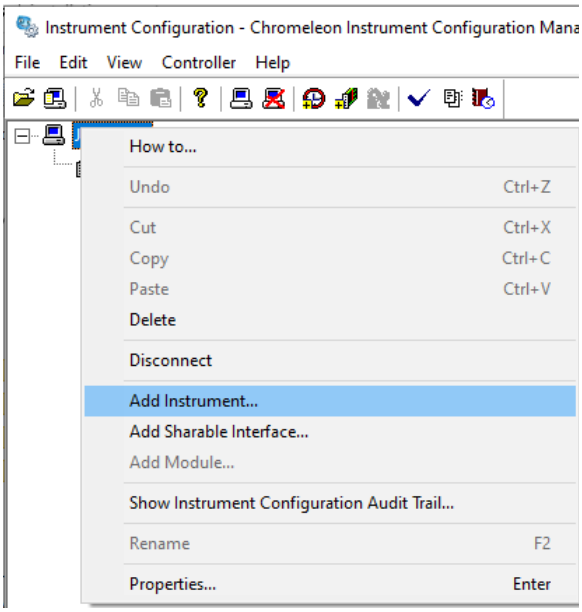
1. From the "Chromeleon Services Manager" window, click "Start Instrument Controller".



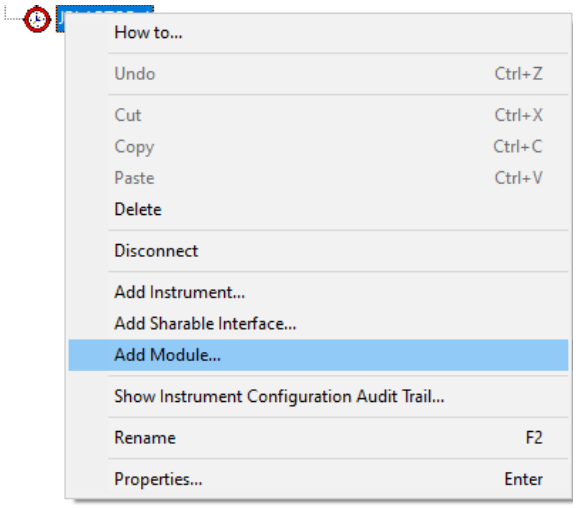
2. Click "Configure instruments".



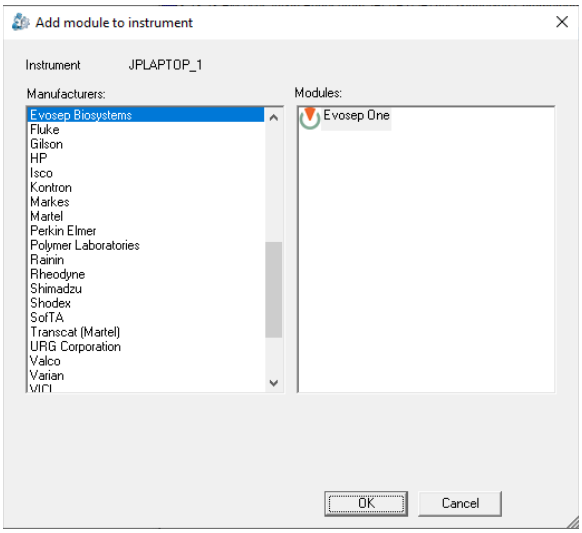
3. Right-click the controller instance and click "Add Instrument…".



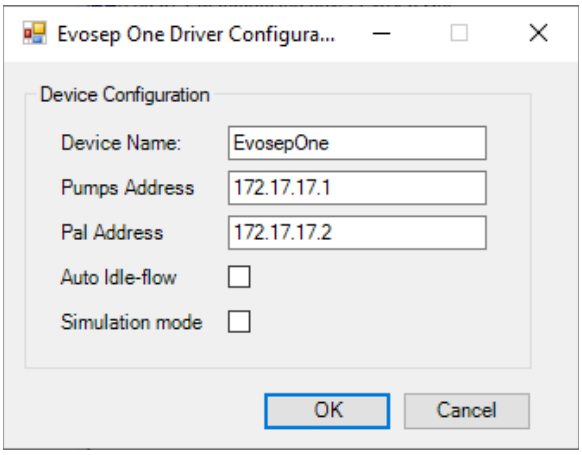
4. Enter desired name, or leave at default, then click "OK".
5. Right-click the added instrument and click "Add Module…".



6. Select “Evosep Biosystems” in the “Manufacturers” list and “Evosep One” in “Modules”. Click “OK”.



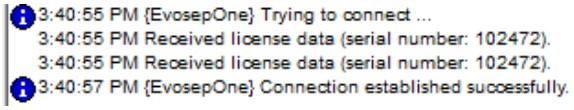
7. On the Evosep One Driver Configuration dialog, you can set the basic instrument settings:



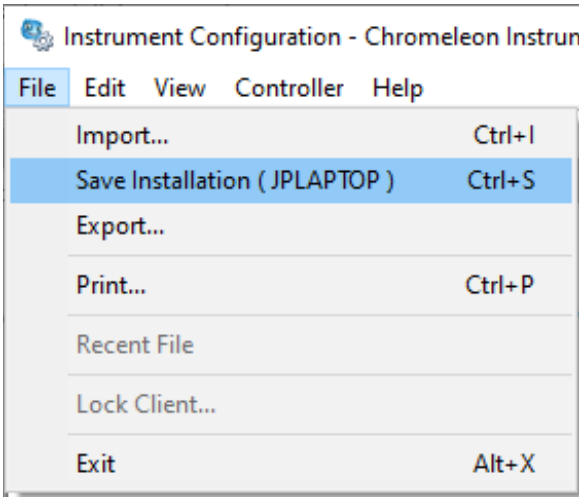
- Name: Used for display, leave at default.
- Pumps address: Communication setting, leave at default.
- Pal address: Communication setting, leave at default.

- Auto idle-flow: Start idle-flow after a few minutes of inactivity.
- 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.

8. When satisfied with the configuration, click “OK” to close the dialog.
   - The message window should show something like this:



9. Click “File”, then “Save Installation”.



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

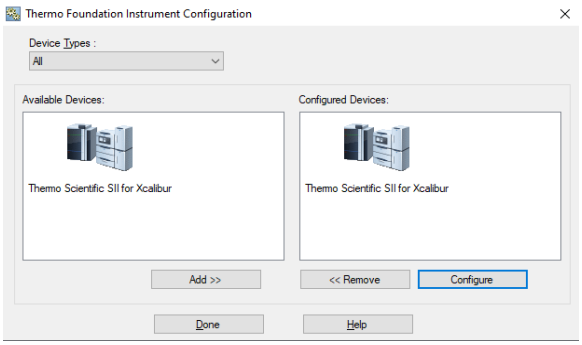
4.5.8 Configuring Thermo Scientific SII for Xcalibur

Important note:

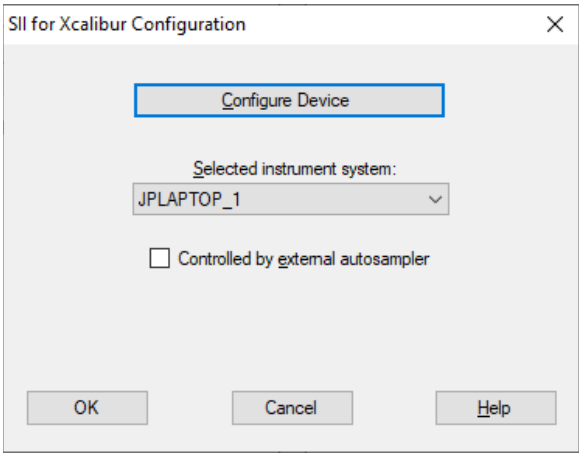
This is only necessary if using the Evosep One driver in Xcalibur, through SII. If using the driver directly in Chromeleon, you do not need to perform this instrument configuration.

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

2. In the “Available Devices” section, click “Thermo Scientific SII for Xcalibur” and click “Add >>”.



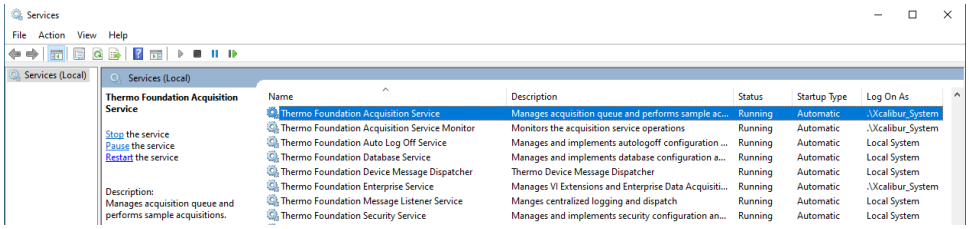
3. In the “Configured Devices” section, click “Thermo Scientific SII for Xcalibur” and click “Configure”.
4. In the “SII for Xcalibur Configuration” window, click “Configure Device”.



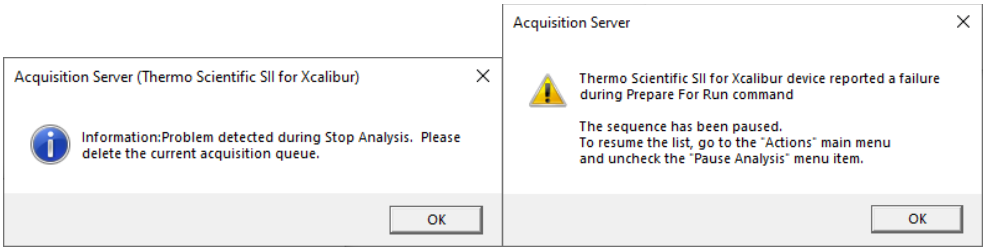
5. Close the window that opened, then click “OK”.
   - This may seem unnecessary, but the configuration will not be initialized properly if this step is not performed!
6. In the “Thermo Foundation Instrument Configuration” window, click “Done”.

4.5.9 Important – When Upgrading from an 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 prior to starting Xcalibur.



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

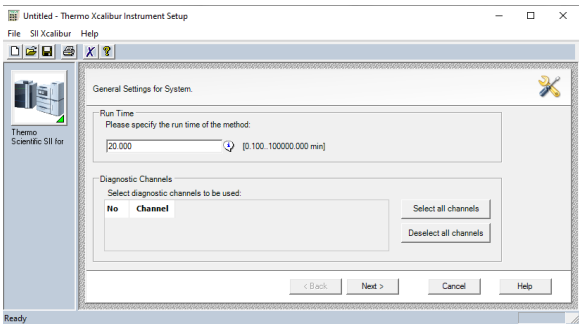


4.5.10 Create LC Methods for Evosep One

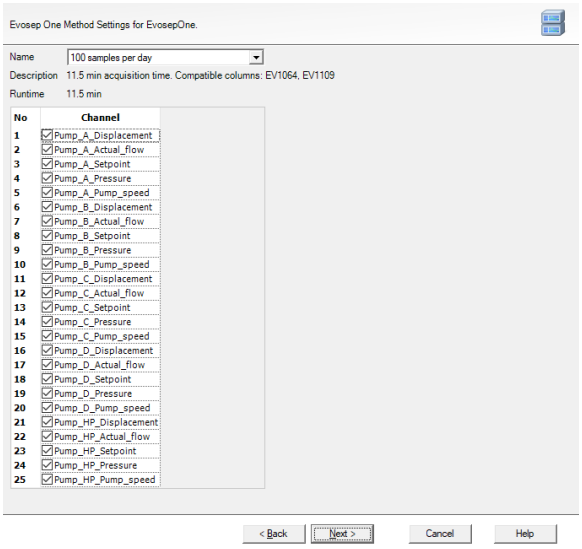
This section describes how to create LC methods through Xcalibur.

1. In Xcalibur, click “Instrument Setup”.

2. Click “Next”.
   - The run time can be ignored here as 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…”.
    - 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.

4.6 Adding Specialized Applications to the Evosep One

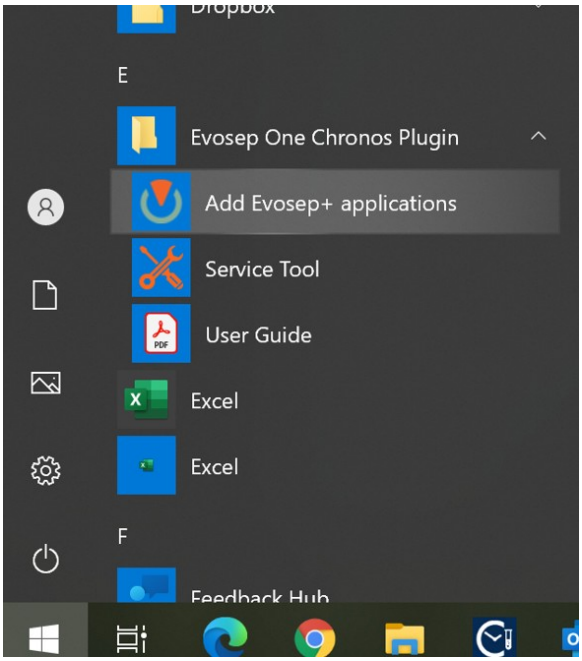
The Evosep One is preconfigured with 6 standard methods with throughput ranging from 500 to 30 samples per day. In addition, it is now possible to add several specialized methods.

Important note:

Whisper Zoom methods will require manual reconfiguration of the system plumbing. For more information, please see Section 7.

Methods must be manually enabled by the following procedure:

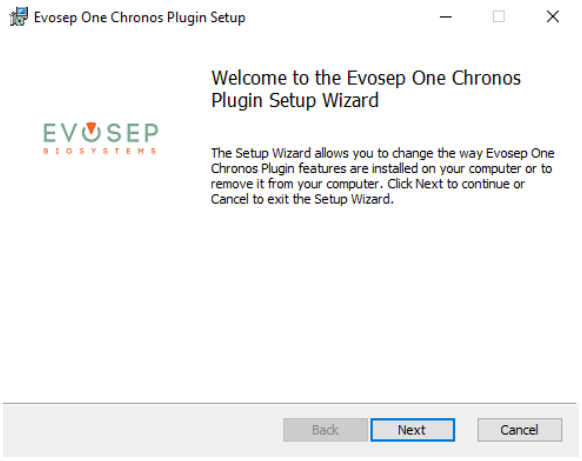
1. Close the Evosep One control software (Chronos/HyStar).
2. Depending on what software is being used on the Evosep One, choose the appropriate folder from the Windows Start menu. I will be one of:
    - Evosep One Chronos Plugin
    - Evosep One HyStar Driver
    - Evosep One SCIEX OS Driver
    - Evosep One MassHunter Driver
    - Evosep One Chromeleon Driver



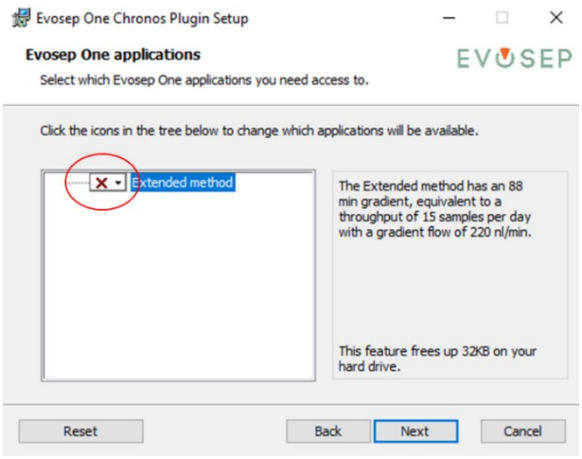
3. Expand the folder and click “Add Evosep+ applications”.
4. Click next in the Evosep One Setup window.

Important note:

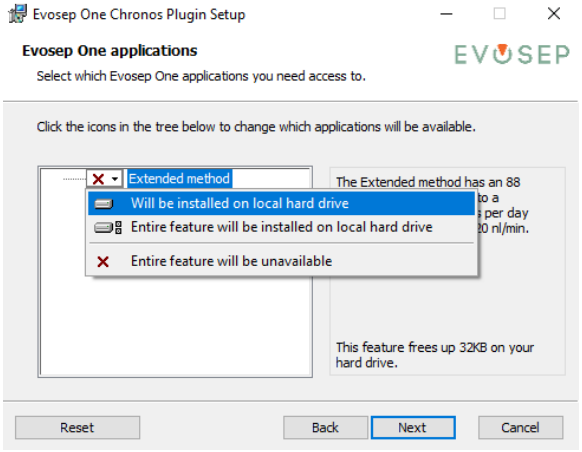
The following example is for the Extended method. Other specialized methods follow the same procedure.



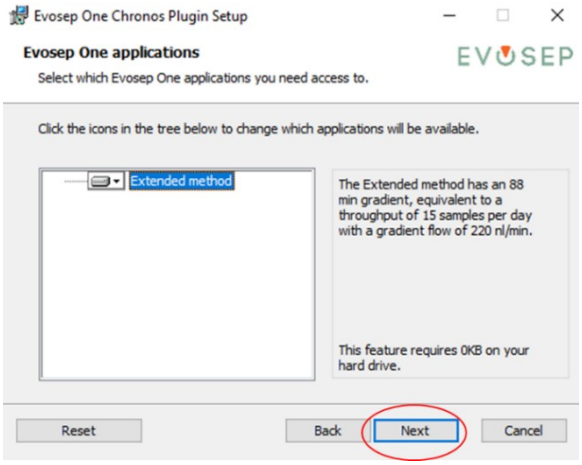
5. Click the “Extended method” icon.



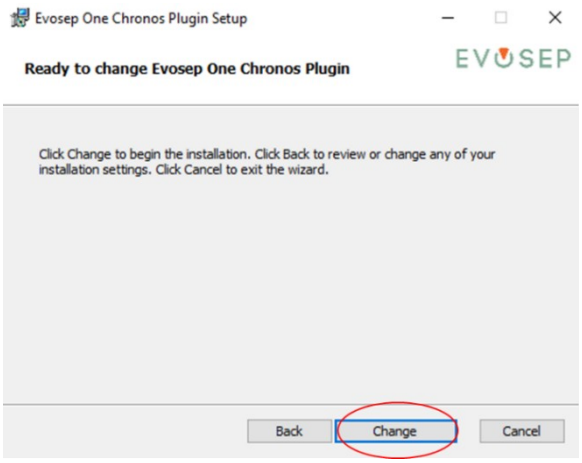
6. Choose “Will be installed on local hard drive”.



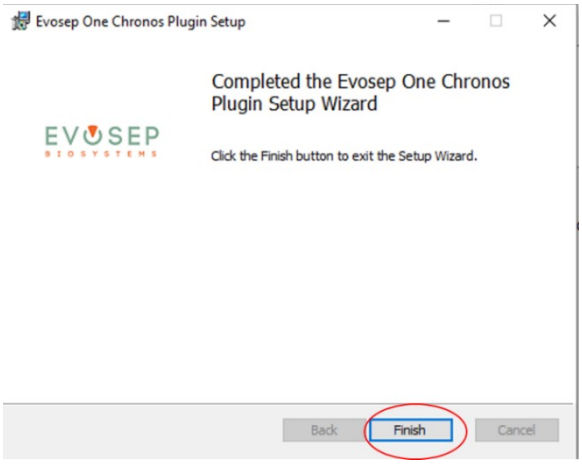
7. Click “Next”.



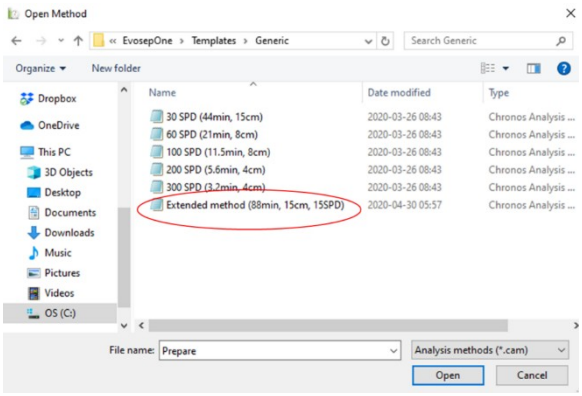
8. Click “Change”.



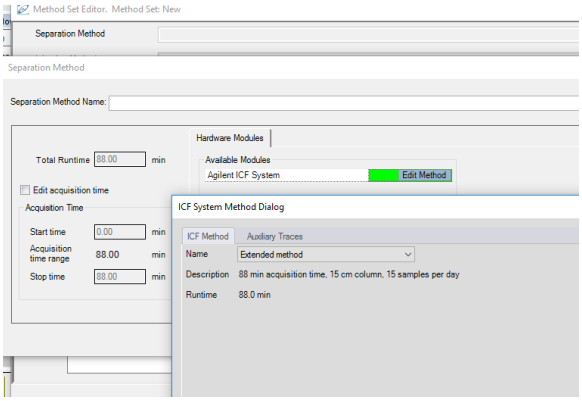
9. Click “Finish”.



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 Section 4.3.4 “Create Evosep Separation Methods” and follow the procedure for creating a separation method for the 88 minute Extended method.

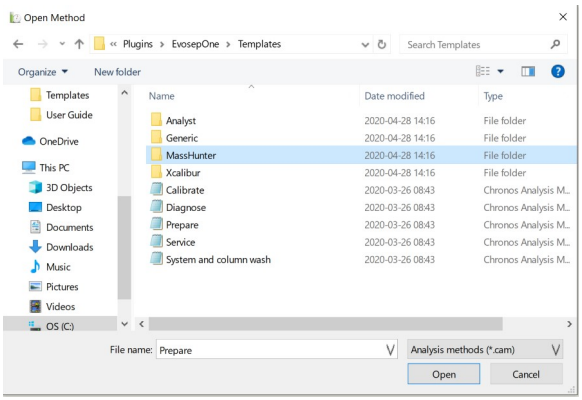

Method Set Editor. Method Set: New
Separation Method
Separation Method
Separation Method Name:
Hardware Modules
Total Runtime 88.00 min
Available Modules
Agilent ICF System
Edit Method
Edit acquisition time
Acquisition Time
Start time 0.00 min
Acquisition time range 88.00 min
Stop time 88.00 min
ICF System Method Dialog
ICF Method
Auxiliary Traces
Name Extended method
Description 88 min acquisition time, 15 cm column, 15 samples per day
Runtime 88.0 min

5 Instrument Software Control

Please refer to Chapter 8 “Running Samples Using Evosep One” to view all safety warnings, cautions, and concerns.

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). This enables Chronos to 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. 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 diagnostic 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

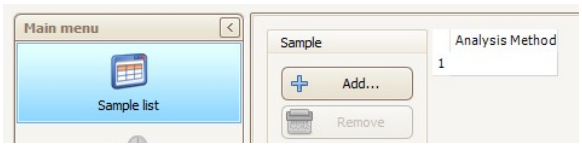
The Evosep One instrument software is configured with several predefined methods for maintenance, diagnostics, service tasks, and sample acquisition. All methods are specifically optimized for the instrument

hardware and cannot be modified by the user. The individual methods are described in detail in the following sections.

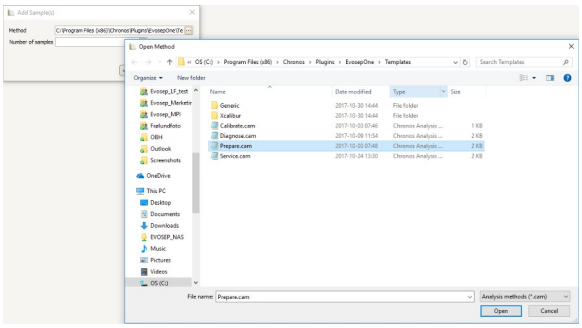
5.1.3 Sample Lists

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

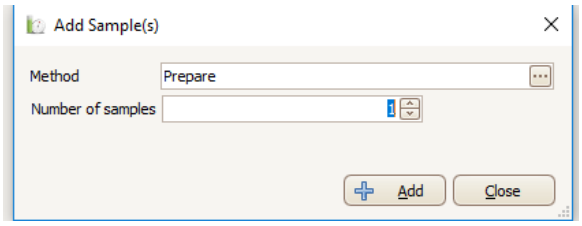
1. Click the “Add” button.



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.

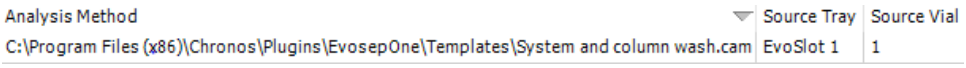


4. The method file will be entered in the sample list. Depending on which method was chosen, a range of columns will be displayed.
   - Sample methods:
     - Source Tray (1-6), Source Vial (1-96), and Xcalibur Method, Filename, and Output Directory must be specified.
     - Sample Name, Xcalibur Post Acquisition Program and Comment are optional.

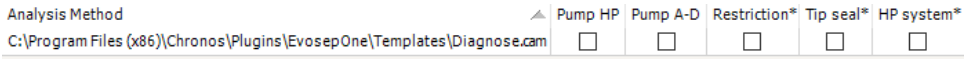
	Analysis Method	Source Tray	Source Vial	Sample Name	Xcalibur Method	Xcalibur Filename	Xcalibur Post Acquisition Program	Xcalibur Output Dir	Comment
1	C:\Pro...9).cam	EvoSlot 1	1		C:\X...\Methods				

   - Prepare methods:
     - Select one or more methods to run using the checkbox(es).

- System and column wash method:
  - Source Tray (1-6) and Source Vial (1-96) must be specified.



- Diagnose methods:
  - Select subsystem test, e.g. “Pump HP” and/or “HP system” method, by using the checkbox(es).



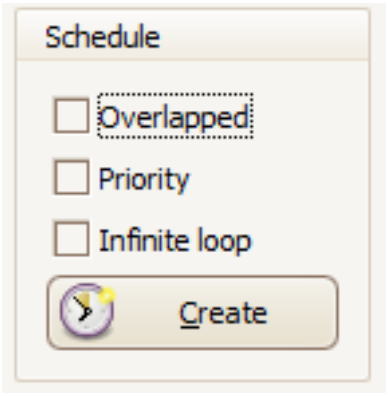
- Calibrate methods:
  - Select method(s) by using the checkbox(es).
  - The “Flow sensor ABCD” calibration script performs a multipoint flow sensor calibration of the low pressure pumps A, B, C and D.
  - The “Flow sensor HP” calibration script performs a multipoint flow sensor calibration of the high pressure 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.

- Service methods:
  - Select one or more of the options by ticking the checkbox(es).

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

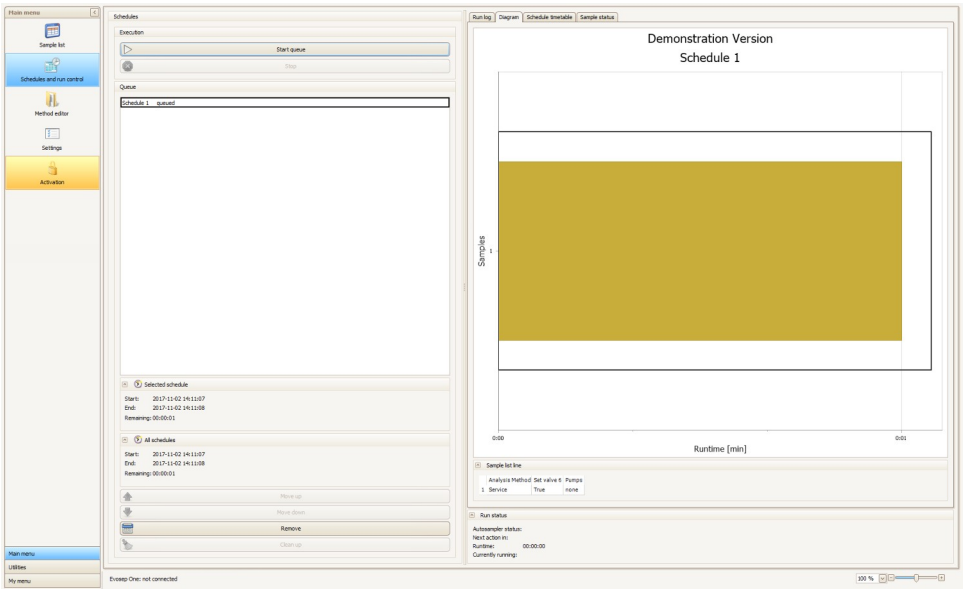
5.1.4 Creating a Schedule

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

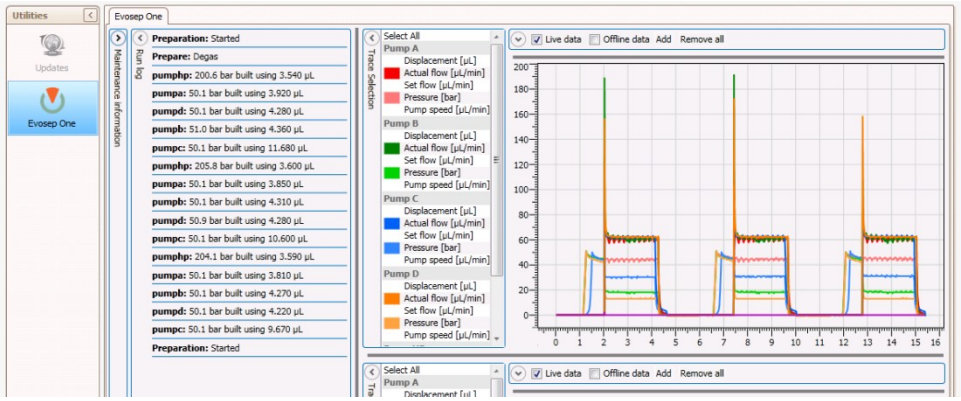


5.1.5 Running a Schedule

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

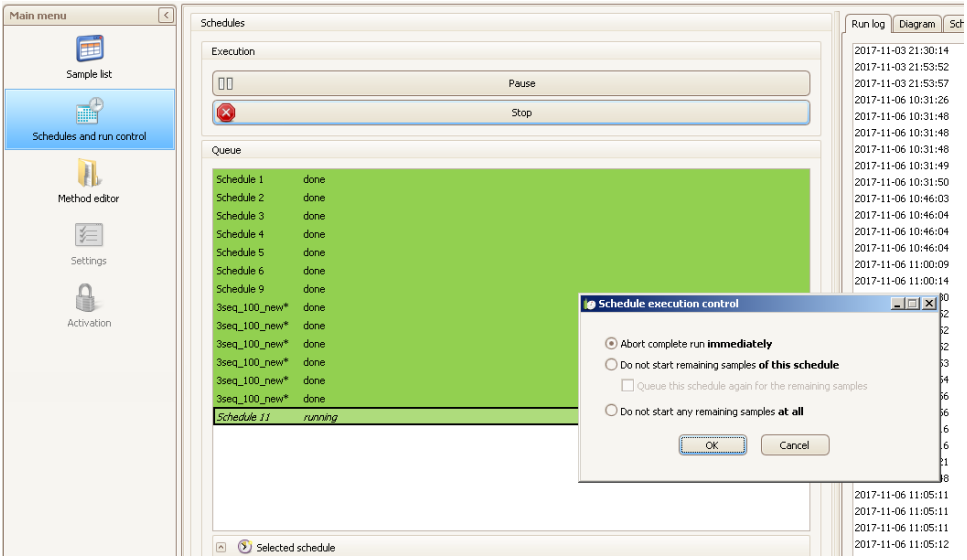


2. Start the sample queue by pressing the “Start queue” button.
3. Additional schedules can be entered in the sample queue. The execution order of the schedules can be shuffled up and down using the arrow buttons.
4. Information about current and previous analyses, such as diagnostic leak test, etc., is shown in the Evosep One Run log tab.

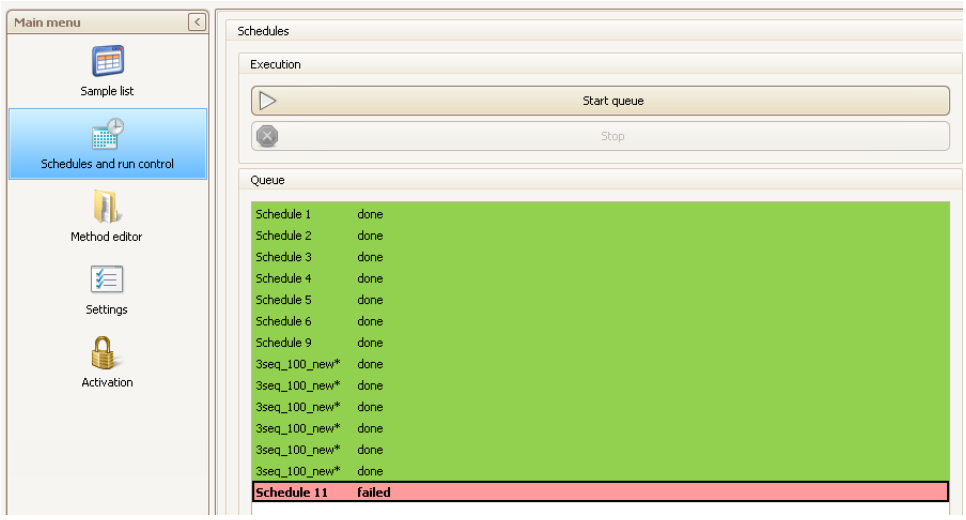


5.1.6 Aborting Samples

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



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



3. The user can choose to restart the failed schedule by pressing the “Start” button. The failed schedule can be removed from the list using the “Remove” button.

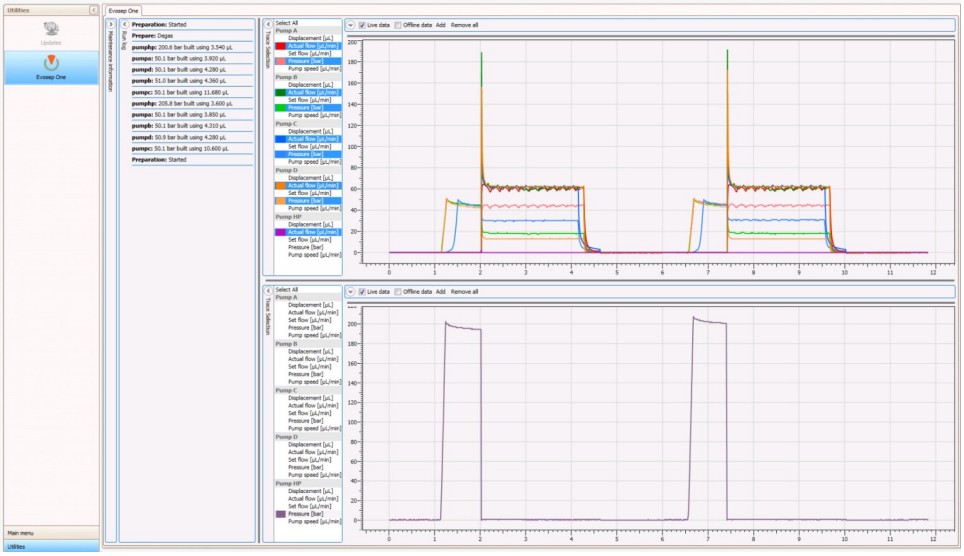
Important note:

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

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

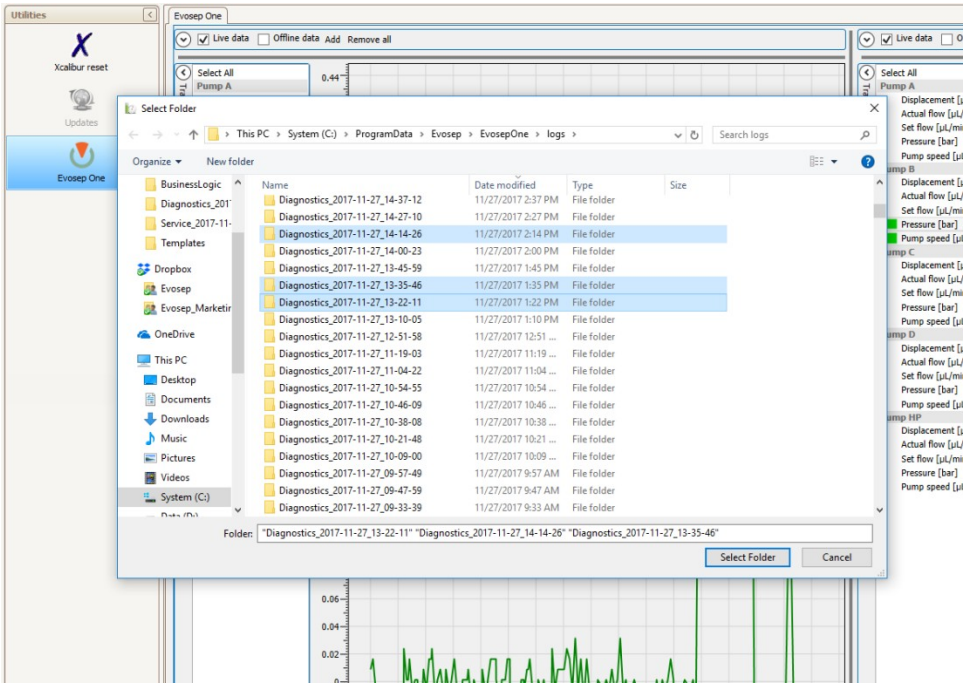


2. The following traces can be selected for each pump by a single left click on the side bar:

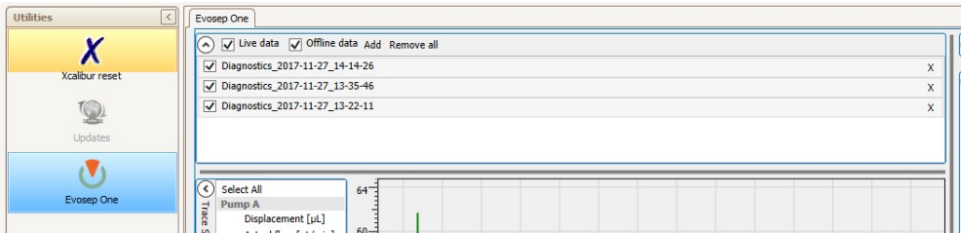
Name	Description
Displacement [µl]	Pump piston position. Pumps are full when Displacement is 0 µl and empty when displacement is 55 µl and 138 µl for Pump HP and Pump A/B/C/D, respectively.
Actual flow [µl/min]	The actual flow is measured in the liquid stream for each pump using an individual flow sensor for each pump.
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 an individual pressure sensor for each pump. 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 velocity. It may differ from the set point 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 that can be resized by left mouse clicking the intersection and dragging. 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-in by “mousing over” the area of interest while holding down the “Shift” button + left mouse click.
3. It is possible to view previous pump traces by clicking the “Offline data” check box. Select one or more sample folders and click “Select Folder”. Alternatively, you can drag-and-drop files from Windows File Explorer to the “Offline data” section.

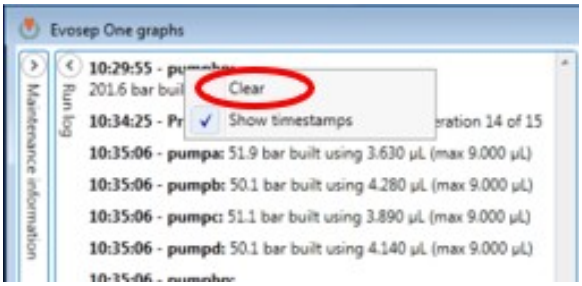


4. The opened sample folders are visible in the “Offline data” list. Individual data files can be selected/unselected using their respective checkboxes.

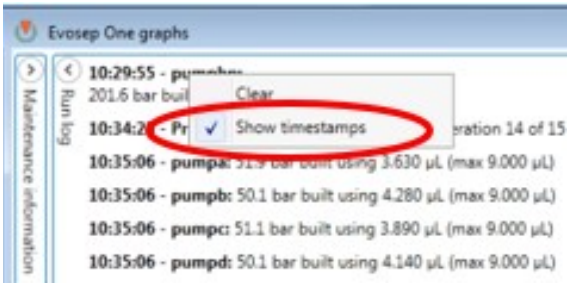


5.1.8 Run Log

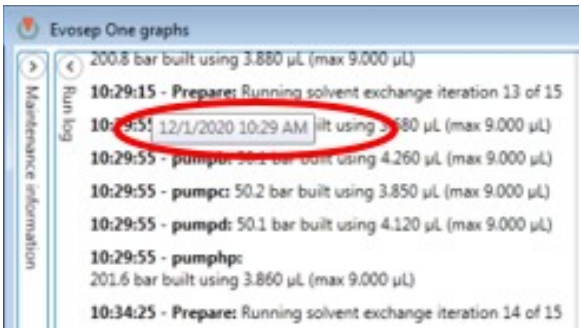
1. The run log displays information to the User regarding the Sample Methods, Calibration, Diagnostics, and Preparation procedures being executed. The log will show which, and when, each program was started and finalized, including pass/fail criteria.
2. The run log can be flushed by right clicking the log and pressing “Clear”.



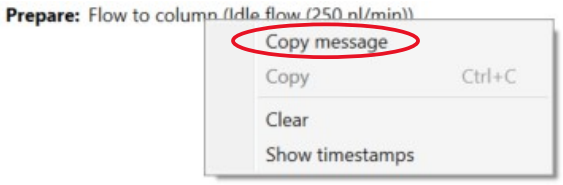
3. The User can choose to enable/disable timestamps in the log by right clicking the log and selecting/deselecting the “Show timestamps” option.



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



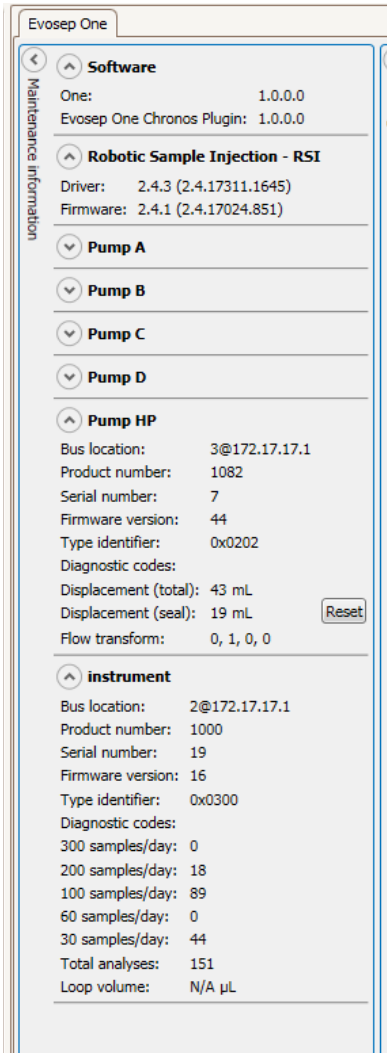
5. The User can also select and copy text from the Run log by right clicking and selecting “Copy message”.



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.

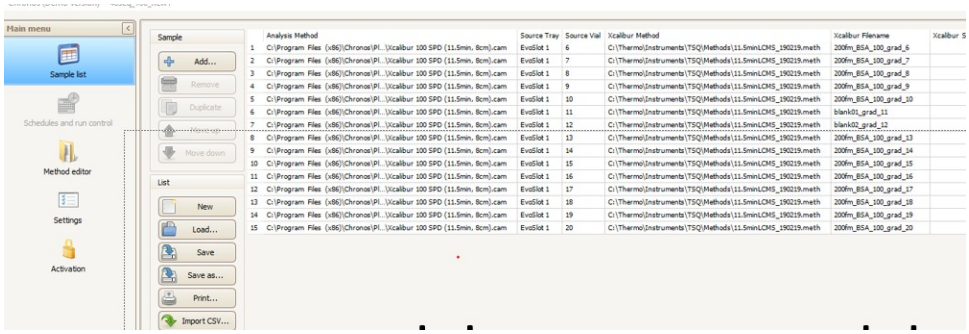
1. Software
   - Plugin software version
2. Pump
   - Serial number
   - Firmware version
   - Displacement (total): Total pumped volume
   - Displacement (seal). Pumped volume since reset (if a pump seal was replaced)
3. Instrument
   - Serial number
   - Firmware version
   - Analysis completed (by type)



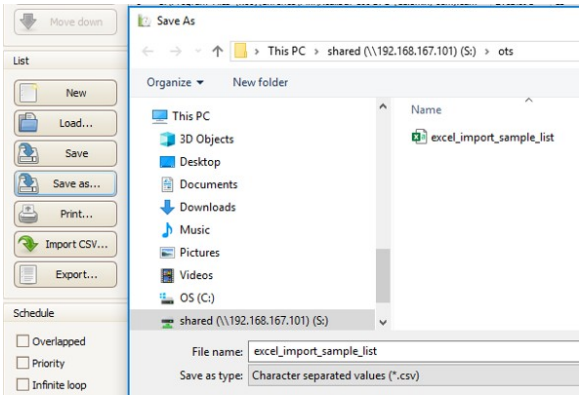
5.1.10 How to Import CSV Files into Chronos

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 if it contains the values to fill-in the columns described in the Chronos .cam file.

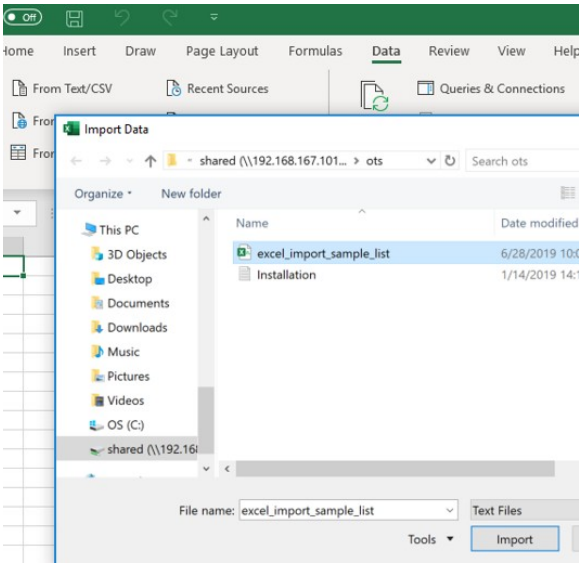
1. Create a sample list in Chronos (this can then be edited in Excel, for example).



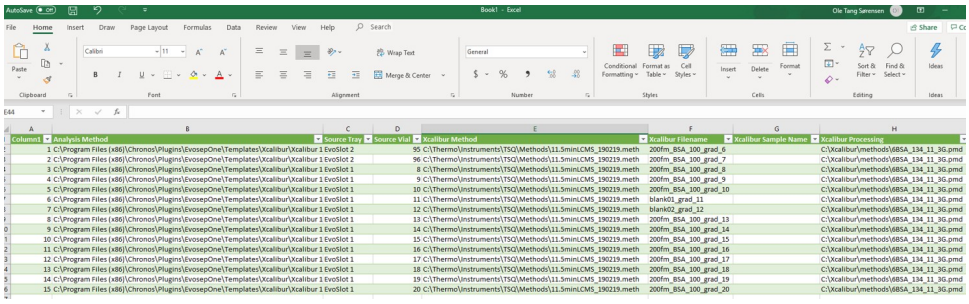
2. Click “Save as…” and choose “Character separated values” as type.
    - The extension CSV typically (and by some standards) denotes “Comma Separated Values”. Depending on the Windows regional settings, another character may be used as separator, such as a semicolon or tab. Keep this in mind when you import the data later!



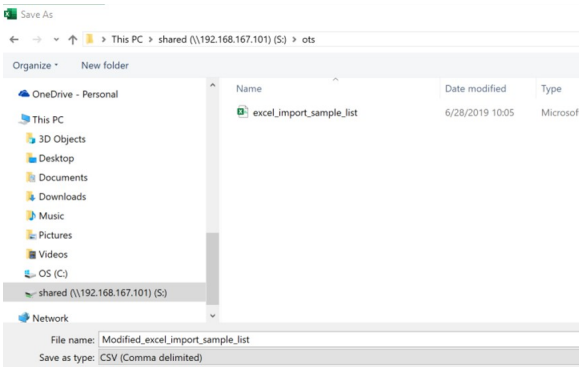
3. In Excel, click “Data” and choose to open “From Text/CSV”.



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

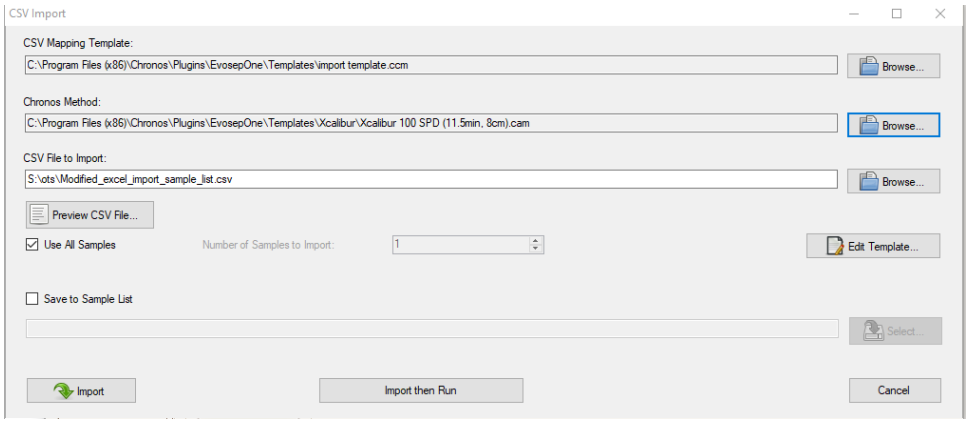


5. Click “Save As” to save the modified sample list and select type “CSV (comma delimited)”.

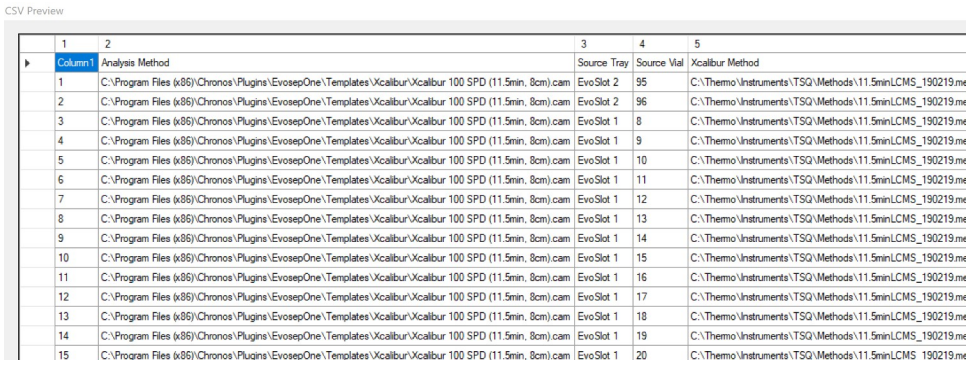


6. To import sample list into Chronos, click “Import CSV…”
   - The first time you perform this operation in Chronos, you will be taken directly to the following step.

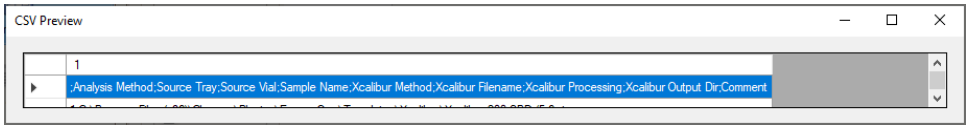
7. In the CSV import window, do the following.
   - Choose “Browse…” to select the desired “Chonos Method”. This ensures the correct format of the sample list columns (e.g. for Xcalibur, use one of the Evosep Xcalibur methods).



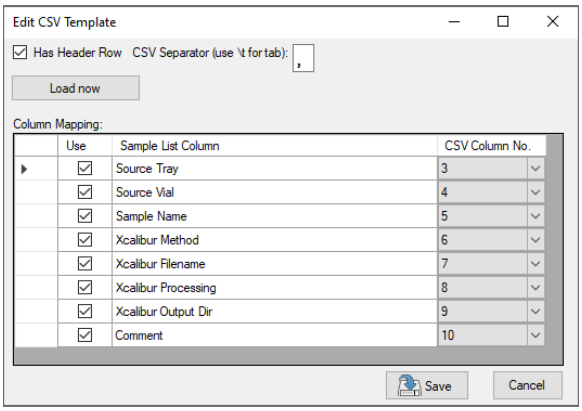
- Choose “Browse…” to select the “CSV File to Import”.
- Click “Preview CSV File…” to verify the correct number of column in the sample list.



- If all the columns shown are merged as a single column, you must adjust the CSV Separator, save the template, and preview the file again.

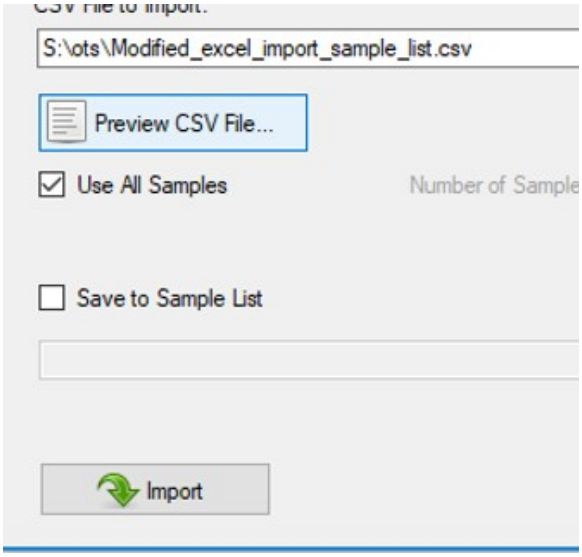


- Click “Edit Template…” to ensure the CSV Separator is set to the character used in your CSV file.



- Match the sample list columns to the headers. Ensure to check the “Use” column, otherwise that data will not be included!

- Click “Save” to save the template. This can be used again for other imports.
- Click “Import” to add the sample list to Chronos.

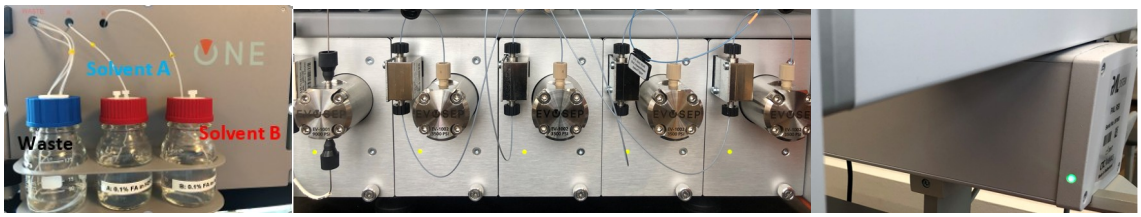


5.2 Evosep Driver for Control of Bruker MS

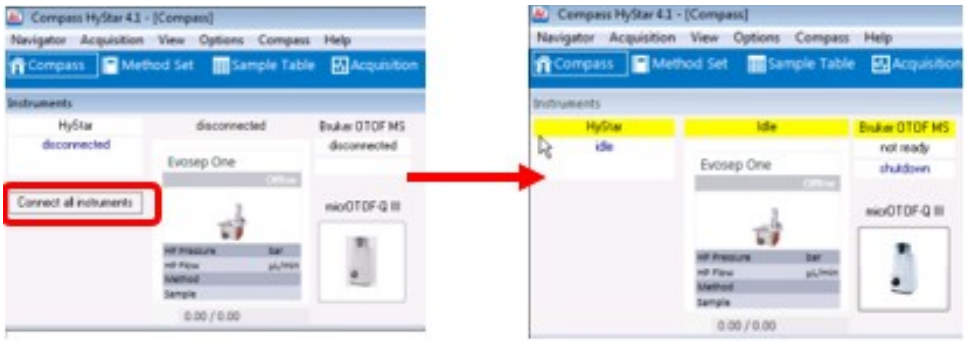
5.2.1 Instrument Preparation with Compass HyStar

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

1. Check the Evosep solvent levels (waste, solvent A, and solvent B; left picture) and LEDs displaying power for the pumps (middle picture) and autosampler (right picture).
   - The LEDs must be green.



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

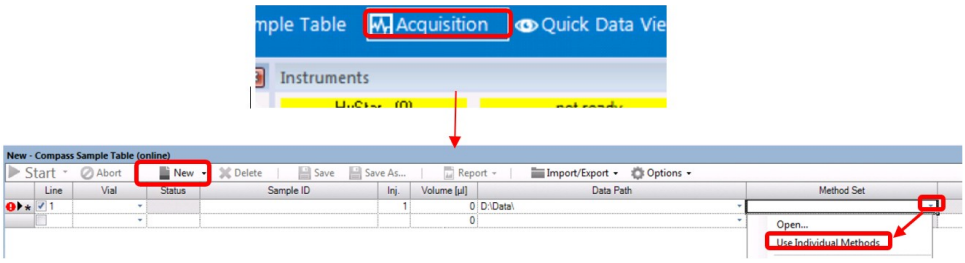


3. Verify the column and emitter are connected to the MS ion source. If not, connect the column and emitter, and run the Preparation “flow to column” script to check spray.

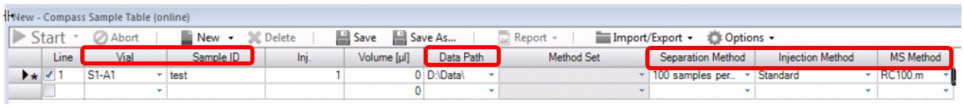
5.2.2 Sample Acquisition with Compass HyStar

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

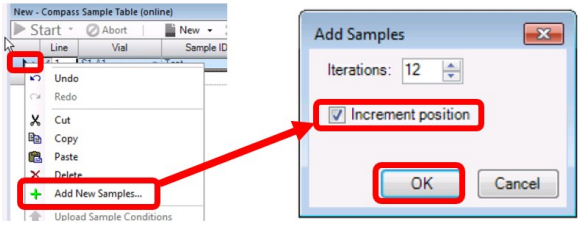
3. In HyStar, click the “Acquisition” Icon to open the HyStar Sample Table. Click “New” and click the small arrow in the first line under “Method Set”. Choose “Use Individual Methods”.



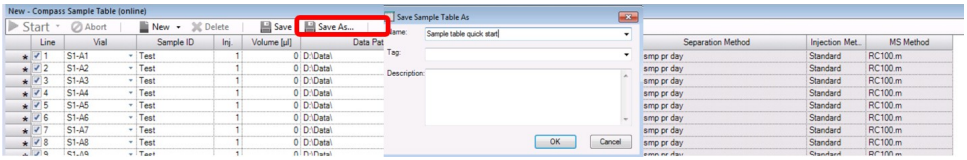
4. In the Sample Table, populate the following fields in Line 1:
   - Vial
   - Sample ID
   - Data Path
   - Separation Method
   - Injection Method
   - MS Method



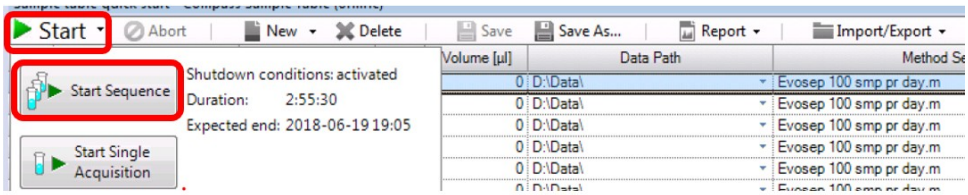
5. Right click the black triangle on the left-most side of sample Line 1 and choose “Add New Samples”. Set the number of “Iterations” (samples) you wish to add and tick the “Increment position” checkbox for incremental increase of the subsequent Vial positions. Click “OK”.



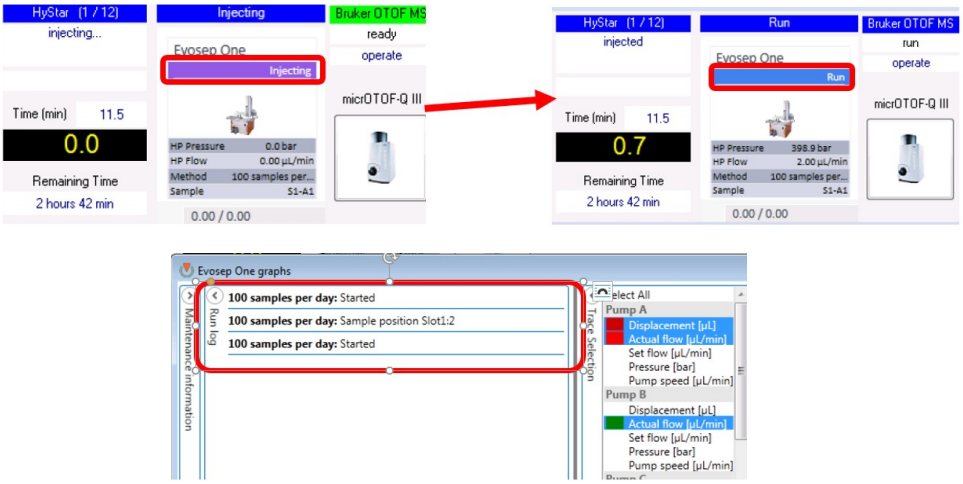
6. Click “Save As” and save the Sample Table with an appropriate name.



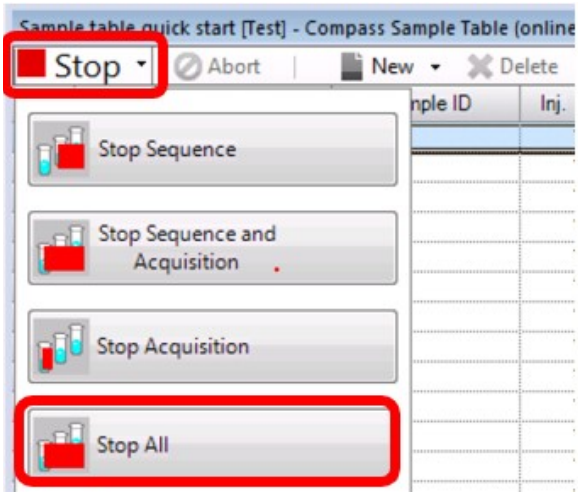
7. Mark the first line in the sample table by clicking the black triangle in Line 1. Click “Start” and select “Start Sequence”.



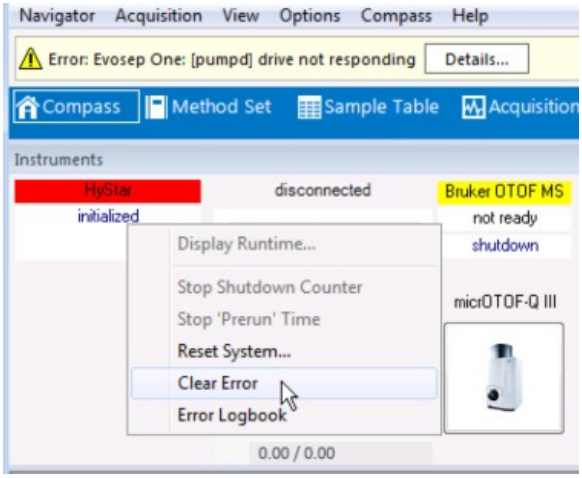
8. The Evosep One status will change from “Idle” to “Prerun” to “Injecting” to “Run” when the gradient starts.
   - More information during the run can be found in the “Run log” of the Graphs window.



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



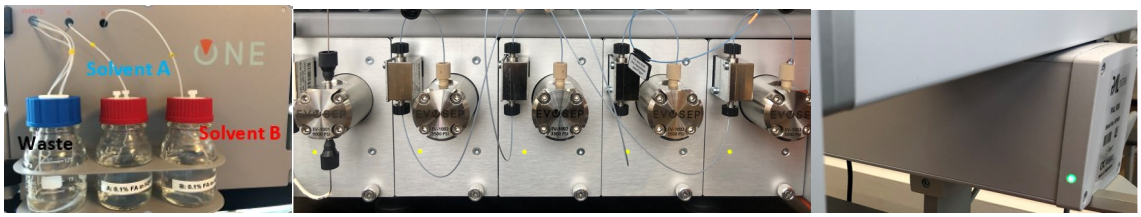
10. If something unforeseen happens during a maintenance script or a method, error messages can be cleared by right clicking the HyStar status view and selecting “Clear Error”.



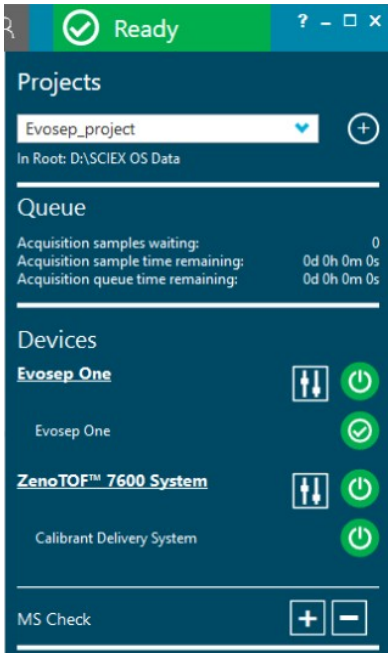
5.3 Evosep Driver for Control of SCIEX OS

5.3.1 Instrument Preparation with SCIEX OS

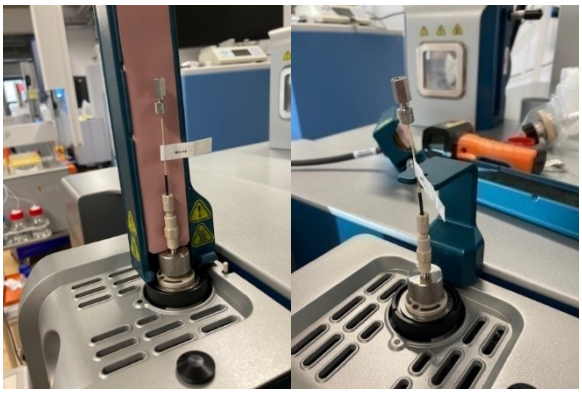
1. Check the Evosep solvent levels (waste, solvent A, and solvent B; left picture) and LEDs displaying power for the pumps (middle picture) and autosampler (right picture).
   - The LEDs must be green.



2. Open SCIEX OS. Check the status ribbon to verify the Evosep and MS are configured and ready.



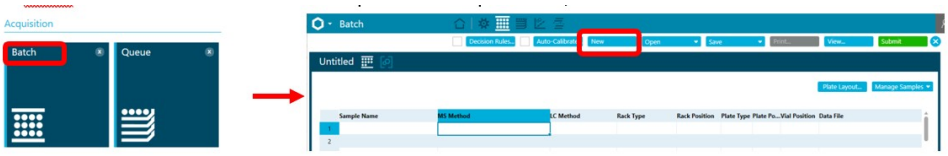
3. Verify the column is connected to the micro-probe on the Optiflow ion source. Then connect the transfer line from Evosep to the column and close the column oven.
   - If the column oven is not used, the “high voltage enable switch” needs to be held down as shown on the right. For low flow applications, the Nanoprobe configuration can be selected (see the “Optiflow Operator Guide” document for details).



5.3.2 Sample Acquisition with SCIEX OS

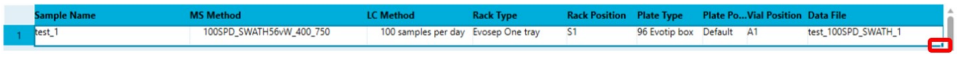
1. Ensure that Instrument Preparation has been performed.
2. Prepare samples according to the SOP for sample loading. Remove lid from Evotip box and place box in Slot 1.

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

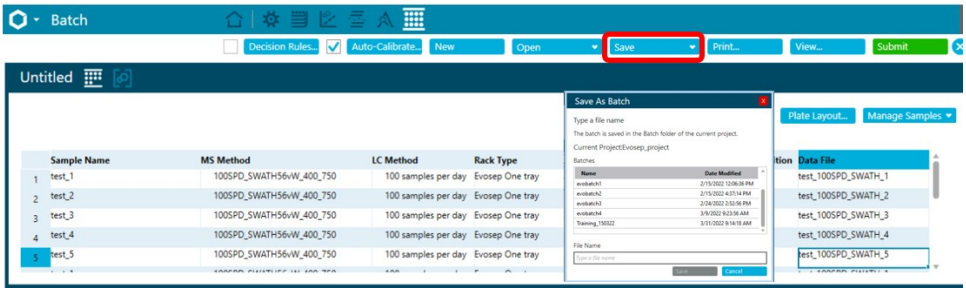


4. In the Sample table, populate the following fields in line 1:
   - Sample Name
   - MS Method (the length should match the LC method)
   - LC Method
   - Rack Type
   - Rack Position
   - Plate Type
   - Plate Position
   - Vial Position
   - Data File

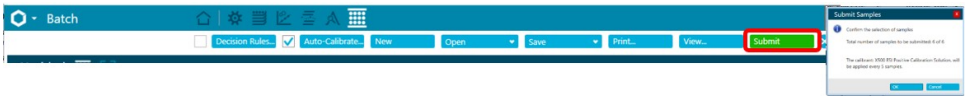
5. Select line 1 and drag down to add samples. Adjust Sample Names, Vial Positions, and Data File values.



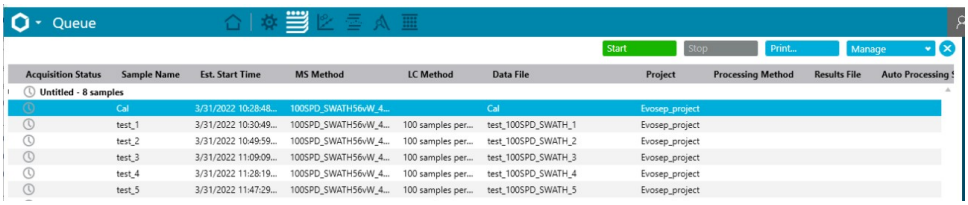
6. Click “Save” and save the Batch Table with an appropriate name.



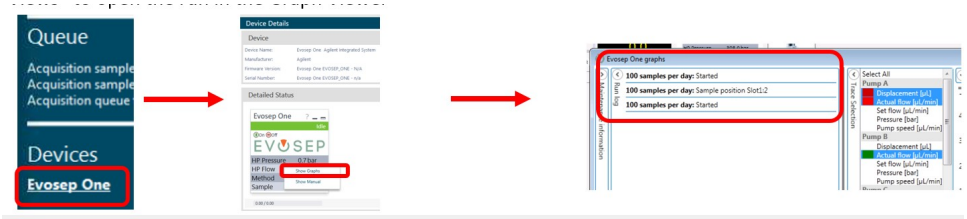
7. Press “Submit” to submit the Batch to sample queue.



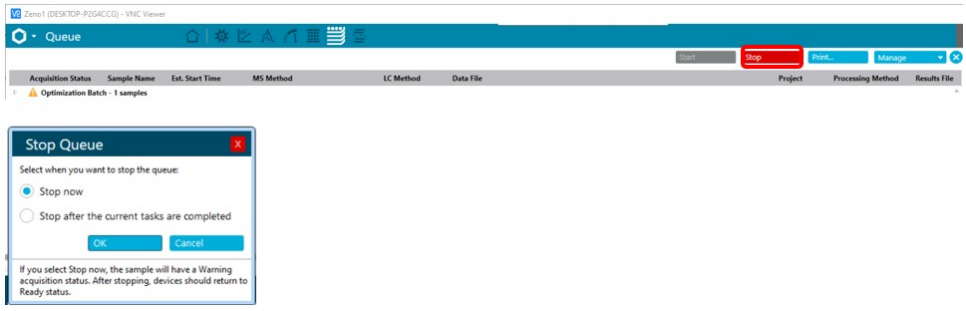
8. Go to the “Queue” and click “Start”.



9. The Evosep One status will change from “Idle” to “Prerun” to “Injecting” 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.



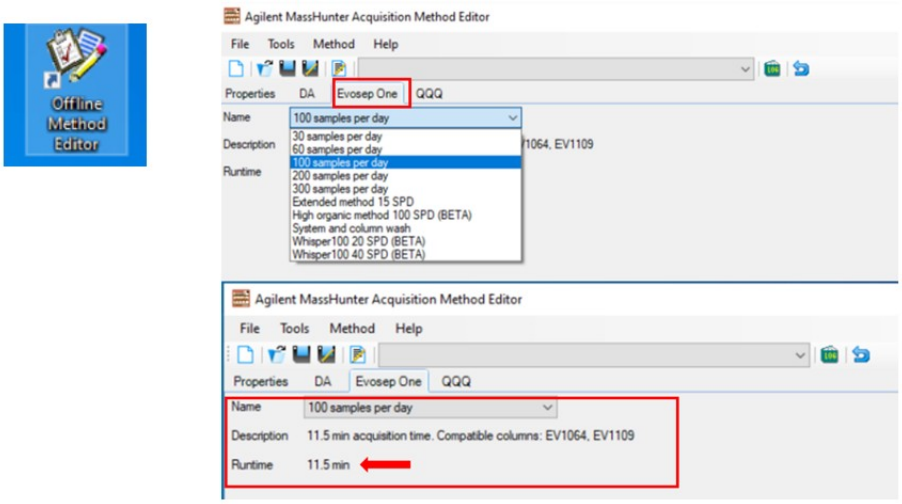
10. To stop an acquisition, click "Stop" in the sample table and select your preferred option (see below).



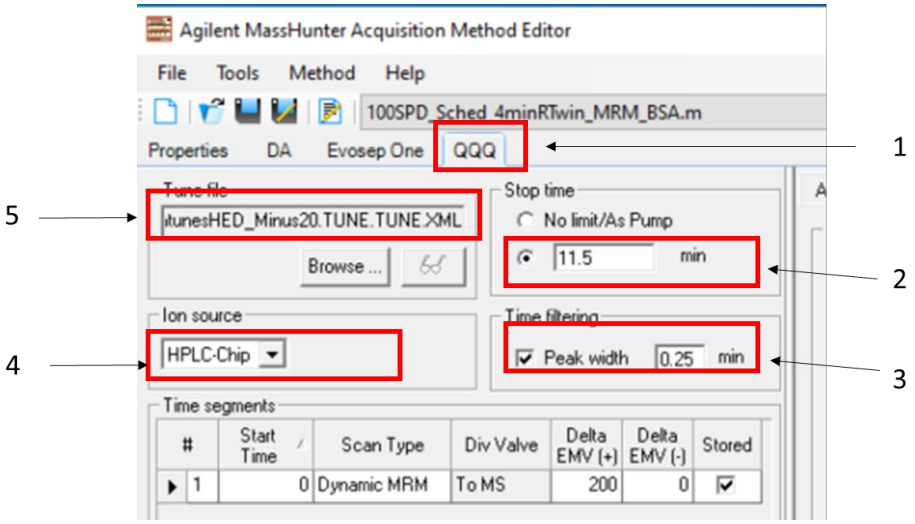
5.4 Evosep Driver and Sample Acquisition with MassHunter Software

5.4.1 Create MS Method with MassHunter

1. To create a method, open “Offline Method Editor” and select the desired chromatography method on the Evosep One tab.
   - Note the LC method run time for MS method timing.



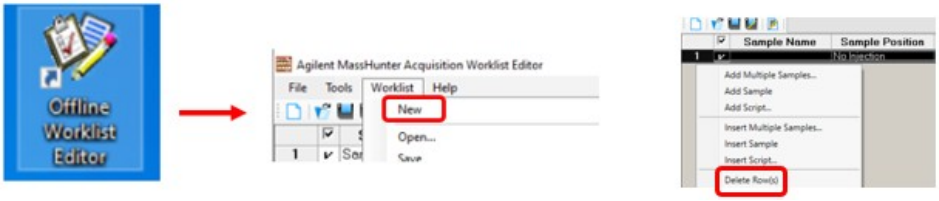
2. Set the following parameters:
   - Select the MS tab – QQQ (1)
   - The MS method should equal the time of LC method (2)
   - Enter the estimated peak width. For example, 15 second peaks provide 0.25 min (3)
   - Select the ion source appropriate for use with the Evosep One – HPLC-chip (4)
   - Ensure the current MS Tune file is incorporated into the method (5)



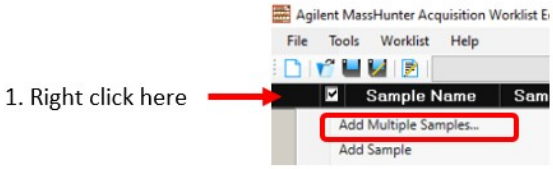
3. Save method by selecting “Save As Method” and navigate to D:\MassHunter\Methods.

5.4.2 Sample Acquisition with MassHunter

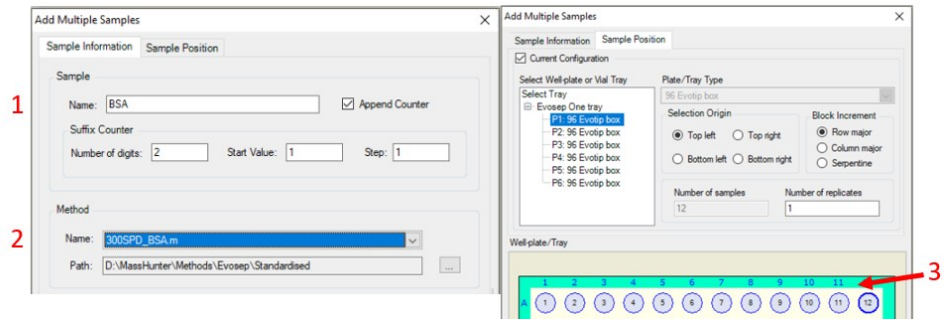
1. To prepare an MS run list, open the MassHunter Acquisition Worklist Editor, click on the "Worklist" drop-down menu, and select "New". Delete the first row.



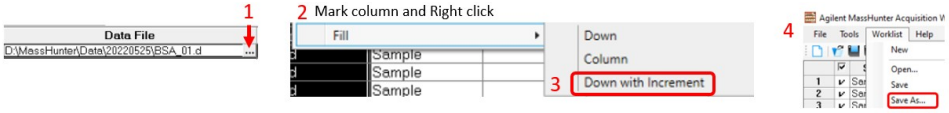
2. Right click and select "Add Multiple Samples…".



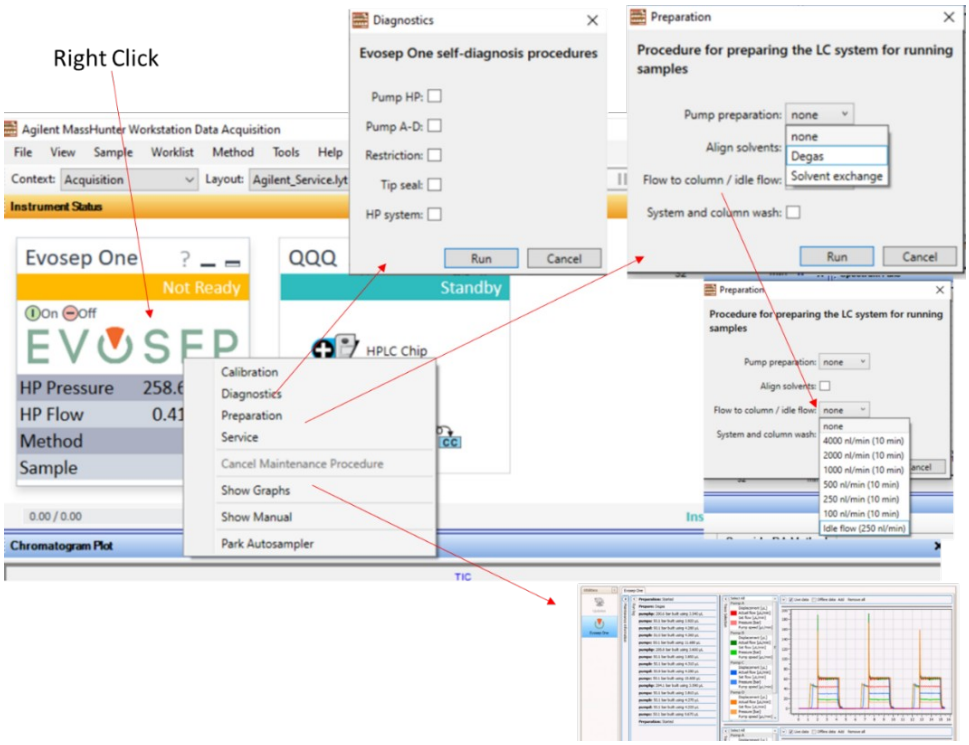
3. Enter the Sample Name (1) and choose the method (2). Select the Sample Positions (3). Click "OK".



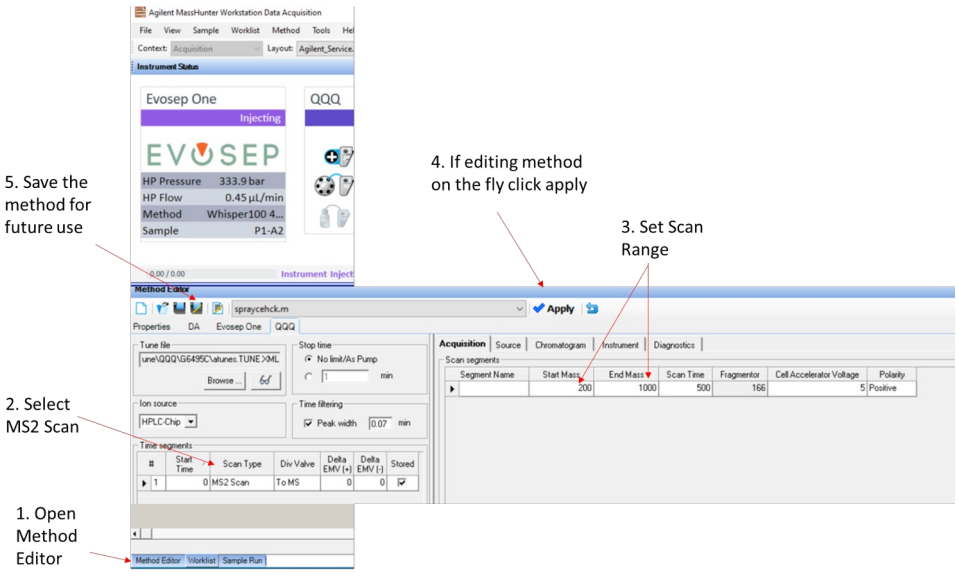
4. Create a Data File folder and enter the file name for first sample (1). Select the "Data File" column (2). Right click, select "Fill", and choose "Down with Increment" (3). Save Worklist (4).

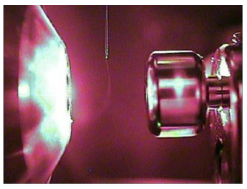


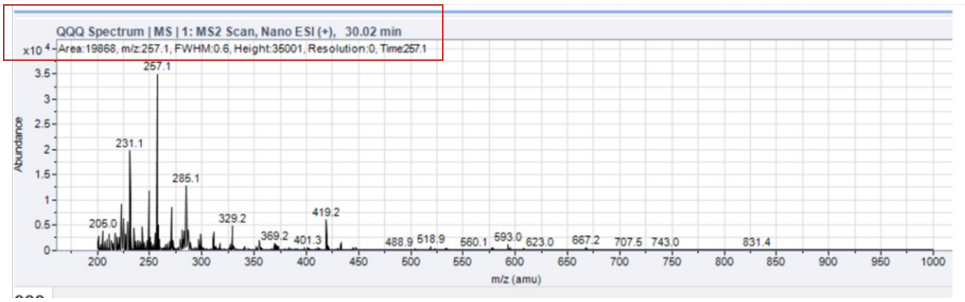
5. To run LC checks/preparations prior to the MS run, right click for drop down method.
   - For example, use "flow-to-column" at 1000 nl/min and check the spray for 100 SPD LC method.



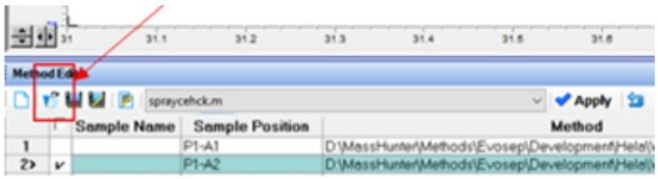
6. Check the emitter position when the flow is running at a stable pressure. Switch on the MS and perform an MS2 scan to check stability and intensity of the spray.



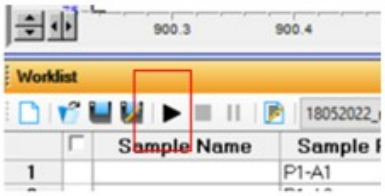


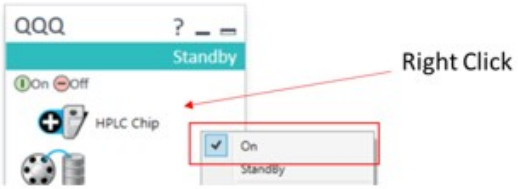


7. To run the worklist, upload the worklist in MassHunter Data Acquisition.

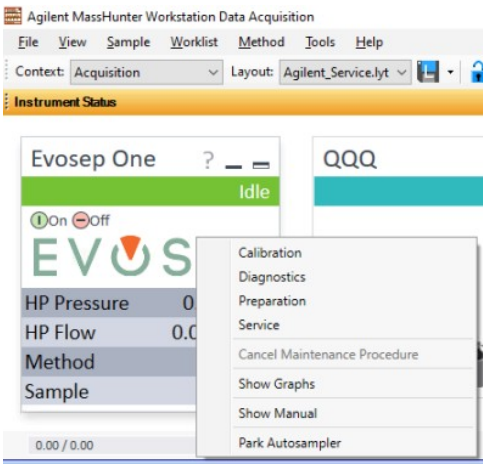


8. Ensure that the MS is “On” to launch a run.





9. The Evosep One status will change from "Idle" to "Prerun" to "Injecting" 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.



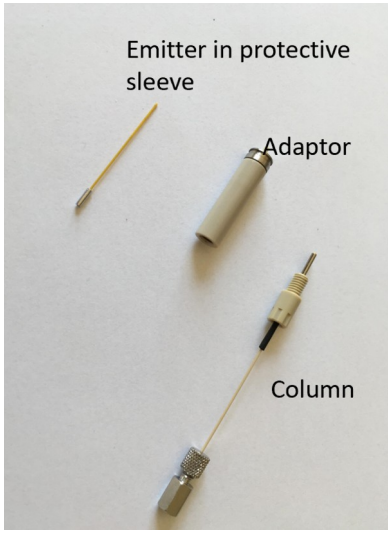
6 Configuration, Source, Column, and Emitter

Warning/Caution	
	**Risk of danger: the use of proper Personal Protective Equipment (PPE) is essential to protect oneself from harm and to minimize contamination from entering the Evosep One instrument. Always exercise caution when dealing with potential health and safety risks.**
	**Electrical shock hazard: Risk of transfer of high voltage from MS to the Evosep One instrument through liquid connection. Do not touch the column, emitter, column/emitter connection, or MS inlet when the MS emitter is at high voltage. Turn off the MS inlet voltage before adjusting or changing the column or emitter.**
	**Sharp objects: the various emitters used in conjunction with the adaptor/column are extremely thin. Exercise caution when handling the emitter and avoid touching the ends as it poses a threat to a puncture wound. The use of appropriate PPE is especially important when working with dangerous and/or toxic materials.**

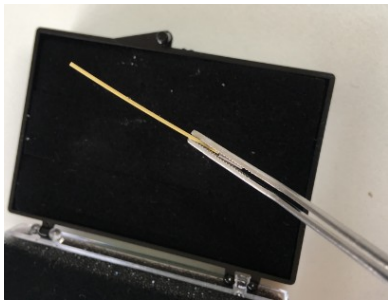
The following Sections describe how we recommend connecting the Evosep One LC to various mass spectrometers and ion source configurations.

6.1 Thermo Scientific EASY-Spray Source

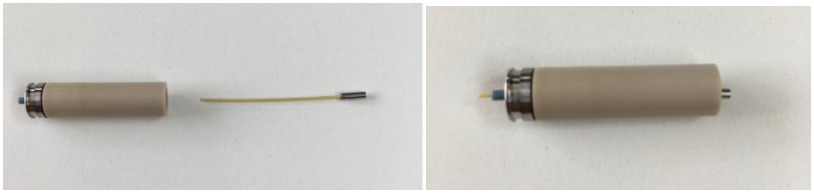
The column, spray adaptor, and emitter used to run the Evosep One with an EASY-Spray source. Follow the procedure below to set up this connection.



1. Carefully remove emitter from the box by using your fingers or a pair of tweezers.



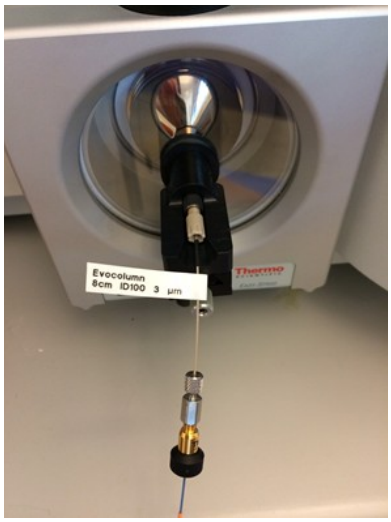
2. Slide the emitter with the sleeve into the spray adapter.



3. 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 off the emitter.

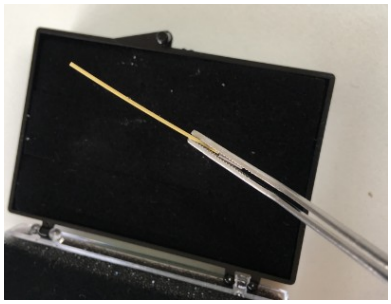
5. After removing the sleeve from the emitter, the spray adapter can be gently pushed into the Easy-Spray source. To avoid damage to the emitter, move the Easy-Spray Z-axis back by using the manipulator before inserting the adaptor.



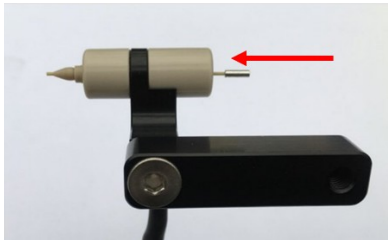
6. Connect Evosep One transfer line to the column and adjust emitter position with the manipulator.
7. To remove the adaptor, gently loosen it with your fingers and slide it outwards.
    - Do not pull on the connecting union to remove the adaptor.

6.2 Thermo Scientific Flex Source

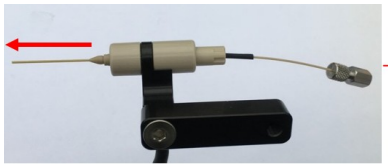
1. Carefully remove emitter from the box by using your fingers or a pair of tweezers.



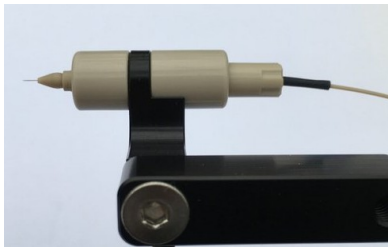
2. Slide the emitter with the sleeve into the flex source spray adaptor.



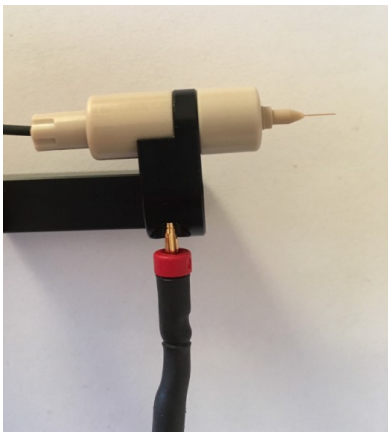
3. Connect the column to push the emitter into the correct position.

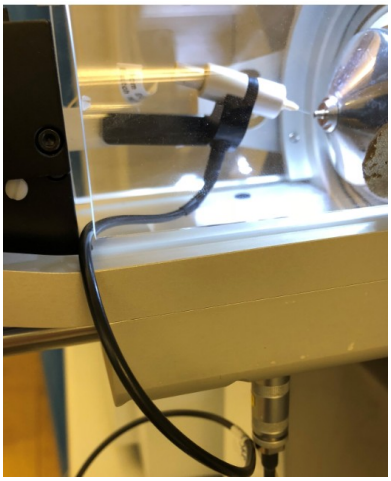


4. Remove the protective sleeve, connect the transfer line, and position the sprayer in the source with the flex source manipulator.



5. Please use the Nanospray Flex HV cable PN# EV1092 for supplying voltage to the spray adapter.

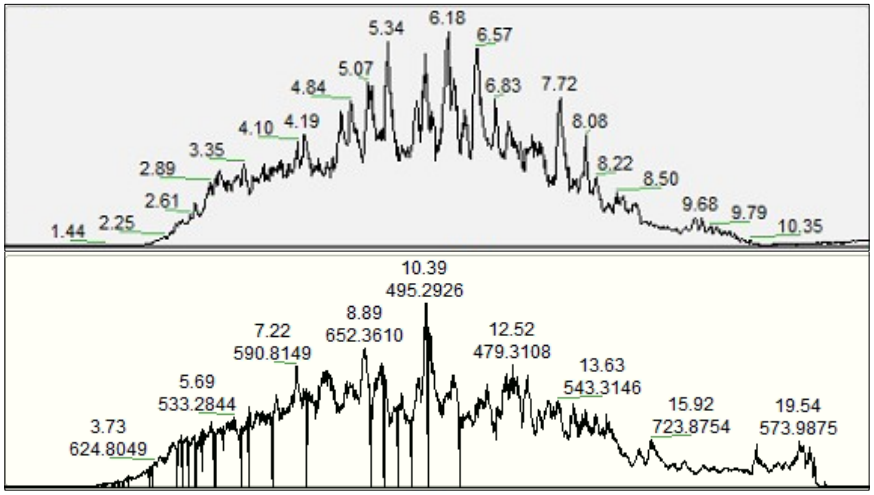




6.3 Thermo Scientific FAIMS Pro Interface

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.

In the image below, the top chromatogram was obtained with a clean FAIMS. The bottom chromatogram was obtained with a dirty FAIMS, which features many dropouts and spray instability.



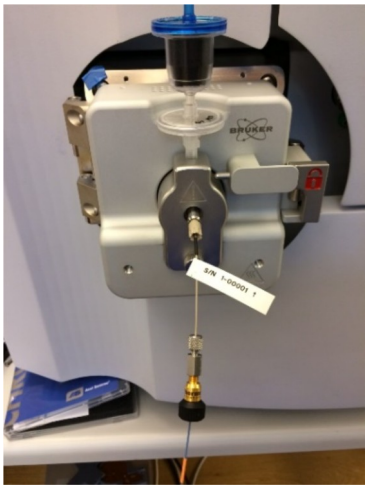
The following set of recommendations is devised to help you use your Evosep One in combination with FAIMS Pro Interface.

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 standard applications, 4-5 mm away is optimal; however, the optimal distance may vary depending on the method used and the sample used. 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/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. More or less frequent cleaning might be needed, depending on the type of samples analyzed.
3. Ionization voltage should be ~300 V higher when FAIMS Pro Interface is used.

6.4 Bruker Daltonics CaptiveSpray Source

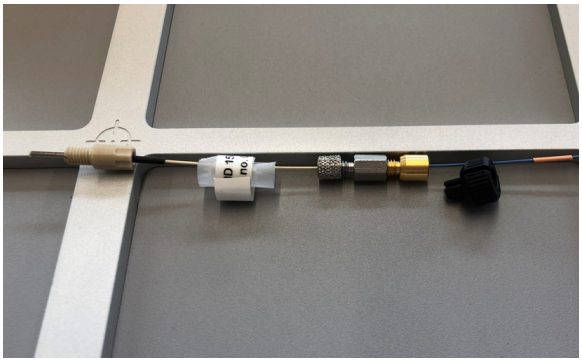


1. Run Connect the column to the CaptiveSpray source and connect the transfer line to the column.

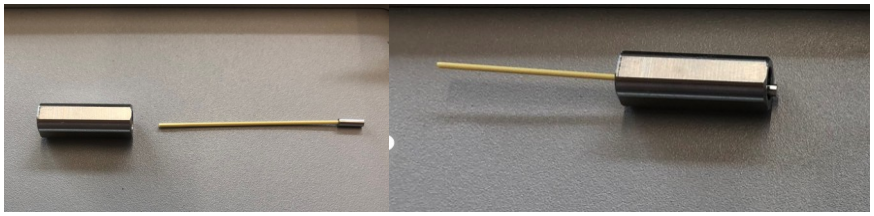
6.5 Agilent Nanospray Source

The Evosep column, Agilent Needle emitter (PN EV1117), Agilent sleeve adapter (PN EV1116), and Agilent Needle sleeve assembly is shown below.

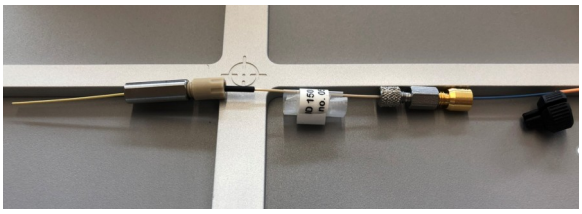
1. Connect the column to the Evosep One transfer line and remove the black knurl from the nanoViper fitting.



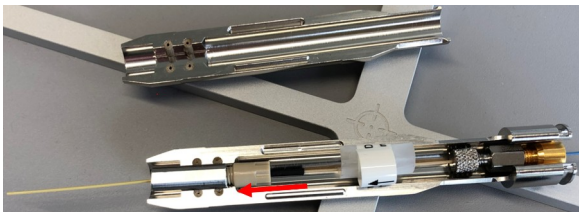
2. Slide the emitter with the sleeve through the sleeve adapter.



3. Connect the column to the sleeve adapter. This creates a ZDV connection between the emitter and column inside the sleeve adapter.

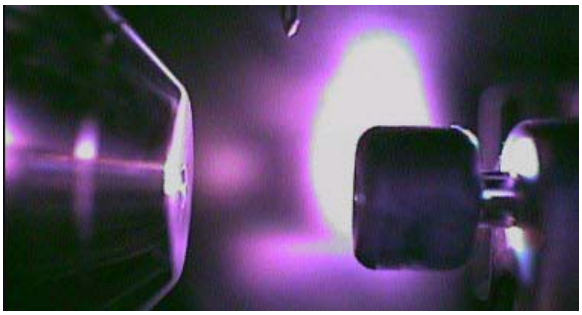


4. Insert the sleeve adapter with the column into the needle sleeve assembly. To ensure the correct position of the sleeve adapter and emitter, push the sleeve adapter towards the end of the needle sleeve assembly until the column peek nut is stopped by the small narrowing in the needle sleeve assembly,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

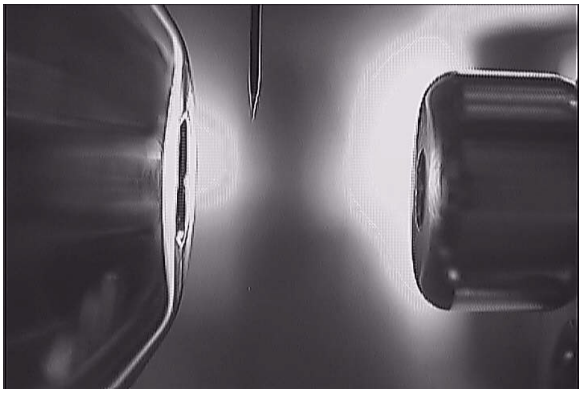


5. Now assemble the needle sleeve assembly and remove the protective sleeve from the emitter.

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 transfer line. 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.

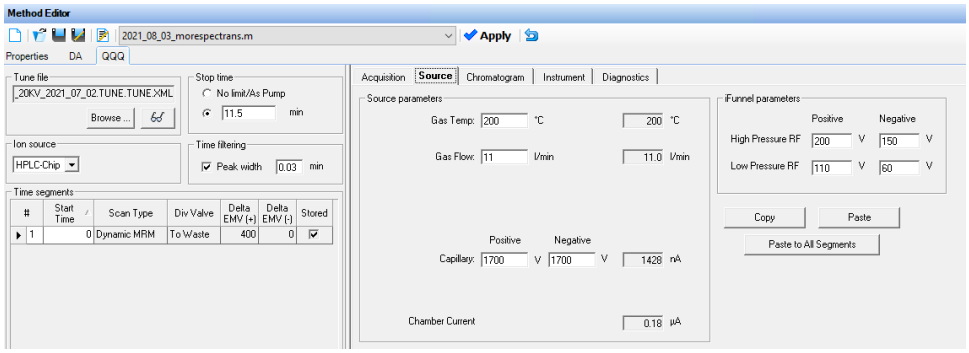


7. Use the adjustment knobs on the source to position the needle as in below picture.



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

9. The recommended source conditions are shown below:
   - Gas Temp: 200 °C
   - Gas Flow: 11 L/min
   - Capillary: 1700 V

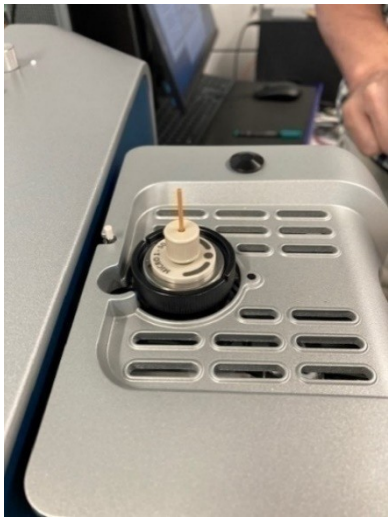


6.6 Sciex Optiflow Ion Source in Microflow Regime

This section outlines how to run samples with the microflow probe on the Optiflow ion source and the Evosep One.

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

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



3. Put on the upper steel 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 transfer line 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

Please refer to Chapter 8 “Running Samples Using Evosep One” to view all safety warnings, cautions, and concerns.

7.1 Hardware Configuration for Standard and Zoom Mode

Important notes:

Before running samples on the Evosep One system check the instrument configuration.

Running the system with the wrong configuration will result in loss of instrument performance!

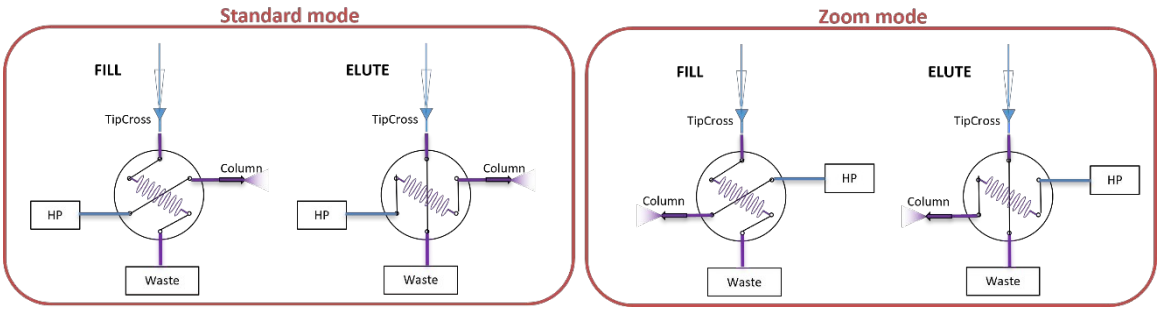
Whisper Zoom methods improve on the existing Whisper methods with significant chromatographic improvements in terms of peak shapes, intensity, reproducibility, and throughput. Running the system in Zoom mode requires a hardware change.

Standard mode:

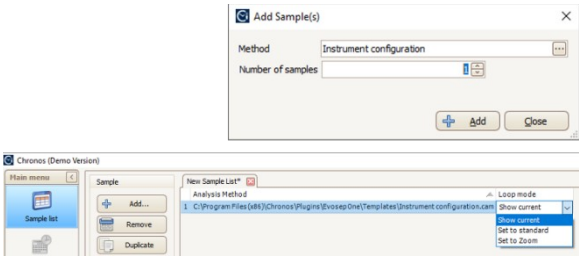
- The transfer line must be mounted in port 6 of the Loop valve
- The HP flow sensor line must be mounted in port 3 of the Loop valve

Zoom mode:

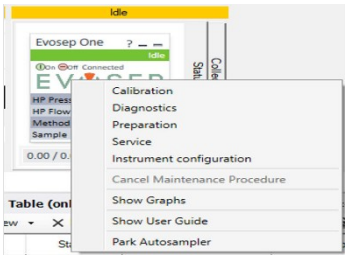
- The transfer line must be mounted in port 3 of the Loop valve
- The HP flow sensor line must be mounted in port 6 of the Loop valve

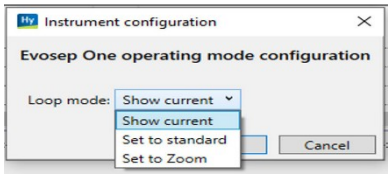


1. Change or check the active instrument configuration by selecting the instrument configuration section in the user interface of the driver. Choose one of the options as shown below.
2. If prompted, please follow the instructions for hardware reconfiguration.
   - Show current: checks the current loop configuration mode
   - Set to standard: sets the instrument to Standard mode loop configuration
   - Set to Zoom: sets the instrument to Zoom mode loop configuration
3. In Chronos, select the “Instrument configuration” method.

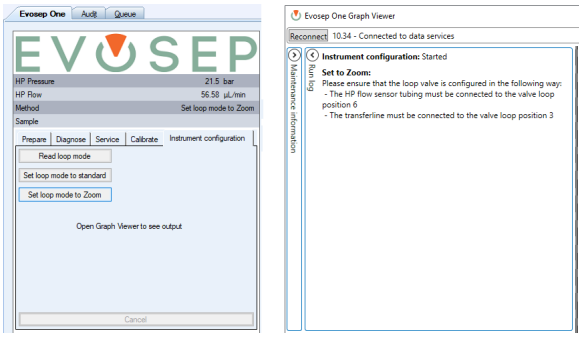


4. In RC.Net versions of the driver, right click the Status view and select the “Instrument configuration” option.





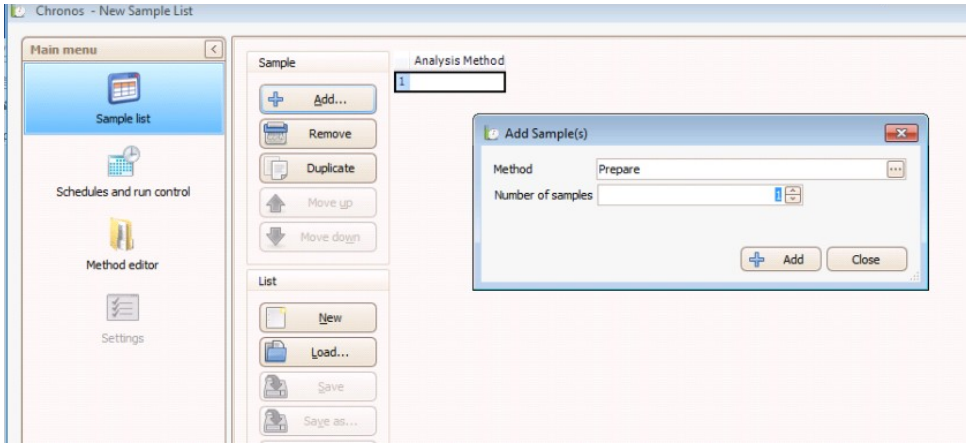
5. In Chromeleon/SII for Xcalibur, select the “Instrument configuration” tab.



6. Before running samples on the Evosep One system, check solvent and waste levels.
   - Solvents:
     - Solvent A: 0.1% formic acid in water
     - Solvent B: 0.1% formic acid in acetonitrile
     - Only use LC-MS grade solvents
     - Exchange the solvents on a weekly basis
   - Waste:
     - Check the waste bottle solvent level and empty if necessary
     - Check the Evotip waste bin and empty if necessary
7. The Evosep One is preconfigured with preparation programs. They are used to prepare the system for running samples and to help maintain the system performance.
   - Pump preparation:

    - Degas: prepares the pumps to run samples if the instrument has been idle for more than 6 hours
    - Solvent exchange: purges the pumps
- Align solvents:
    - Prepares the system flow lines if the instrument has been idle for more than 6 hours
- Flow to column:
    - Sets a flow to the column. This is useful when setting up the MS spray conditions
- System and column wash:
    - Cleanses the system and analytical column of contaminants

8. The Evosep One automatically assesses the need for doing preparative actions to ensure optimal instrument performance without the need for User interaction. Under these circumstances, the system autonomously initiates the proper system preparation tasks immediately preceding sample analysis.
    - Degas is initiated if the system has been idle for more than 6 hours.
    - Align solvents is initiated if:
        - The system has been idle for more than 6 hours
        - The previous procedure was aborted
        - The previous procedure caused the solvents in the flow lines and the ceramic needle to be unaligned
9. Additionally, all the preparation programs can be manually executed by adding a sample using the "Prepare" method (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Prepare.cam) in the "Sample list" panel.



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

11. Start queue in the "Schedules and run control" panel.

7.2 Degas Pumps

When the instrument is idle for an extended period, gas penetrates the pumps and tubing. The increased solvent gas level has a negative impact on pump responsiveness and mass spectrometer electrospray stability. Consequently, peak retention time and area reproducibility are lowered.

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

If one or more of the pumps fail to reach 200 or 50 bar within the maximum volume of 9 µl, proceed to the “Troubleshooting” section for guidance.

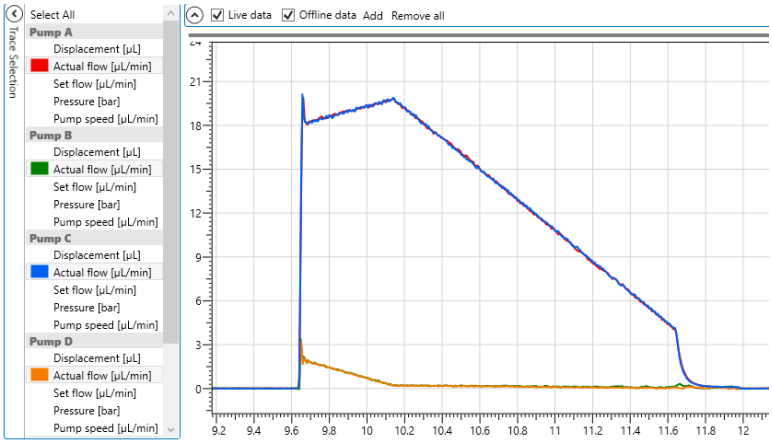
7.3 Solvent Exchange of Pumps

The “Solvent exchange” program is an automated pump purge procedure that runs 15 cycles of total pump solvent exchange. This should be ran weekly or if the instrument has been idle for an extended period of time.

7.4 Align Solvents

When the instrument is idle for extended periods of time, the solvent will be subject to diffusion and evaporation in areas with contact to the ambient air. This causes 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 to the wash station, whereas the CD gradient flushes the flow lines going to the tip cross.



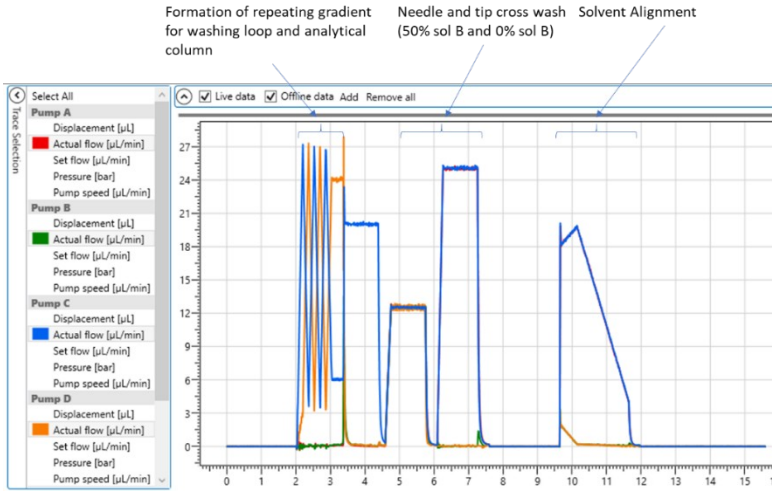
7.5 Flow to Column

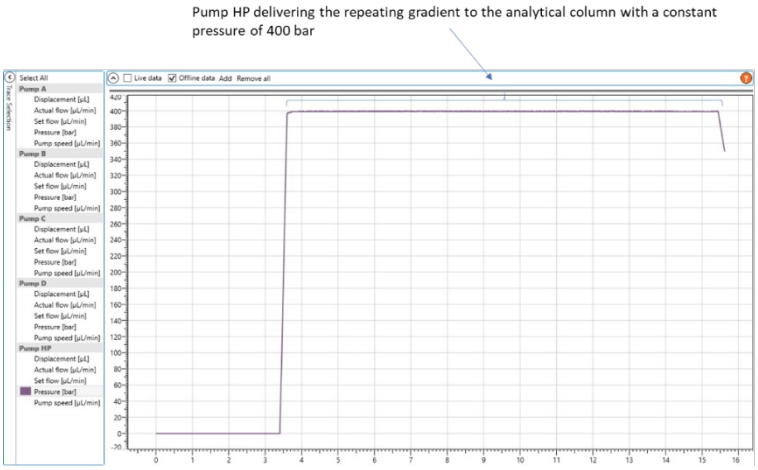
Before running the first sample or a sequence of samples, it is recommended to tune the MS ion source conditions, according to MS manufacturer guidelines, to secure a stable electrospray. For this purpose, use the “Flow to column” program, which encodes an automated procedure for delivering solvent A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

7.6 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 program occurs in four steps and the duration varies according to the backpressure of the connected analytical column (typically 10 – 15 minutes).

1. The program automatically creates a gradient of repeated increments of solvent B from 10-90%, which are 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 solvents in the low pressure pump flow lines and the autosampler needle.



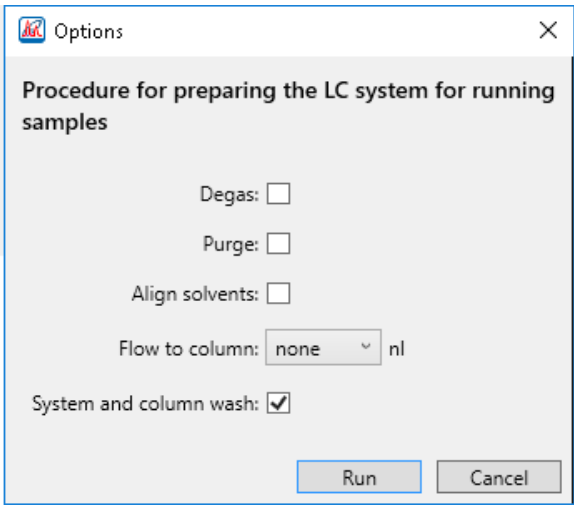


The “System and column wash” procedure can be started in two ways:

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 can be 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. It’s required that a blank Evotip is present in the autosampler tray 1, pos 1 (A1).

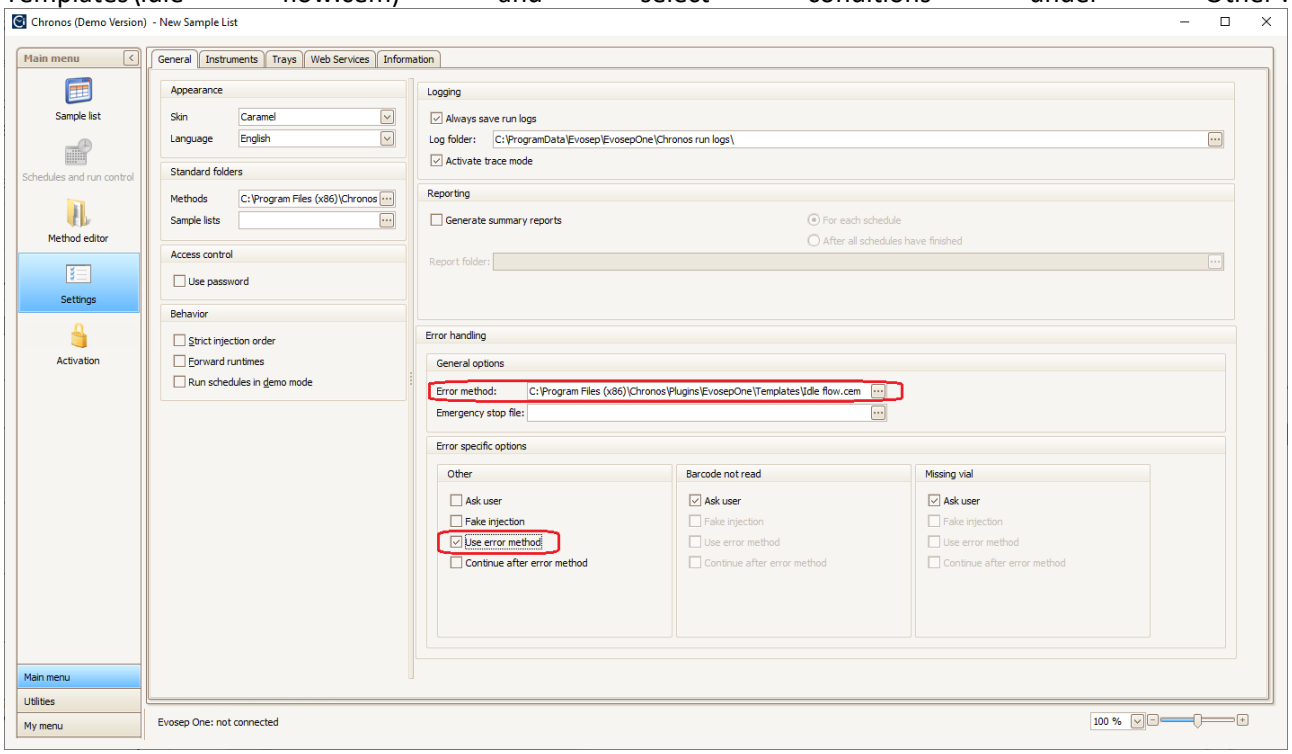


7.7 Idle Flow

The “Idle flow” method 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 dle flow must be manually stopped from the Schedules and run control window before another preparation or sample method can be started.

In Chronos, idle flow can be manually run by using the “Idle flow” method (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Idle flow.cam).

In Chronos, a method can also be selected to run in case of an error. Under ‘Settings/General/Error handling’, set ‘Idle flow.cem’ as an error method (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Idle flow.cem) and select conditions under ‘Other’.



8 Running Samples using Evosep One

Warning/Caution	
	**Risk of danger: the use of proper PPE is essential to protect oneself from harm and to minimize contamination from entering the Evosep One instrument. Always exercise caution when dealing with potential health and safety risks.**
	**Electrical shock hazard: Risk of transfer of high voltage from MS to the Evosep One instrument through liquid connection. Do not touch the column, emitter, column/emitter connection, or MS inlet when the MS emitter is at high voltage. Turn off the MS inlet voltage before adjusting or changing the column or emitter.**
	**Risk of fire: take care when handling flammable solvents to ensure they do not come in contact with, are spilled on, or are exposed to the Evosep One instrument electronics or sources of sparks, open flames, heat, or hot surfaces as they may cause a fire. Follow MSDS and local safety guidance when handling flammable solvents.**
	**Risk of infection: microbiological agents added as biological samples to the Evotips may lead to infection if handled incorrectly. Ensure all hazardous biological sample material is inactivated/decontaminated before placement into an Evotip.**
	**Corrosive hazard: exercise caution when handling corrosive solvents. Contact with the skin and/or eyes, inhalation, and/or ingestion may be harmful. Follow MSDS and local safety guidance when handling corrosive solvents. The use of proper PPE is essential to protect against skin and eye contact, inhalation, and ingestion.**
	**Broken glass: handling of broken glass may result in cuts. Take care not to drop or break glass containers. Avoid handling broken glass to reduce the risk of cuts. The contents of the glass containers when spilled may impose additional healthy and safety risks.**
	**Toxic fumes: handling of toxic solvents imposes a risk of toxic solvent evaporation, inhalation, and causing harm. Exercise caution when handling toxic solvents and their vapor. Minimize vapor production by avoiding heat sources and prepare samples and solutions in a well-ventilated area. Follow MSDS and local safety guidance when handling toxic solvents. The use of proper PPE is essential to protect against skin and eye contact, inhalation, and ingestion.**
	**Risk of impact: autosampler acceleration or deacceleration may lead to table movement, depending on the table stability, imposing a risk of impact. Do not stand or place items too close to the autosampler and/or table during operation, other than the Evotips in the designated locations. It is recommended to have the Evosep One instrument on a stable table with clear space on and around the instrument when operational.**

	**Risk of entrapment: during operation, movements of the autosampler arm can cause impact or trapping. Do not place or move your hand/body parts within the safety rail of the autosampler during operation. As an additional precaution, an alarm sounds prior to autosampler arm movement. Do not intercept the autosampler arm during movement. Placement of Evotips in the autosampler area should only be done when the autosampler is stationary and nonoperational.**
	**Sharp objects: during operation, the autosampler needle is raised and lowered within the safety rail. This has a blunt point but poses a risk of puncturing the skin if a hand/body part were to be trapped when the needle is lowered. Additionally, needle contact with skin leads to exposure of small amounts of potentially hazardous solvent/sample. Do not place or move your hand/body parts within the autosampler safety rail during movement or operation. Exercise caution when handling and/or replacing the needle and avoid touching the ends as it poses a threat to a puncture wound. The use of appropriate PPE is especially important when working with dangerous and/or toxic materials.**

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., have been reimagined 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 integrating 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, and resuspending the samples from the tips are completely omitted. Instead, the tips are loaded directly into the Evosep One for analysis. This new process leads to significantly less sample loss and variation, as well as 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. See Figure 1 for the Evosep One plumbing diagram.

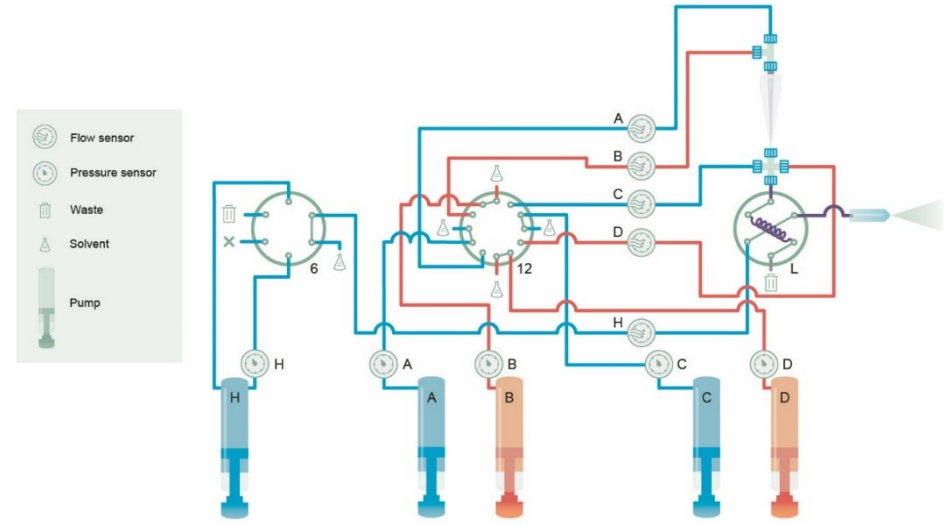


Figure 1. Evosep One plumbing diagram. H: High-pressure Pump, A/B/C/D: Low-Pressure pumps, 6: 6 port high-pressure solvent valve, 12: 12 port low-pressure solvent valve, L: Loop Valve.

Upon starting an analysis, the autosampler places one, pre-loaded tip at the time in line with the solvent system (Tip Pickup). See Figure 2 for the high-level sample acquisition process diagram.

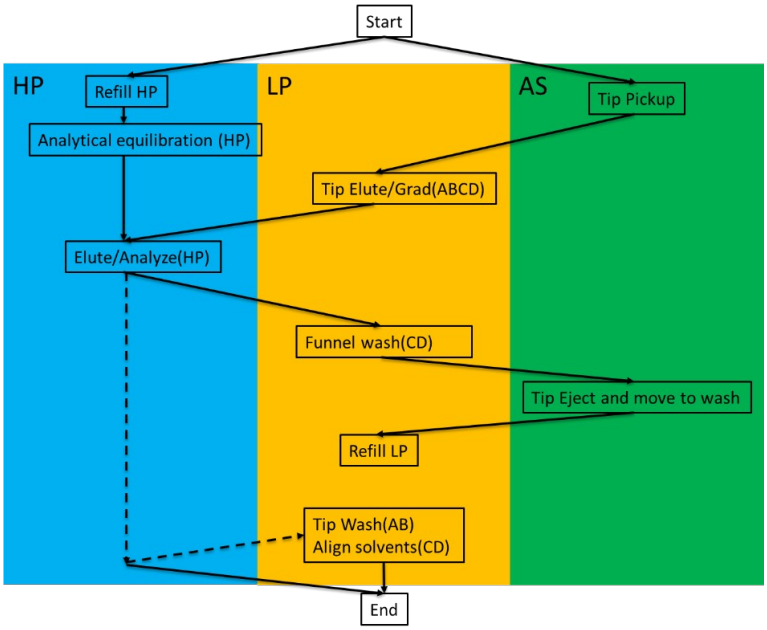


Figure 2. High level sample acquisition process diagram. The acquisition cycle can be divided into three sections: Blue – High pressure, pump HP; Yellow – low pressure, pump A,B,C,D; and, Green: autosampler actions.

Once the Evotip is sealed in line with the solvent system, a gradient from pumps A and B runs through the Evotip and sequentially elute 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 3a. Pumps A+B deliver a partial gradient sufficient to sequentially elute the analytes of interest, but filter out all the high-molecular contaminants to be 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 3b.

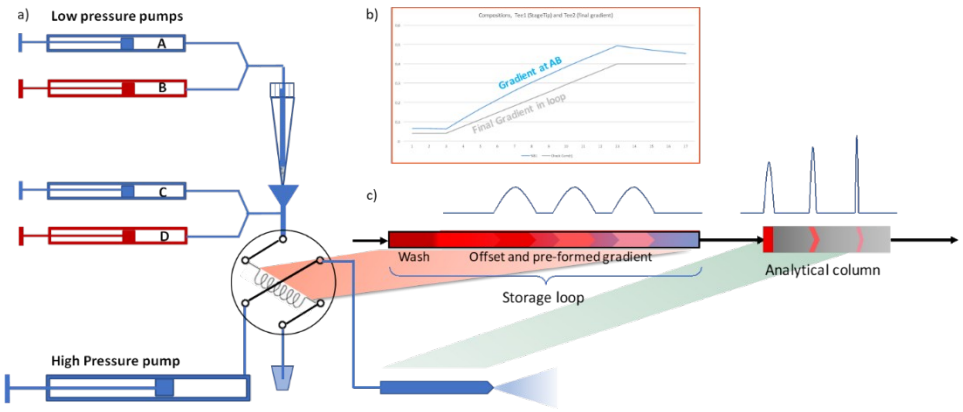


Figure 3. Evosep One a) Simplified plumbing diagram of Evosep One, b) Illustration of the A/B gradient running through the Evotip and the following C/D modified gradient resulting in an offset gradient for optimal focusing at the analytical column, and c) Illustration of the preformed and offset gradient 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 increase the capacity and chromatographic performance, see Figure 3c.

The instrument comes with preset 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.

Table 1. Evosep One Methods

Throughput	Cycle time	Gradient length	Flow rate	Column (length/ID/C18 bead size)
Samples/day	Minutes	Minutes	µl/min	cm/um/µm
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

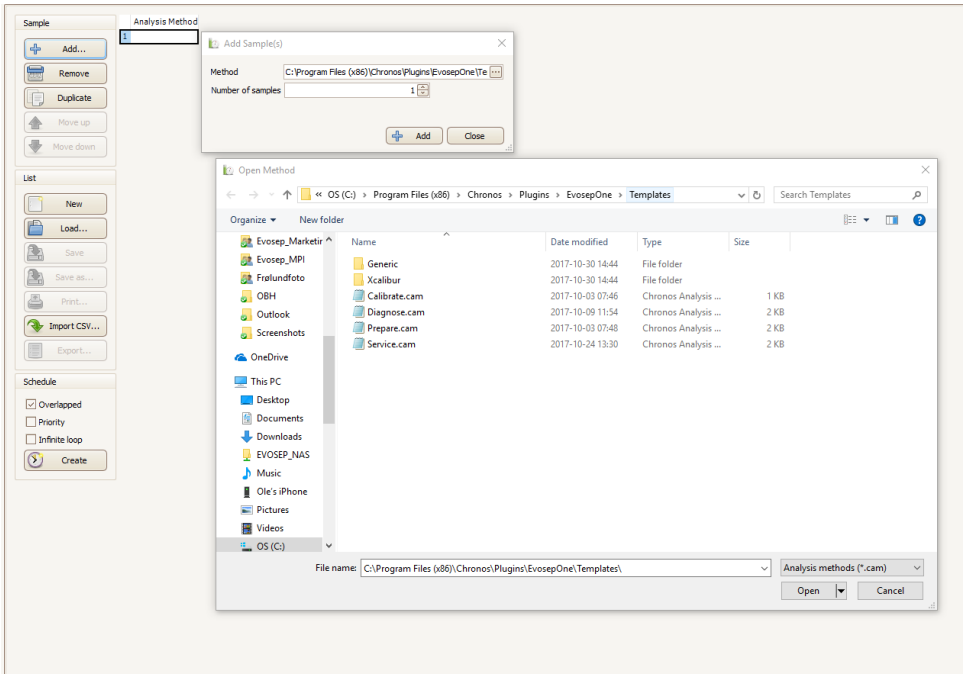
8.2 Sample Acquisition

Chromatographic Data System (CDS) and generic methods will be described in this section.

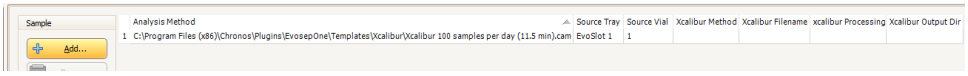
The sample acquisition methods are executed using Chronos software, as described in the SW section. The instrument can be operated in two distinct fashions.

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 and 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).

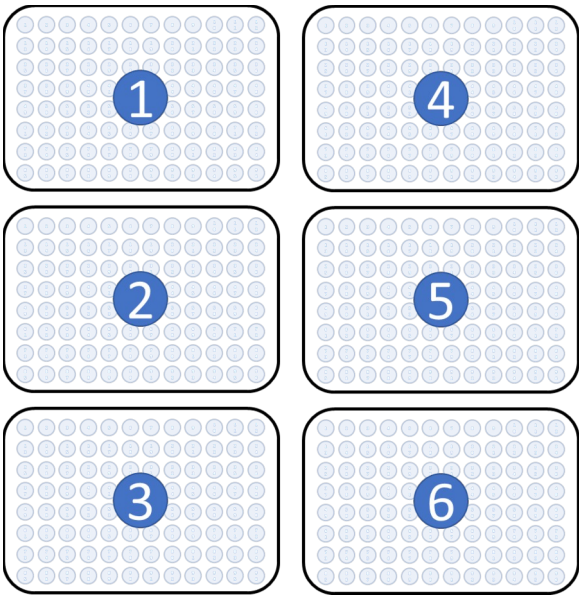
The sample methods for both modes are stored in the "C:\Program Files(x86)\Chronos\Plugins\EvosepOne\Templates" folder. Standalone mode methods are stored in the "Generic" folder, whereas LC-MS integrated methods are stored in a MS CDS specific folder, e.g. methods for Thermo mass spectrometers are saved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



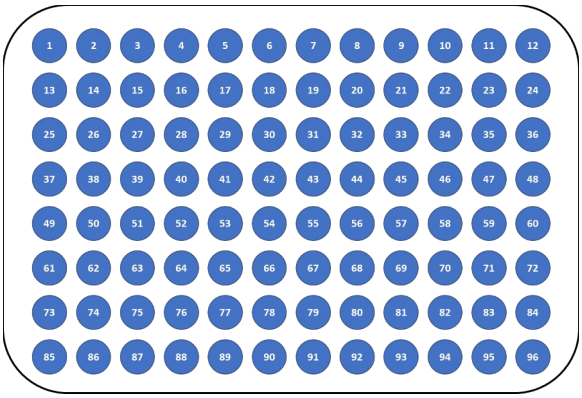
1. Integrated LC-MS mode:
   - The appropriate sample method is chosen based upon the MS CDS vendor and the required sample/day throughput (Table 1).



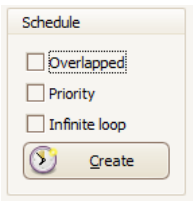
   - Source Tray (Slot 1-6) must be specified using the dropdown menu.

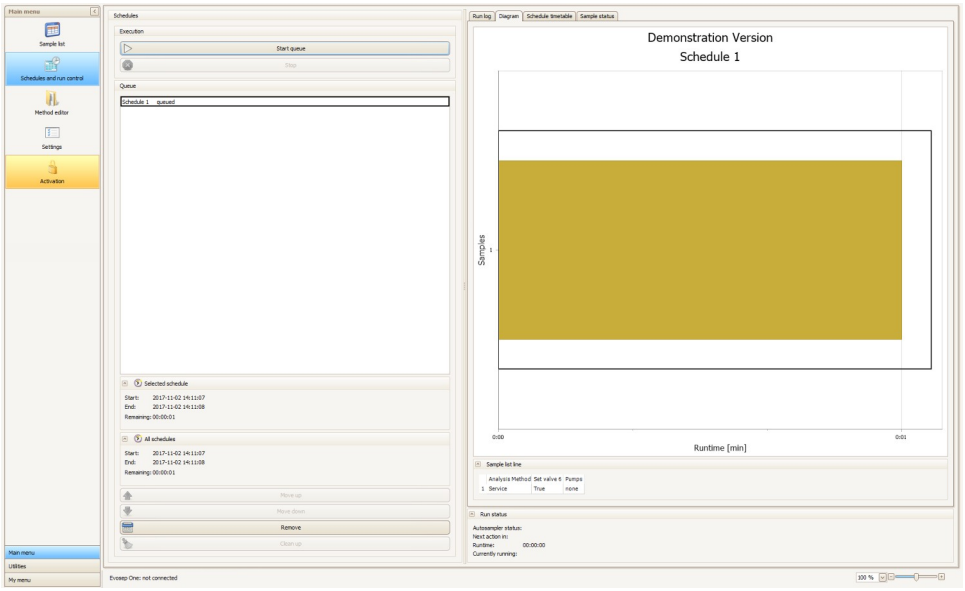


- Source vial position (1-96) must be specified using the dropdown menu.

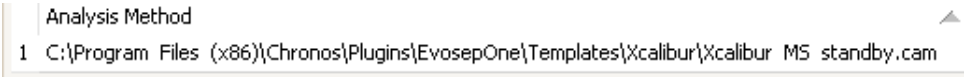


- For Thermo MS: “Xcalibur Method” – the MS acquisition method must be specified. The MS acquisition time must correlate with the individual LC-MS methods, as specified in Table 1.
- For Thermo MS: “Xcalibur Filename” – the MS data filename(s) must be specified.
- For Thermo MS: “Xcalibur Post Acquisition Program” – the MS data post-acquisition executable or batch file can be specified.
- For Thermo MS: “Xcalibur Output Dir” – the MS data directory must be specified.
- 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” mode, the Evosep One sample separation will start.



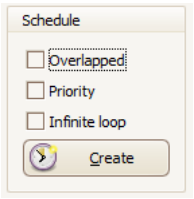


- For Thermo MS: Running the Xcalibur MS standby program will set the MS in standby, even if time remains in the chosen MS method. Typically, this method is chosen as the last sample in a batch.

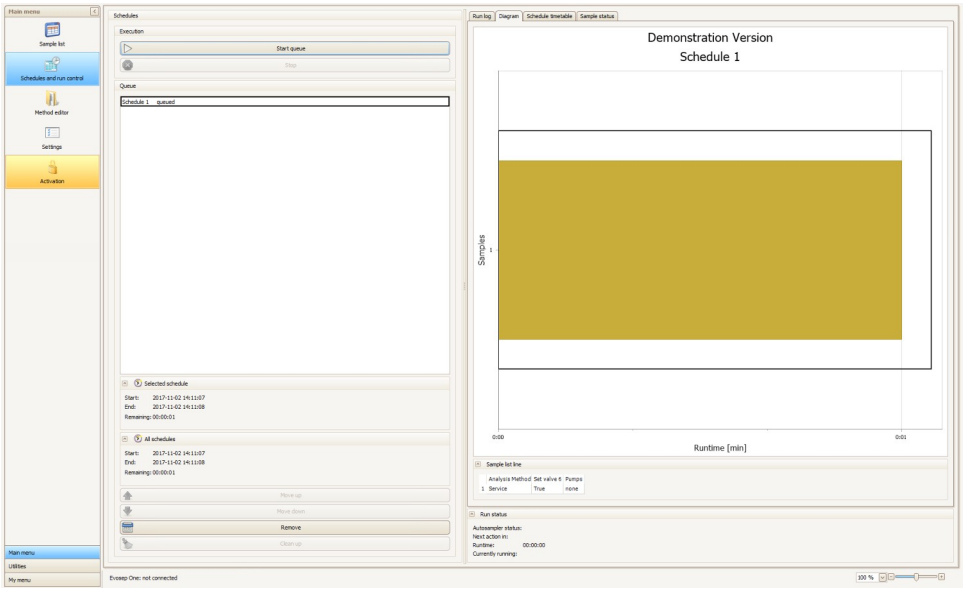


2. Standalone LC mode:
   - The appropriate sample method is chosen based on the required sample/day throughput (Table 1).

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



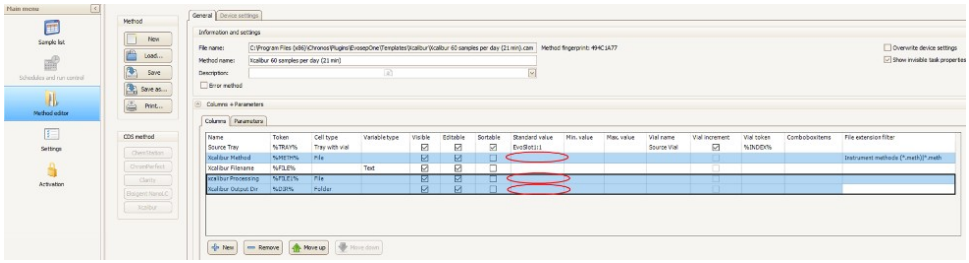
   - For MS CDS, create a synchronous sample list, queue the sample list for acquisition, and wait for the MS to be in "waiting for contact closure" mode.
   - Start the Evosep One schedule queue.



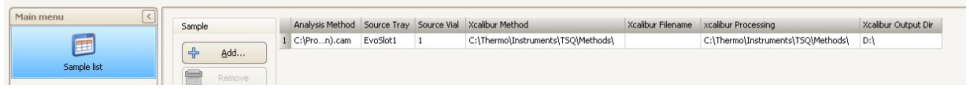
- The Evosep One starts the sample separation procedure and sends a contact closure signal to the MS CDS when the sample elution begins.

Tips and tricks:

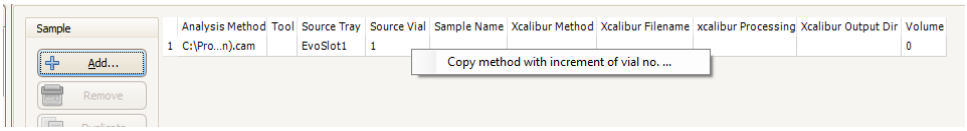
- When running integrated LC-MS methods, e.g. for Thermo mass spectrometers, the user can set default values for:
  - "Xcalibur Method": folder or file name
  - "Xcalibur Post Acquisition Program": folder or file name
  - "Xcalibur Output Dir": folder
  - These values are set in the "Method editor" section.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

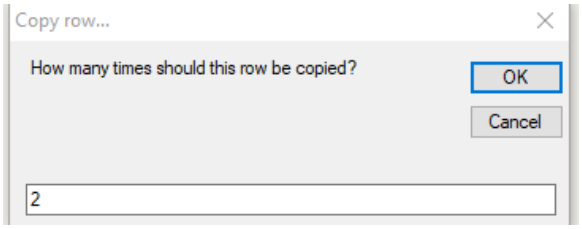


  - Save the method with the same name.
  - When submitting new samples with the updated method, the user can now browse from the specified default directory.

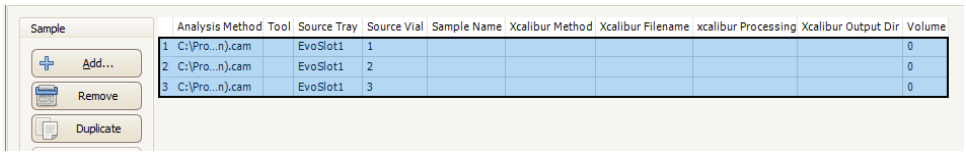


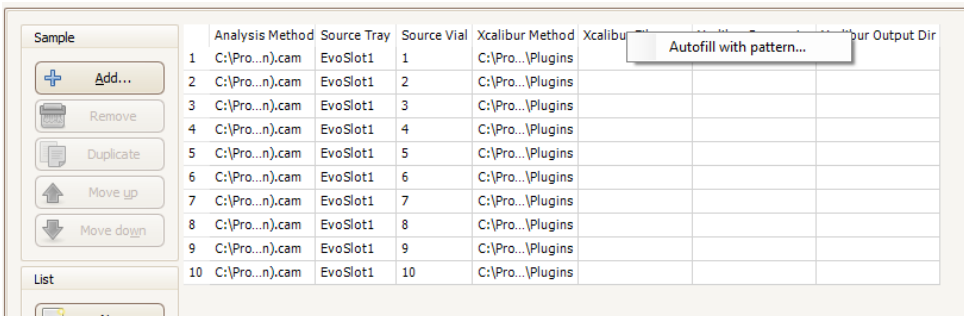
- If several samples in consecutive autosampler positions are to be processed using the method, right-clicking the "Source Vial" column will open a "Copy method row…" dialog. Define how many samples must be added to the sample list and press "OK".

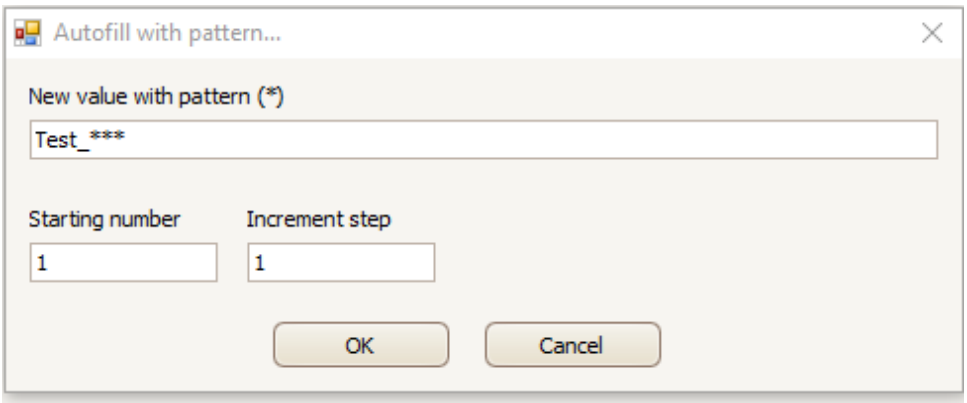


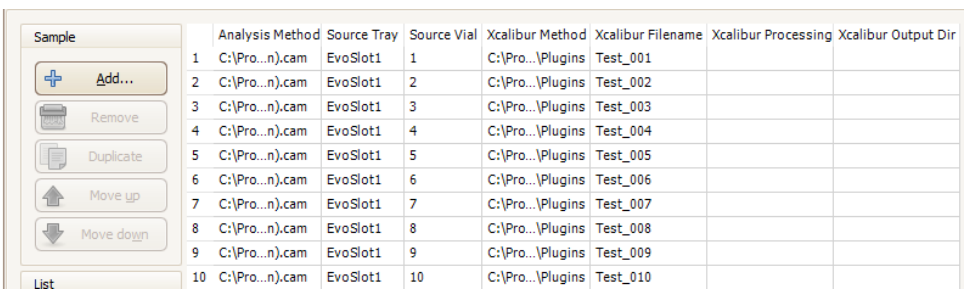


- If the sample name of several samples only vary by a suffix, right clicking the "MS Filename" will open a "Autofill with pattern…" dialog. Fill in the sample "base name" followed by one or more asterixes (*). When you press "OK" the sample names will be filled down in the sample list with the starting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





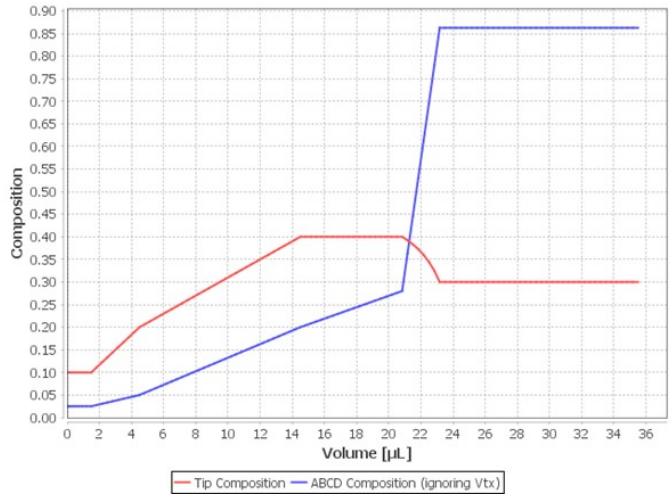




- If you often run the same analysis using the same methods, the sample list can be saved and reloaded instead of created from scratch.

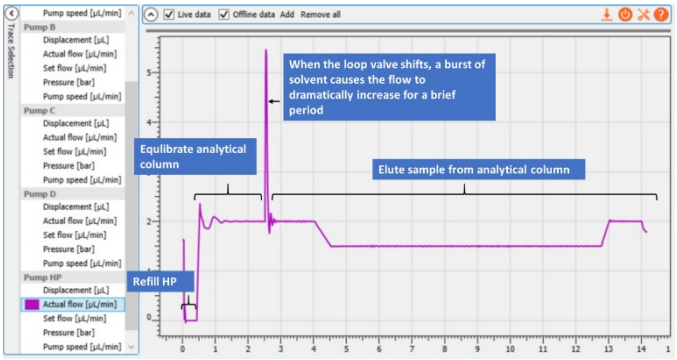
8.3 Example Pump Data

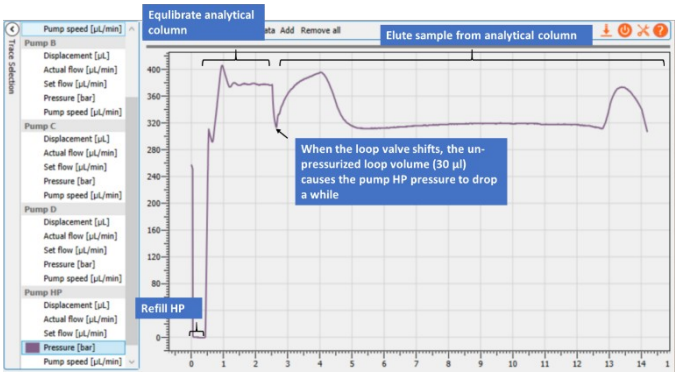
1. The pump graphs shown below are from a standard “100 SPD” chromatographic method on an Evosep One system using the installation analytical column (length 8 cm, ID 100 µm, 3 µm C18 beads).
   - Gradient



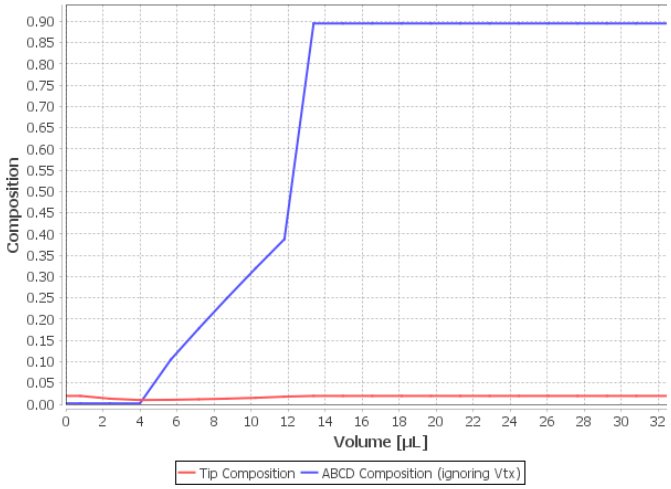
   - The actual flow rates (µl/min; top) and pressure (bar; bottom) for the LP pumps A/B/C/D

   - The actual flow rate (µl/min; top) and pressure (bar; bottom) for the HP pump

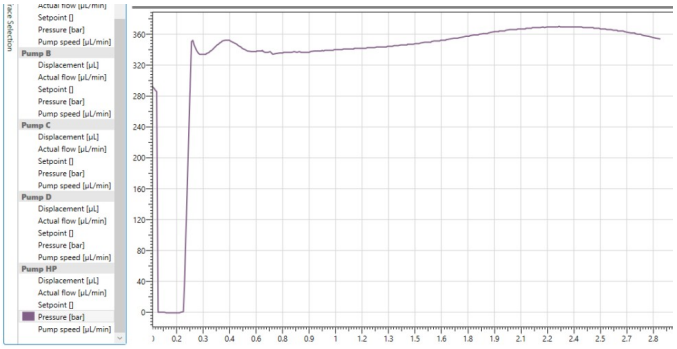




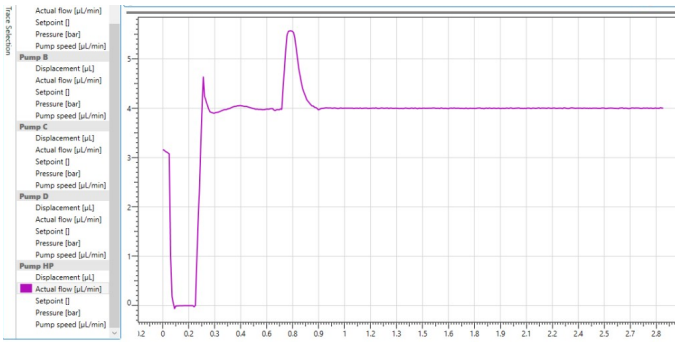
2. The standard “500 SPD” gradient and HP pump pressure and flow profile are shown below.
    - The 500 SPD method is optimized for speed and is unique to the other standard methods in several ways:
        - The ABCD flow rate is higher during the gradient formation.
        - The pump refill speed 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 quicker.
        - The HP pump is pressure controlled when the loop valve shifts to “elute” position.
    - Gradient:



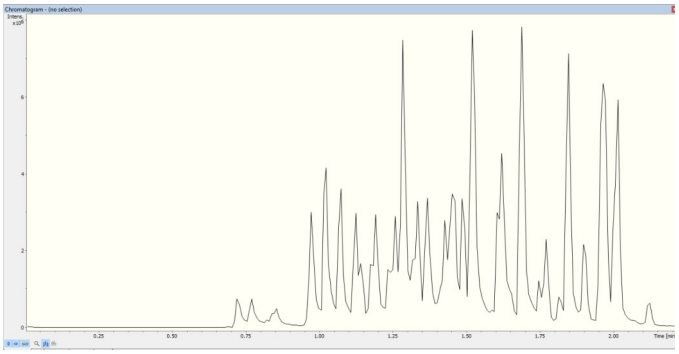
- Pump HP pressure (bar):



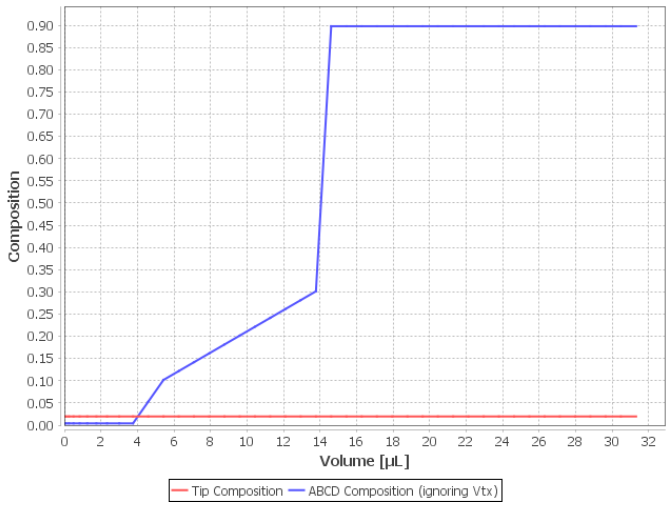
- Pump HP flow (µl/min):



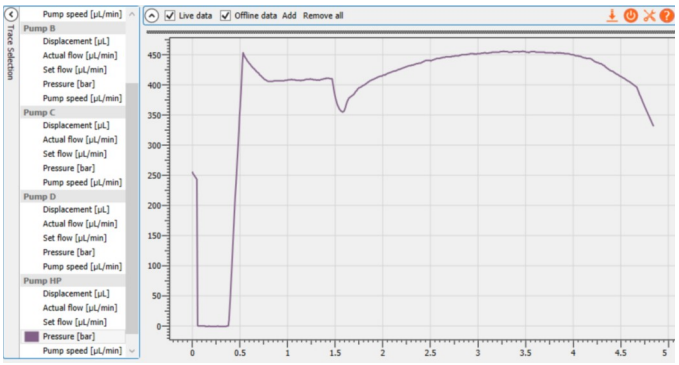
- Example base peak chromatogram of tryptic BSA digest:



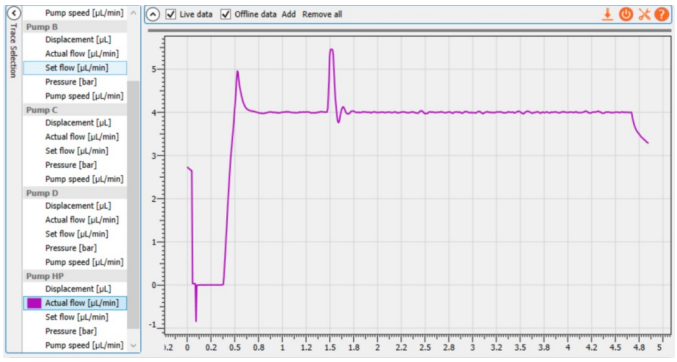
3. The standard “300 SPD” gradient and HP pump pressure and flow profile are shown below.
   - Gradient:



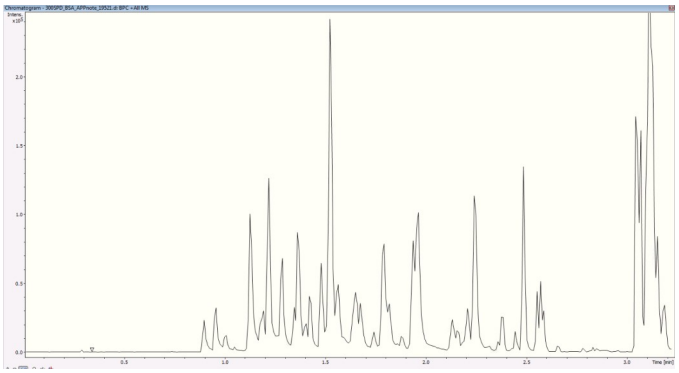
   - Pump HP pressure (bar):



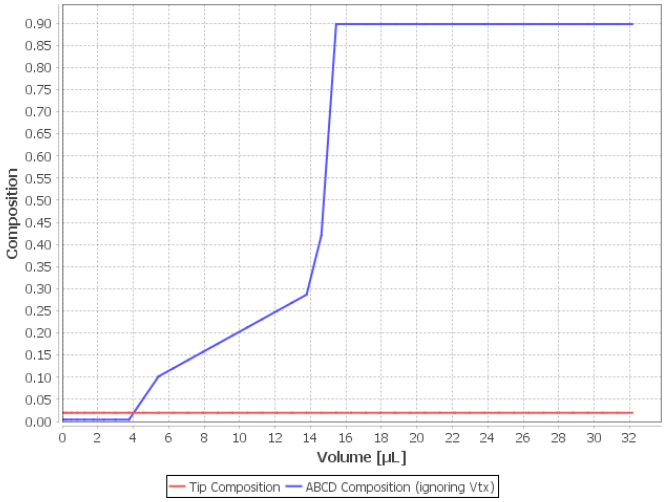
   - Pump HP flow (µl/min):



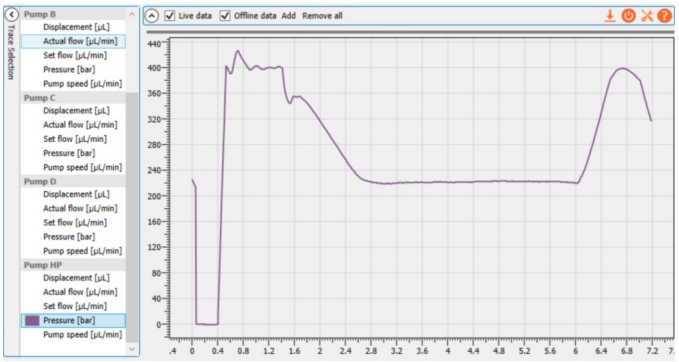
   - Example base peak chromatogram of tryptic BSA digest:



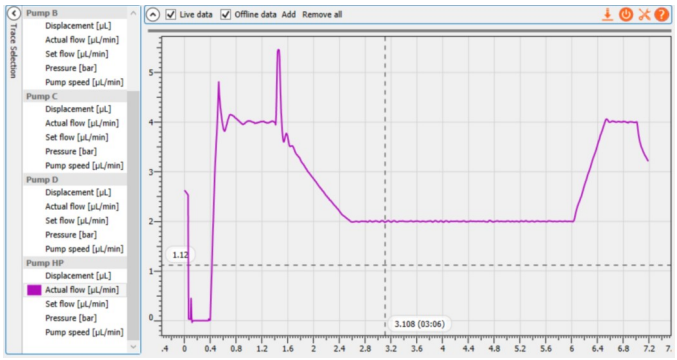
4. The standard “200 SPD” gradient and HP pump pressure and flow profile are shown below.
   - Gradient:



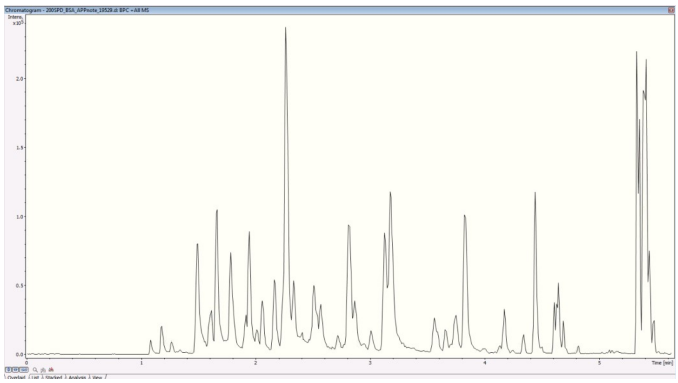
   - Pump HP pressure (bar):



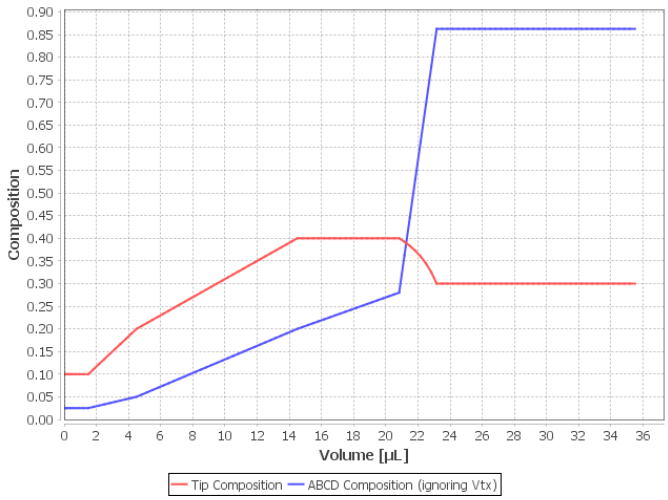
   - Pump HP flow (µl/min):



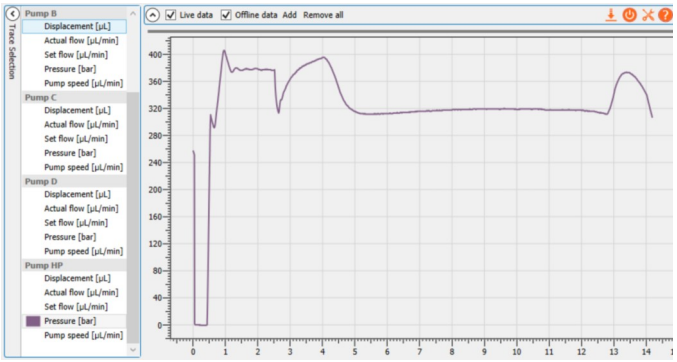
- Example base peak chromatogram of tryptic BSA digest:



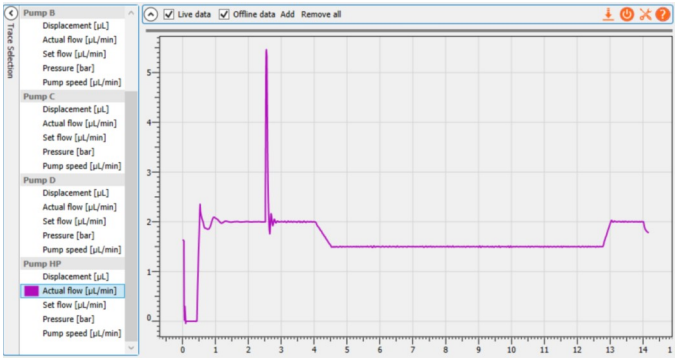
5. The standard “100 SPD” gradient and HP pump pressure and flow profile are shown below.
   - Gradient:



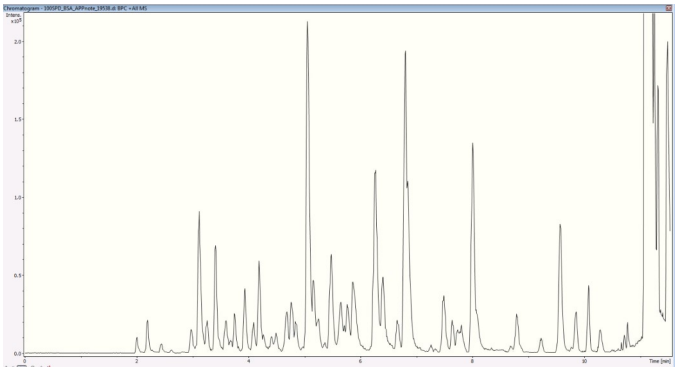
   - Pump HP pressure (bar):



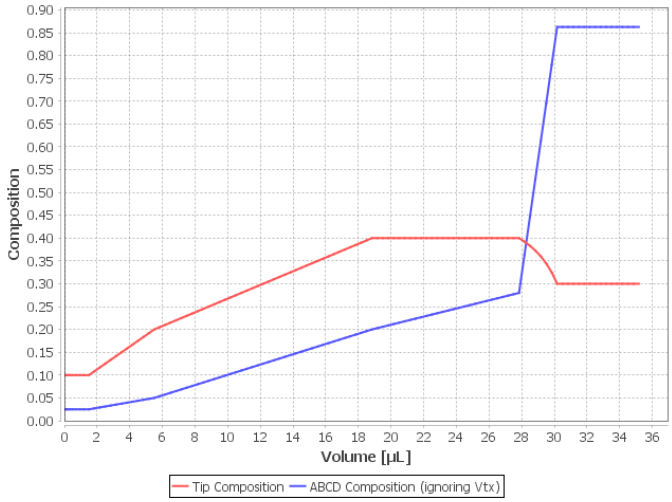
- Pump HP flow (µl/min):



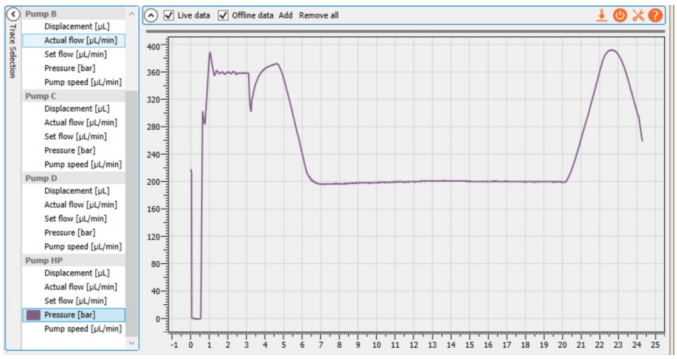
- Example base peak chromatogram of tryptic BSA digest:



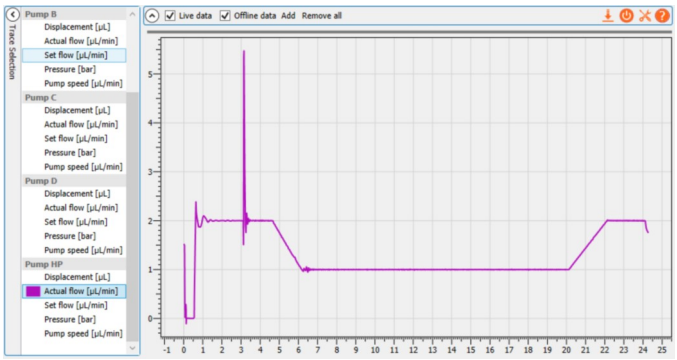
6. The standard “60 SPD” gradient and HP pump pressure and flow profile are shown below.
   - Gradient:



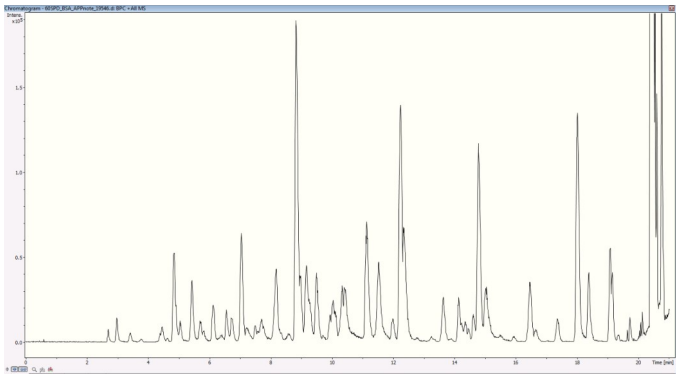
- Pump HP pressure (bar):



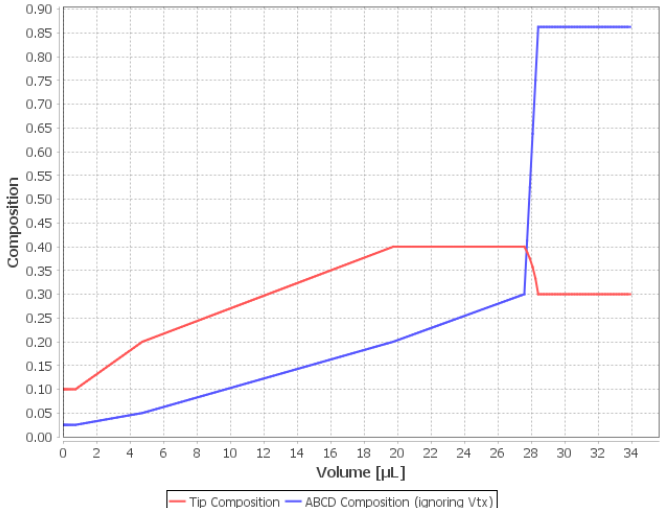
- Pump HP flow (µl/min):



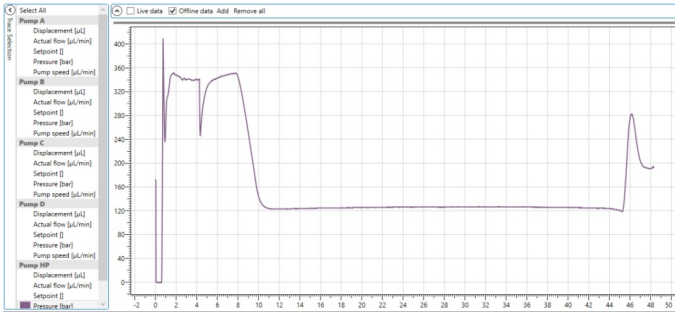
- Example base peak chromatogram of tryptic BSA digest:



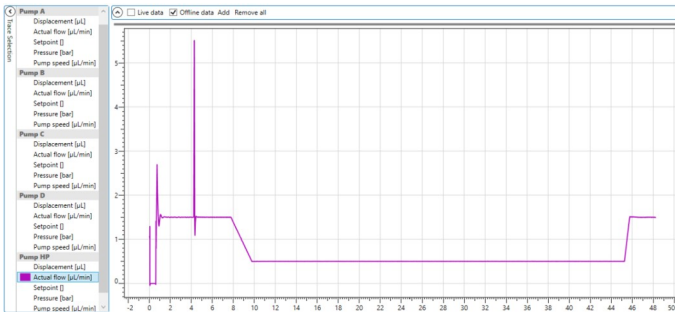
7. The standard “30 SPD” gradient and HP pump pressure and flow profile are shown below.
   - Gradient:



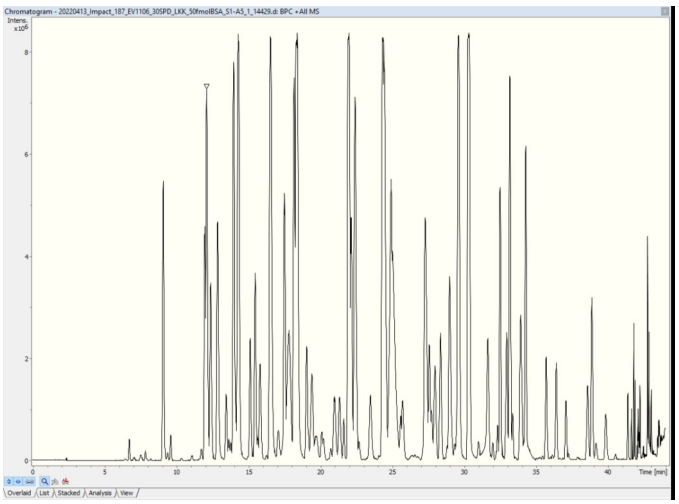
   - Pump HP pressure (bar):



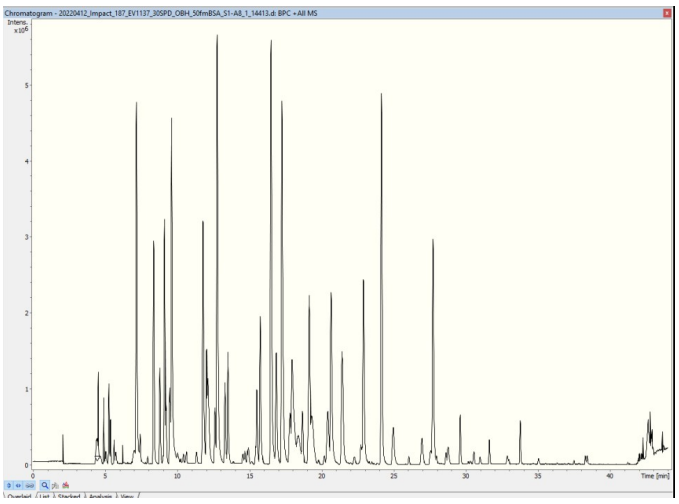
- Pump HP flow (µl/min):



- Example base peak chromatogram of tryptic BSA digest with column EV1106:



- Example base peak chromatogram of tryptic BSA digest with column EV1137:



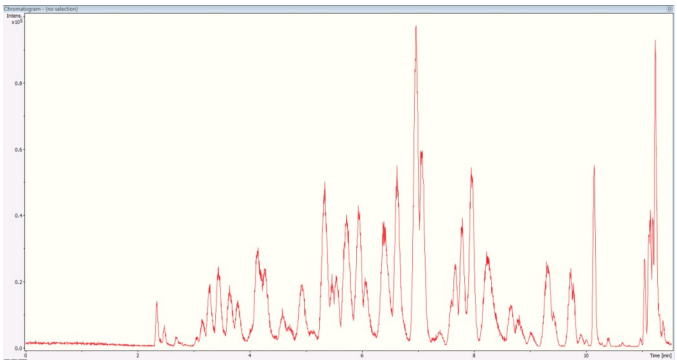
9 Troubleshooting

Please refer to Chapter 8 “Running Samples Using Evosep One” to view all safety warnings, cautions, and concerns.

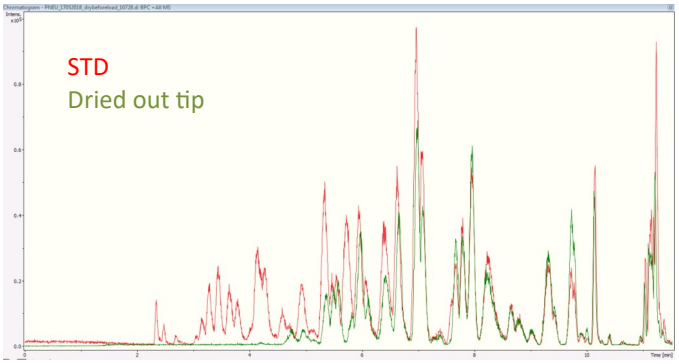
9.1 Evotip Troubleshooting

If the Sample loading onto the Evotips is not done correctly, it can have a negative influence on 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. Some examples of sample loading issues are illustrated below using 200 fmol BSA.

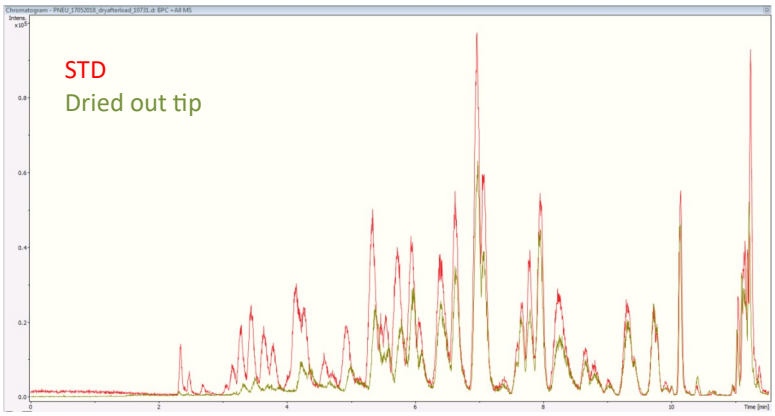
1. BSA correctly loaded on the Evotip according to the SOP.



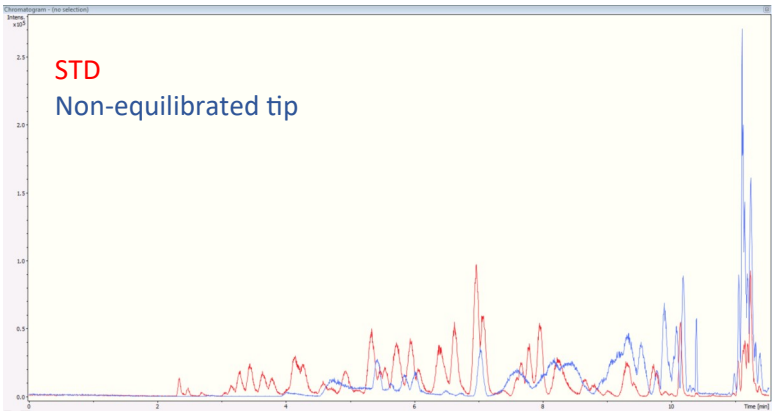
2. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out before loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



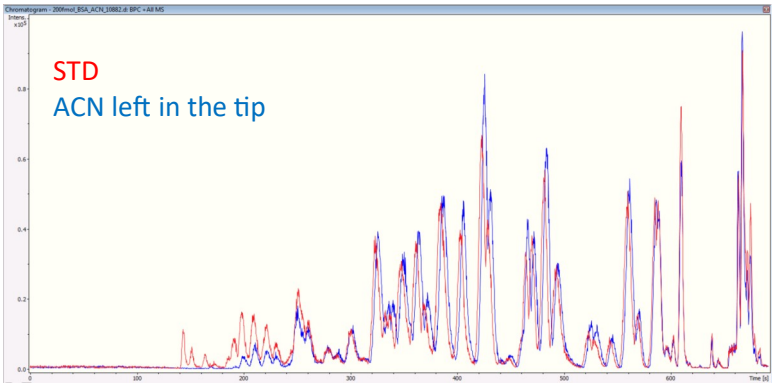
3. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out after loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



4. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which has not been equilibrated correctly before loading the sample (blue).
    - This can happen if activation is accidentally done with water instead of 1-propanol. Weak binding of the peptides leads to poor separation and low intensity.

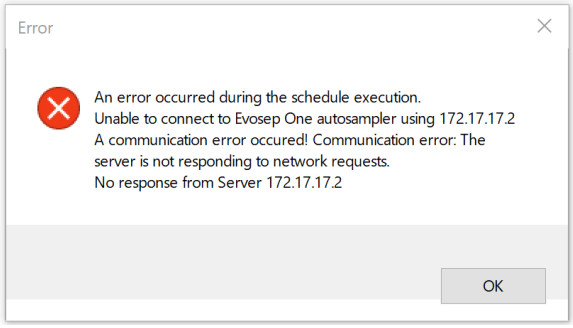


5. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which has residual ACN from the wash step during loading the sample (blue).
    - This can happen if the centrifuge is not correctly adjusted according to the SOP (too low g-force or too short centrifugation time).



9.2 How to Troubleshoot Connection Problems Between PC and Evosep One

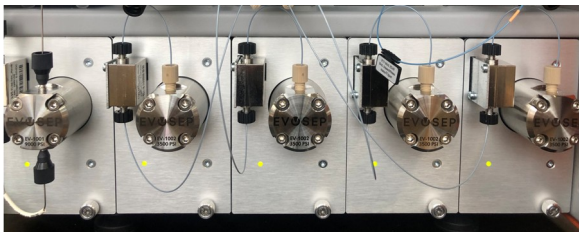
If you are experiencing communication problems between the acquisition PC and the Evosep One, or getting error messages about not being able to connect to the Evosep One instrument, then please follow this guide to work through the most common issues.



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.

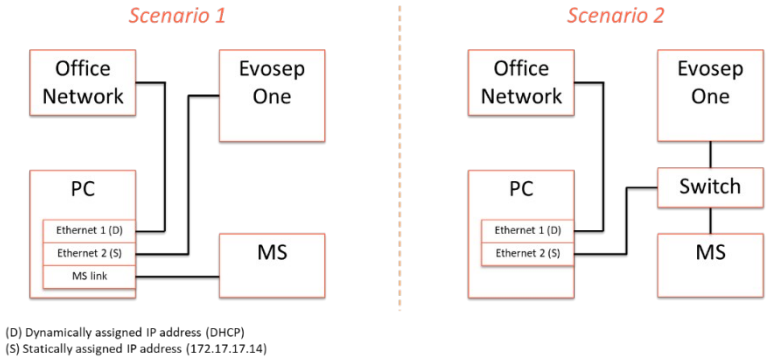


2. Autosampler
   - Check that the LED on the right-hand side of the X-axis is green.

If any of the LEDs are off, please check that power cords are correctly inserted from the power outlet 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.



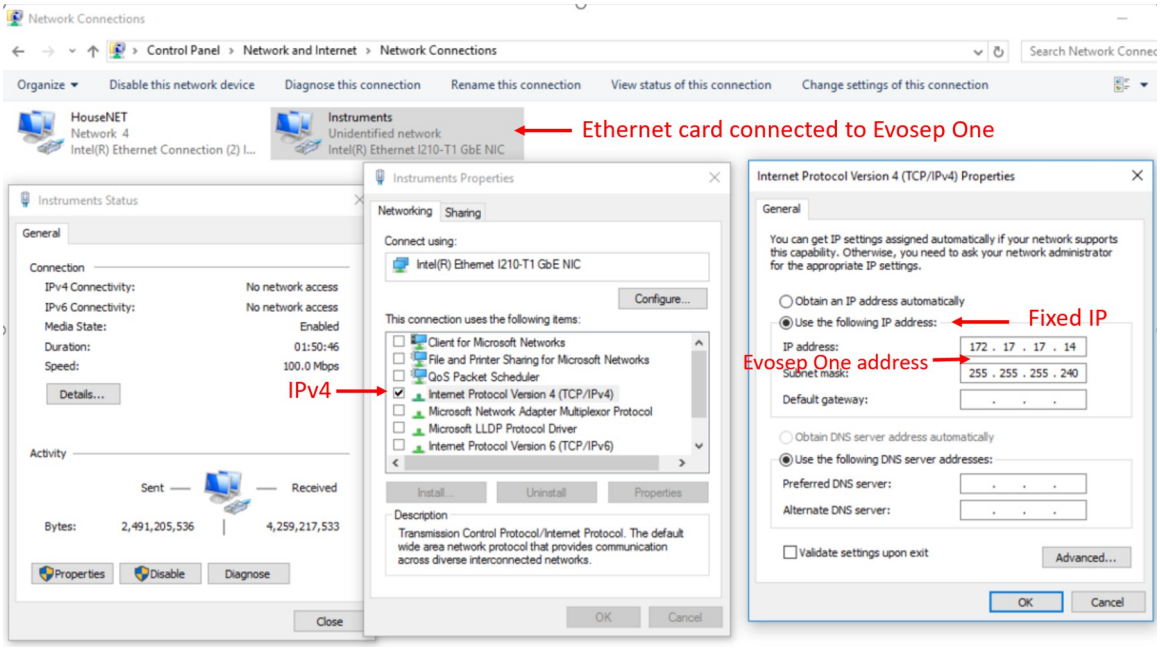
Please verify that the following:

1. The ethernet cables are connected correctly as shown above (normally Scenario 2), and
2. The switch between PC and Evosep One is powered on.

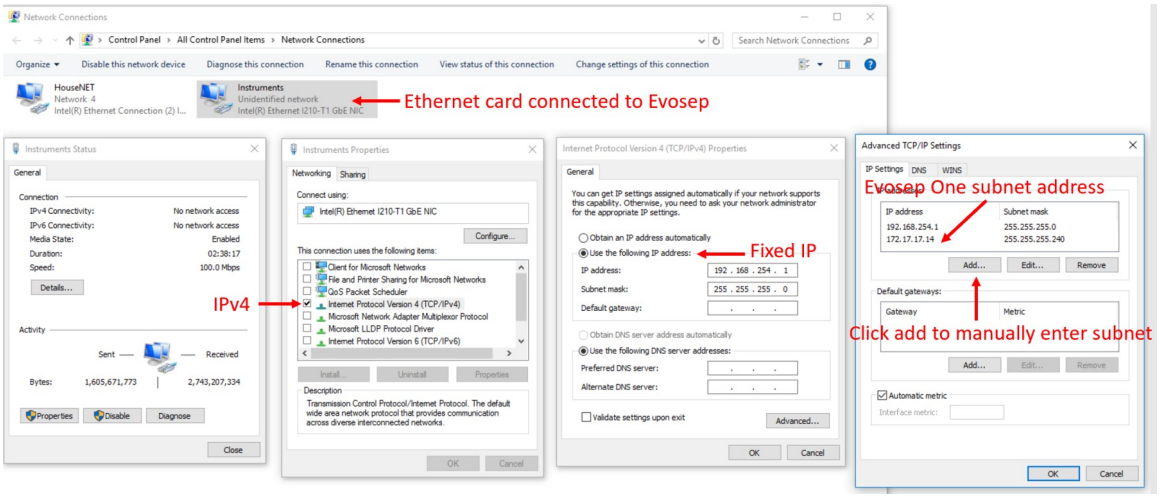
9.2.3 Check Network Adapter Set Up

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

1. The ethernet card is set up to use a fixed IP address.
   - 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)”
2. 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. Change the ethernet card to use a fixed IP address and manually configure its settings.
   - IP address 172.17.17.14 and subnet mask 255.255.255.240



3. 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.

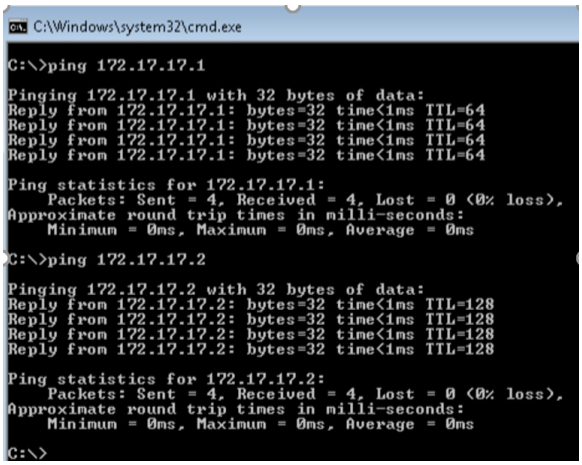


9.2.4 Ping Hardware Units

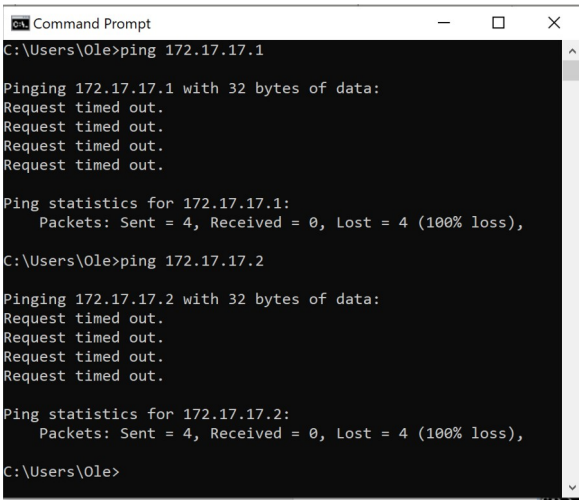
If there is still no connection to the instrument after the network adapter settings have been verified, use the “ping” command from the Windows Command Prompt to check communication with the hardware units.

1. From Windows Start menu, open the “Command Prompt” and type “ping” followed by the IP address of the hardware unit.
   - Pump box: “ping 172.17.17.1”

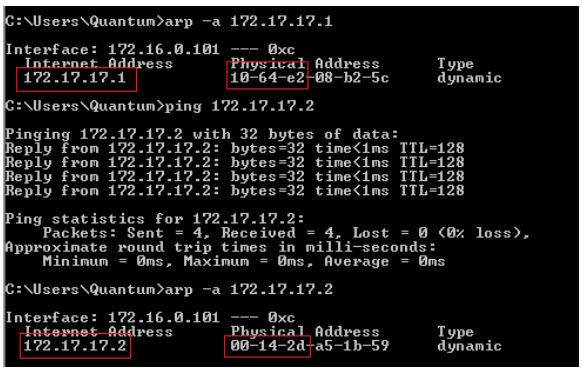
- Autosampler: “ping 172.17.17.2”



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.

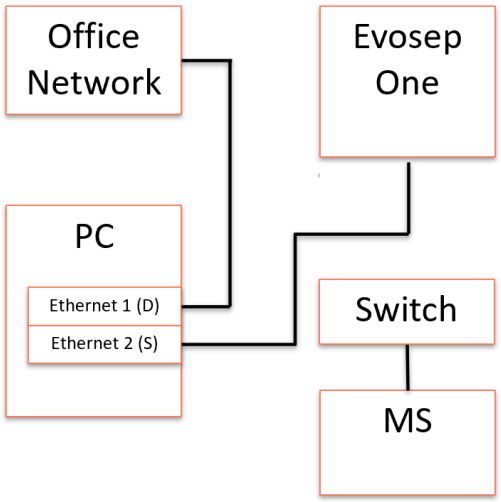


4. To verify that the Ping reply is coming from the Evosep One hardware and not another hardware unit with the same IP address, perform the following commands:
   - Pump box: “arp -a 172.17.17.1”
     - Verify the physical address has prefix “10-64-e2” or “00-90-e8”
   - Autosampler: “arp -a 172.17.17.2”
     - Verify the physical address has prefix “00-14-2d”



On connecting the instrument, the software also logs warnings to the driver/plugin log when encountering unrecognized physical (MAC) addresses.

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.



1. If pinging without the switch is not giving a positive reply, then proceed with removing the ethernet cable from the autosampler, disconnecting the ethernet cable from the pump box, and plugging the pump box ethernet cable into the ethernet connector on the autosampler (this will bypass the built-in gateway of the Evosep One). Redo the ping to IP address 172.17.17.2 (autosampler).
2. If still no reply, please repeat with a known working ethernet cable.
3. 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
4. If restarting the PC does not work, then power cycle the pump box and autosampler and try to connect again.
5. 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 the 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 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 connected/inserted.
     - Power cycle the Evosep One, 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 to exchange 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.

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, the autosampler has been controlled with the terminal and the action has not been completed.
    - Action:
      - With the terminal, check and finish pending action (e.g. change tool).
      - Check that the autosampler is powered on and all cables connected.

9.5 Error Messages Regarding Hardware

Consult the list of error messages below 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.

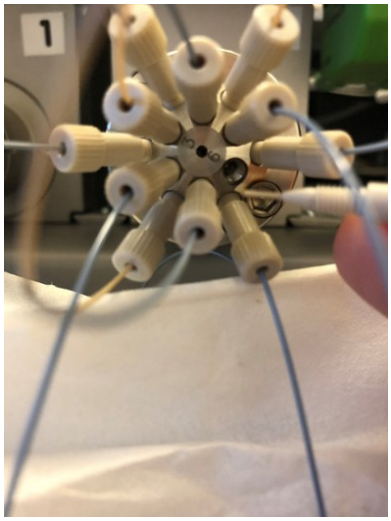
Error Message:

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 using 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 connected 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 (see Section 11.12) and re-run the test.
        - If only one of the four 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. 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. The 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 is obstructing the robot arm. Be aware that the Y-axis needs some room to move 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-down), including the A,B 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 the valve or if valve drive is disconnected.
    - Action:

- Verify in the error message what valve drive has the issue (Valve Drive 1 = Valve 6, Valve Drive 2 = Valve 12, Valve Drive 3 = Valve Loop).
- Verify that the p-bus cables from robot X-axis to valve drive and between valves drives are fully connected into the connector on the 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 (20 µl) to build up pressure during column equilibration, the analysis is stopped. This is done to avoid the risk of the pump emptying completely during the gradient.
    - Action:
        - Ensure the solvent levels in the solvent bottles are adequate and run Prepare – Pump preparation – Degas. If the problem persists, run the Diagnose – Pump HP leak test.

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 Prepare – Pump Preparation – Degas and Solvent exchange scripts, the solvent volume is measured to verify that pump can build up pressure (LP 50 bar, HP 200 bar). If the volume 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:
        - Check the solvent levels in bottle A and B and verify the 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:
        - Pump A/B sample loading pressure is above 50 bar.
    - 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:
  - Pump A/B sample loading pressure is above 50 bar and a tip is not present on the needle.
  - May occur 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:
  - Verify the Evotip position in the autosampler matches the sample list (e.g., check that the same Evotip position was not chosen twice or that a wrong Evotray slot has been chosen).
  - Check that a Tip is not sitting in the Tip inject port.
  - Check needle for damage.
  - Re-run with a 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 is faulty or not connected.
    - Action:
      - Power cycle the instrument and verify if the error is still present and reported for the same pump.
      - Contact support@evosep.com to inform about the 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 the error is still present and reported for the same pump.
      - Contact support@evosep.com to inform about the error message.

Error Message:

23. **Couldn't verify method script authenticity**
    - 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:

- Verify the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile).
- Check that the system is level and free of vibration.
- If both above are correct, then perform a Prepare – Pump preparation – Solvent exchange and run a new sample.
- If the warning continues to appear, please perform Calibrate – Flow sensor ABCD.

Error message:

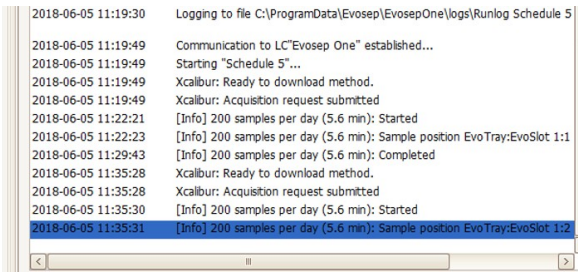
25. **Measured difference in flow between solvent A and B is x /min, please check that the solvents are correct and run Tip seal and HP system tests**
    - Possible cause:
        - To find the loop volume, the system relies on being able to distinguish if solvent A or solvent B is present in the transfer line. It does so by measuring the actual flow rate at 40 bar. 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:
        - Verify the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile).
        - Check that the system is level and free of vibration.
        - If both above are correct, then perform a Prepare – Pump preparation – Solvent exchange and run a new sample.
        - Run Diagnose – Tip seal and HP system scripts to check for leaks.

9.6 Schedule / Sample Not Starting / Contact Closure Problems

9.6.1 Troubleshooting Tips for Xcalibur Set Up

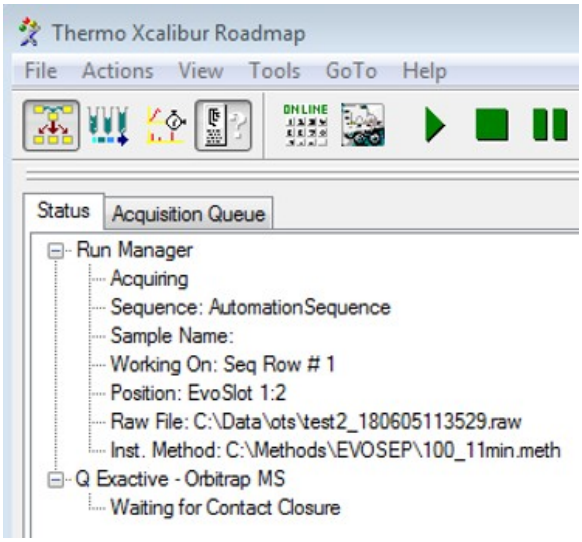
The Evosep One is set up to wait for the MS to be ready before starting a sample run. This feature ensures that the Evosep One does not run any samples if the MS is not ready to acquire data.

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



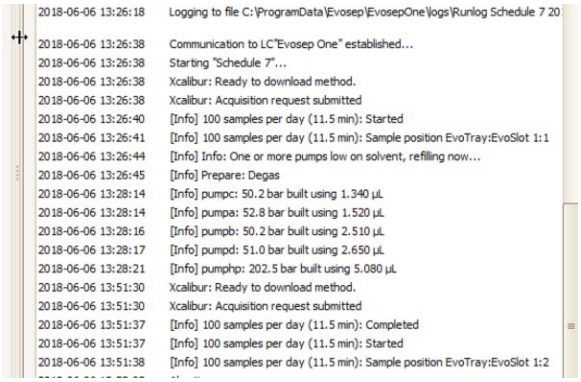
When Chronos has verified that Xcalibur is in the “Ready to download method” state, the acquisition request is submitted, and the sample started.

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.
   -Info: One or more pumps not referenced, referencing now.
   -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 without 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.



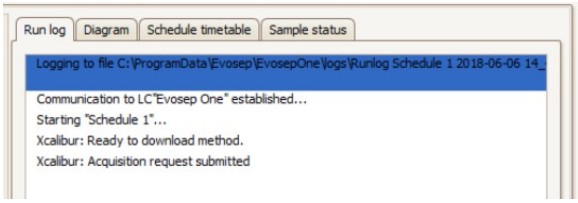
- Action:
  - None, after successful degas and align solvents the instrument will start the sample

Typical issue 2:

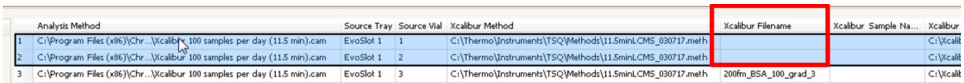
2. Evosep One does not continue the schedule after the first sample, MS does not change status from Waiting for contact closure to Running.
   - Possible cause:
     - Contact closure cable is not connected, faulty, or not configured correctly.
     - When a schedule is started the instrument will check if MS is ready and then start the first sample. If the contact closure cable is not properly 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 for information 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:

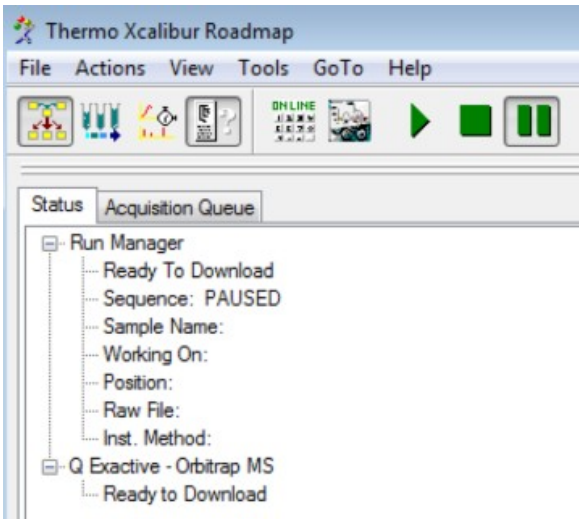
3. The schedule does not start even though the Run log shows Acquisition request submitted.



- 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:
    - In the status view for MS, verify 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:

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 provide information on how to fix the most common errors. This Section describes the diagnostic programs and provides additional background information on the error causes and how to repair the issue.

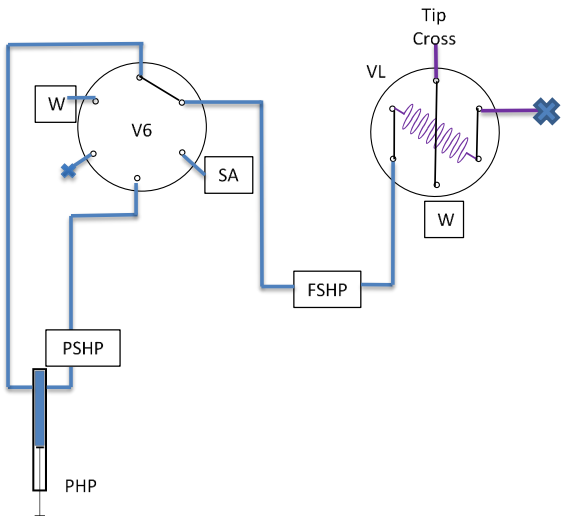
9.7.1 Leak in the HP System

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

When asked to blind the transfer line, 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	☐	☐	☐	☐	☑



1. **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 tubing connections 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.

2. **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.
3. **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, valve, or connecting lines.
   - Check all lines and re-run the script.
   - If the script fails, please run the Diagnose – Pump HP script.

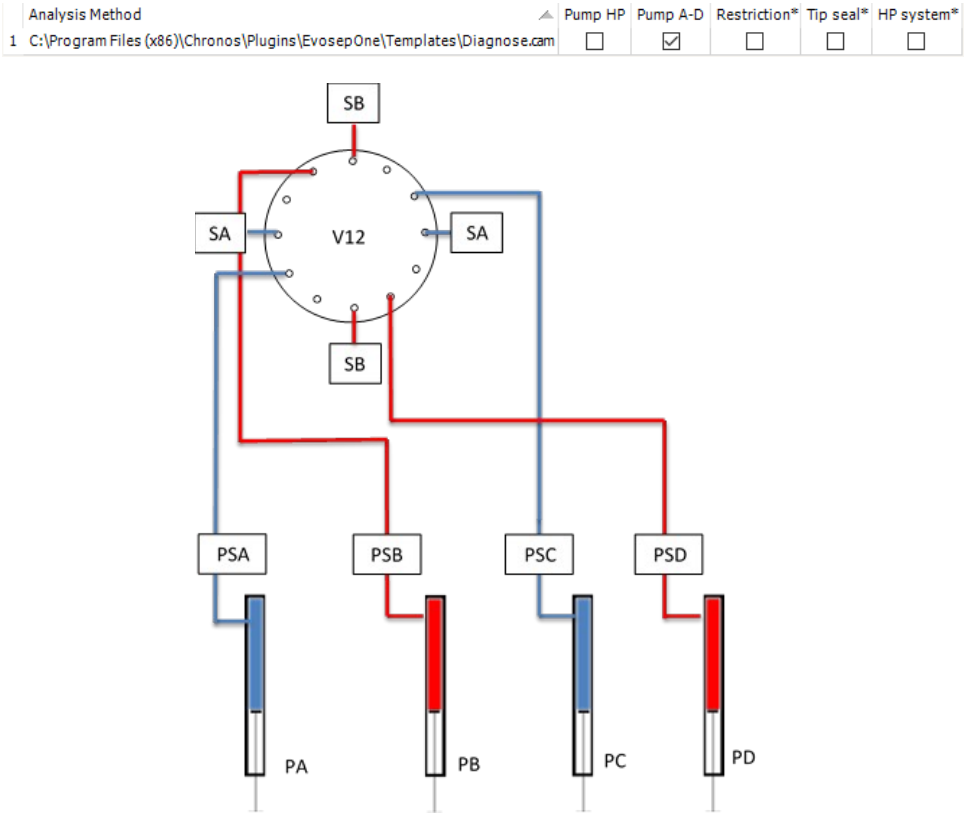
	Analysis Method	Pump HP	Pump A-D	Restriction*	Tip seal*	HP system*
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.cam	☑	☐	☐	☐	☐

- 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 script Diagnose – 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.



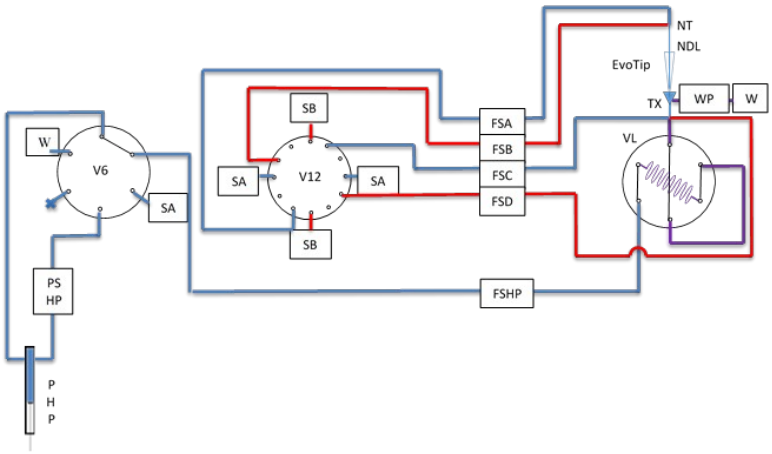
1. **A leak is observed as a flow that is too high on pump speed for PSA, PSB, PSC or PSD, pump speed > 1.0 µl/min.**
   - Tighten the connections in the subsystem displaying a leak and re-run the script.
   - If the script fails, block the line going from the pump pressure sensor to the V12 at the V12 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	☐	☐	☐	☑	☐

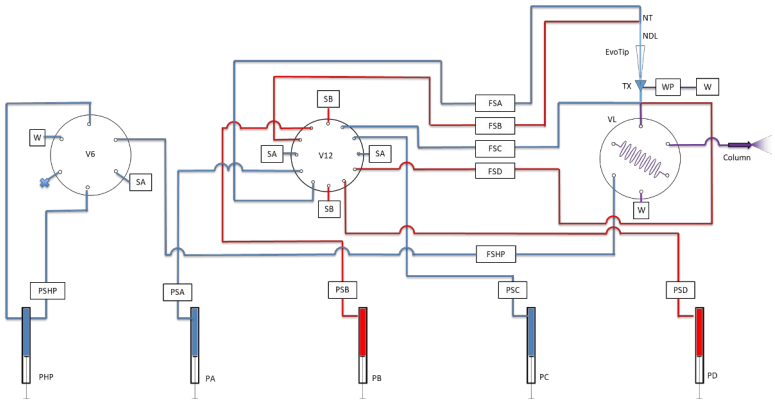


1. **A leak is observed with a flow that is too high on FSHP, flow > 0.10 µl/min and negative flow on FSA, FSB, FSC, or FSD.**
   - Tighten the line connecting the FS to the V12 and re-run the script.
   - If the script fails, place the endcap on the line connecting to the V12 and re-run the script.
   - 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.
2. **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 Diagnose – 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 pressure above 70/50/70/50 bar for pump A/B/C/D respectively is a sign of a partially blocked subsystem.
   - If both pump A and pump B are restricted:
     - This indicates a blocked needle.
       - Please try to rinse the tip of the ceramic needle with Kimwipe or similar, soaked in methanol.
       - Exchange the needle.
     - Re-run the script and if the restriction still is too high, call for assistance.
   - If either pump A/B/C/D are restricted:
     - This indicates a blocked line from V12 to the respective flow sensor.
       - Disconnect the tubing on valve V12, flush the valve port with ethanol to remove residual material, and reconnect the tubing.
       - Exchange the tubing.
     - Re-run the script. If the restriction is still too high, call for assistance.
2. **High pressure system:**
   - A pump HP pressure above 150 bar indicates a partially blocked high-pressure subsystem.
     - Remove the transfer line and re-run the script.
     - 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.
     - If pressure is still > 70 bar, remove the line FSHP to VL and re-run the script.
     - 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.
     - If the pressure is still > 40 bar, replace the line going from the V6 to FSHP and re-run the script.
     - If the pressure remains > 40 bar call for assistance.
3. **Tip interface and loop:**
   - A pressure above 50 bar for either pump A/B/C/D indicates a partial restriction of the tip interface or the loop.
     - Disconnect the loop on the loop valve, flush the valve ports with ethanol to remove residual material, and reconnect the loop.

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

10 Routine Maintenance

Please refer to Chapter 8 “Running Samples Using Evosep One” to view all safety warnings, cautions, and concerns.

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 solvent bottles A and B. Refill if necessary.
- Visually inspect solvent level in waste bottle. Empty if necessary.
- Visually inspect tip disposal container. Empty if necessary.

10.1.2 Weekly Maintenance

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

10.1.3 Prepare Instrument for Storage

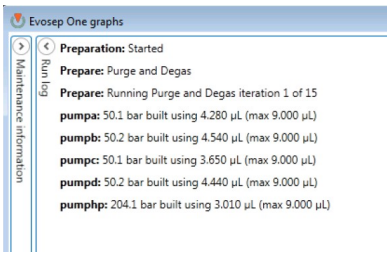
If the instrument is not going to be used for 1-2 weeks, please perform the following tasks.

1. To prevent bacterial growth, it is recommended to switch to organic solvents.
2. To minimize risk of instrument contamination, always wear gloves when handling the tubing going to the solvent bottles.

3. Place both A and B lines in solvent B bottle as illustrated above.
4. Run the Prepare – Pump preparation – Solvent Exchange script.
5. Park the autosampler in lock position.
6. Switch off instrument.

When starting up after storage, please perform the following tasks.

1. 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.
2. Empty, rinse, and refill solvent bottles A and B.
3. Place both A and B solvent lines in Solvent B bottle as illustrated above.
4. Empty waste bottle.
5. Run 3-4 cycles of the "Solvent exchange" script and ensure that values in the run log are within specification.
   - Pump HP is less than 6 µl.
   - Pump A-D is less than 7 µl.



6. Move the solvent A tubing back into solvent bottle A.

7. Check that the solvent lines are inserted in the correct bottles.
8. Run the Prepare – Pump preparation – Solvent Exchange for all 15 cycles.

11 Replacing Spare and Wear Parts

Warning/Caution	
	**Risk of danger: the use of proper PPE is essential to protect oneself from harm and to minimize contamination from entering the Evosep One instrument. Always exercise caution when dealing with potential health and safety risks.**
	**Electrical shock hazard: Risk of transfer of high voltage from MS to the Evosep One instrument through liquid connection. Do not touch the column, emitter, column/emitter connection, or MS inlet when the MS emitter is at high voltage. Turn off the MS inlet voltage before adjusting or changing the column or emitter. Power off the instrument before handling/exchanging electrical components to prevent electrical shock.**
	**Risk of fire: take care when handling flammable solvents to ensure they do not come in contact with, are spilled on, or are exposed to the Evosep One instrument electronics or sources of sparks, open flames, heat, or hot surfaces as they may cause a fire. Follow MSDS and local safety guidance when handling flammable solvents.**
	**Corrosive hazard: exercise caution when handling corrosive solvents. Contact with the skin and/or eyes, inhalation, and/or ingestion may be harmful. Follow MSDS and local safety guidance when handling corrosive solvents. The use of proper PPE is essential to protect against skin and eye contact, inhalation, and ingestion.**
	**Broken glass: handling of broken glass may result in cuts. Take care not to drop or break glass containers. Avoid handling broken glass to reduce the risk of cuts. The contents of the glass containers when spilled may impose additional healthy and safety risks.**
	**Toxic fumes: handling of toxic solvents imposes a risk of toxic solvent evaporation, inhalation, and causing harm. Exercise caution when handling toxic solvents and their vapor. Minimize vapor production by avoiding heat sources and prepare samples and solutions in a well-ventilated area. Follow MSDS and local safety guidance when handling toxic solvents. The use of proper PPE is essential to protect against skin and eye contact, inhalation, and ingestion.**
	**Risk of impact: autosampler acceleration or deacceleration may lead to table movement, depending on the table stability, imposing a risk of impact. Do not stand or place items too close to the autosampler and/or table during operation, other than the Evotips in the designated locations. It is recommended to have the Evosep One instrument on a stable table with clear space on and around the instrument when operational.**
	**Risk of entrapment: during operation, movements of the autosampler arm can cause impact or trapping. Do not place or move your hand/body parts within the safety rail of the autosampler during operation. As an additional precaution, an alarm sounds prior to autosampler arm movement. Do not intercept the autosampler arm during movement. Placement of Evotips in the autosampler area should only be done when**

	the autosampler is stationary and nonoperational.
	**Sharp objects: during operation, the autosampler needle is raised and lowered within the safety rail. This has a blunt point but poses a risk of puncturing the skin if a hand/body part were to be trapped when the needle is lowered. Additionally, needle contact with skin leads to exposure of small amounts of potentially hazardous solvent/sample. Do not place or move your hand/body parts within the autosampler safety rail during movement or operation. Exercise caution when handling and/or replacing the needle and avoid touching the ends as it poses a threat to a puncture wound. The use of appropriate PPE is especially important when working with dangerous and/or toxic materials.**

11.1 Recommended Wear Part List

Evosep recommends having 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

After replacing a wear part, please see below table for what tests to run to verify successful replacement:

Wear part	Test to run after replacement
EV1008 – valve 12 rotor	Pump A-D
EV1011 – valve 6 rotor	Pump HP
EV1014 – valve loop rotor	HP system
EV1103 – valve 12 to flow sensor LP (EV1023 for systems S/N < S00088)	Tip seal + Restriction
EV1018 – needle	Tip seal + Restriction
EV1058 – HP piston seal	Pump HP
EV1060 – LP piston seal	Pump A-D
EV1034 – Transferline	HP system

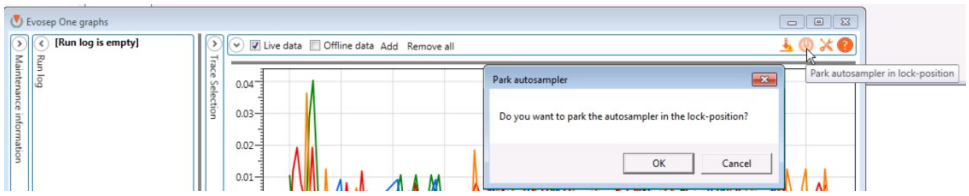
Please find pictures of all parts in our web shop.

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

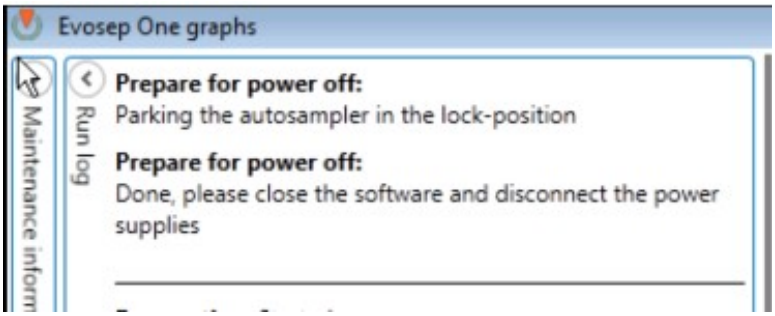
Please follow below instructions on replacing spare and wear parts on the system.

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 power icon and click “OK” in the pop-up window to park the autosampler in the lock-position.



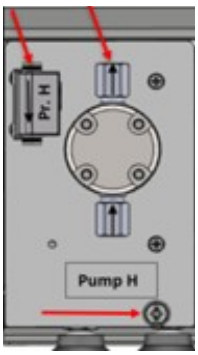
4. The autosampler Z-axis will now be parked in lock position. When completed, a message will be shown in the run log. The software can be closed and the power supplies disconnected.



Note: if for 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 the Viper tubing from the top port on HP pressure sensor.
4. Disconnect the Viper tubing from the top port on HP pump.

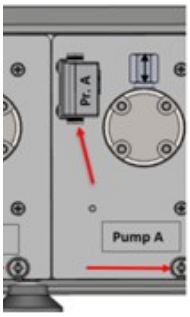


5. From the left side, push out the cable binder holding the waste tubing.

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 the 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.

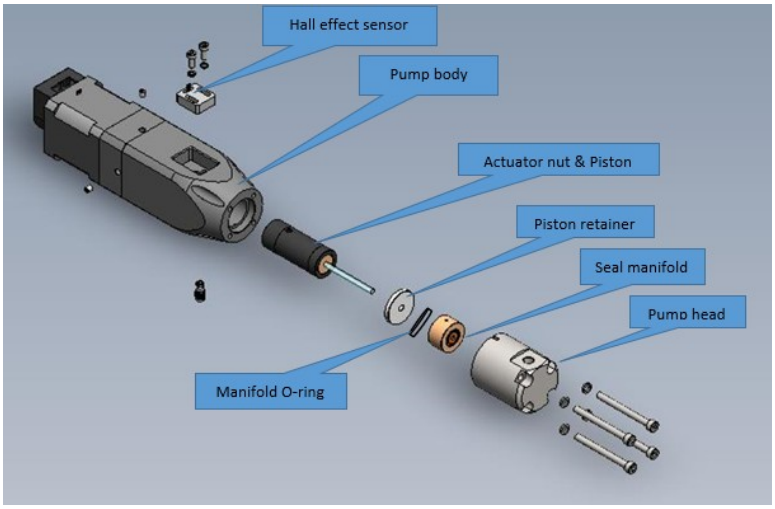


4. Gently pull out the pump cassette from the instrument by pulling the knurled nut. Be careful not to kink or break the surrounding tubing.
5. Insert the pump cassette in reverse order.
6. Run Preparation – Pump preparation – Solvent exchange to ensure the new pump is fully purged and degassed.
7. Run 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 the pump cassette from the instrument as described in Sections 11.5 and/or 11.6.
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 hold the pressure sensor.
4. Gently slide the pressure sensor away from the cassette and disconnect the pressure sensor cable.
5. Install pressure sensor in reverse order. Make sure the pressure sensor cable connects securely into the pressure sensor.

11.6 Replacing Pump Piston Seals Including Seal Manifold



1. Run the Service script to fill pumps. This will fully retract the piston within the pump housing.
2. Disconnect the tubing from the pump head.
3. Use a 3 mm hex key to loosen and remove the 4 pump head screws. 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.

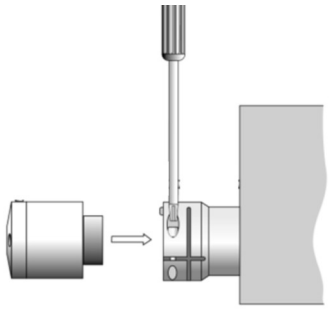
Upon setup or when servicing the seals, wetting the seal and pump head piston bore with acetonitrile will help reduce air bubbles and will allow faster flow stabilization. Use an adjustable pipette with a plastic tip to avoid damaging the sealing surfaces. Flush the spring cavity of the pressure seal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

1. 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.

2. Take the pump head with the manifold and slide the manifold and pump head onto the piston.
3. Tighten the 4 pump head screws finger-tight and then tighten them securely, diagonally.
4. Reconnect the tubing to the pump head.

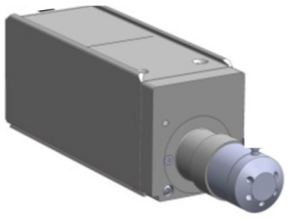
5. Run the degas script to remove air from the pump.
6. Run leak test for that pump to ensure that the new seal is sealing correctly.

11.7 Replacing a Valve Stack (Field Service)

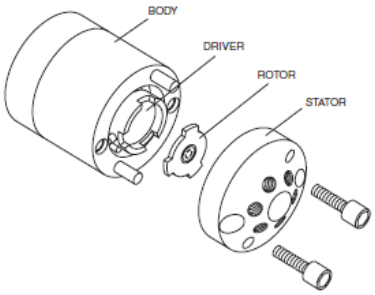


1. Disconnect all tubing from the stator port.
2. For replacement of Valve 6, run the service script called “Set valve 6 pos 2-3”
3. Unscrew the Torx screw at the valve adapter, which holds the valve to the valve drive.
4. Install the replacement valve and reassemble the above steps in reverse order.

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 the load is removed.
2. Slide the stator off the two guiding rods, . Be careful not to stress any tubing connected to the stator.
3. Visually inspect the stator surface within the inner ring.
   - If stator surface is not 100% clean, remove residues with a lint free tissue soaked in appropriate solvent.
   - If the 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. Make 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 the guiding rods with port one pointing upwards. 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 in Section 11.10 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 table indicating tubing positions in valve ports can be found.

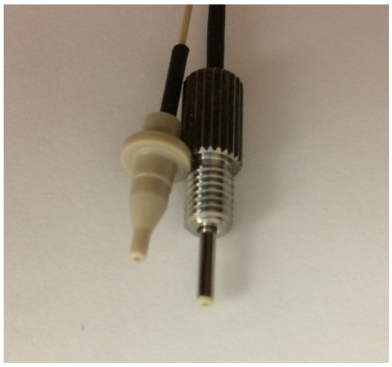
Viper and nanoViper tubing:

Viper and nanoViper tubing are finger tight fitting systems that require little torque to seal. It is essential to follow the below guidelines to avoid damage by over-tightening:

1. Insert the Viper or nanoViper into the receiving port.
2. Tighten the screw until you feel resistance.
3. Turn the screw a maximum of 45 degrees (1/8 of a full turn).
4. Verify that the connection is leak free. Typically, the fitting is tight after an additional 45-degree turn.

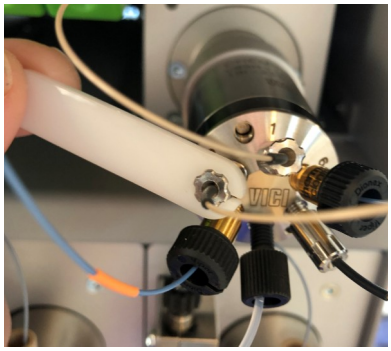
5. If the fitting is leaking, turn the screw up to an additional 45 degrees. Do not turn the screw beyond 90 degrees from where the initial resistance was felt.

NanoConnect tubing:



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

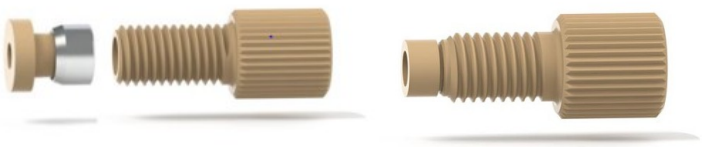
1. Insert the NanoConnect fitting into the receiving port and finger-tighten the nut securely.
2. For male NanoConnect fittings, use the NanoConnect torque wrench.



Peek tubing:

Peek tubing is used for low pressure connections on the system with various fittings. These are all finger tight connections. 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.



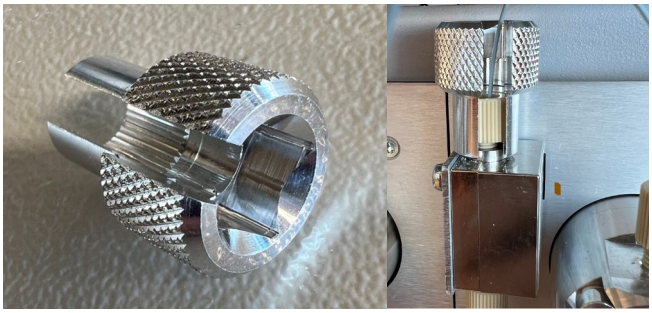
1. Check that the tubing is either level or sticking slightly out of ferrule.
2. Insert into the receiving port and finger-tighten the nut securely.

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

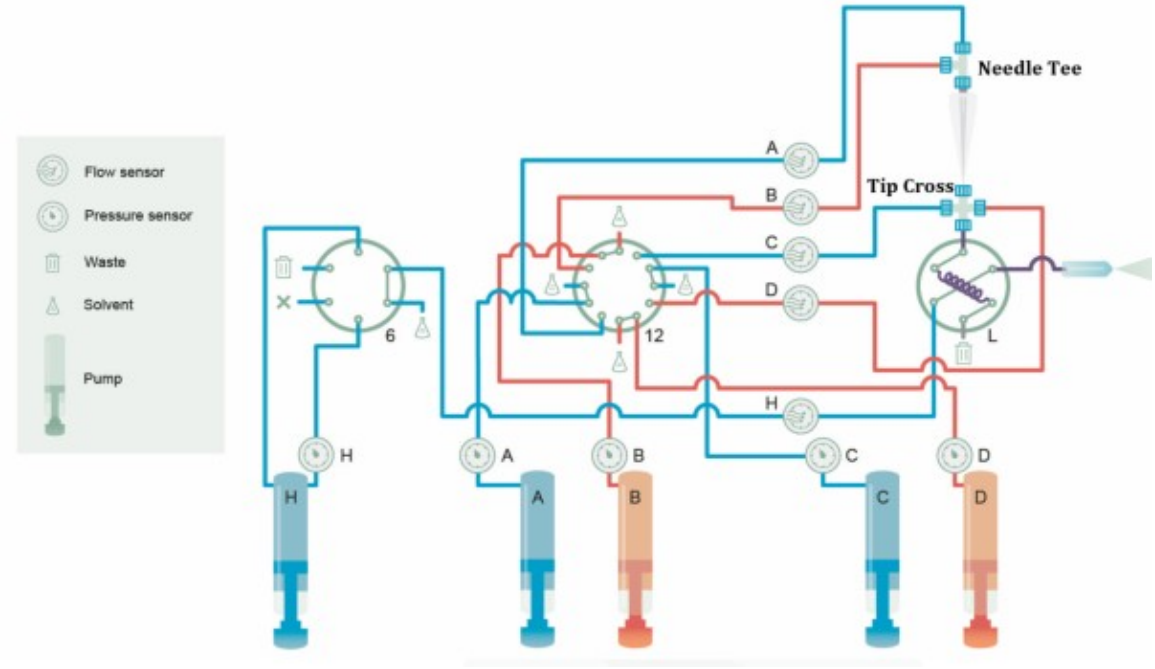
1. Slide the peek nut onto the tubing.
2. Insert the nut and tubing into the receiving port.
3. Finger-tighten the nut securely while making sure that the tubing is bottomed out in the port.
4. When tightened, gently pull the tubing to verify it is seated correctly.

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

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



Tubing diagram and ports valve port positions:



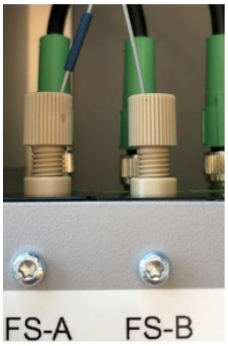
Valve Drive 1 Valve 6 tubing position		Valve Drive 2 Valve 12 tubing position		Valve Drive 3 Valve Loop tubing position	
P1	Pump HP	P1	Solvent B Tee	P1	TipCross
P2	Waste	P2	Pressure sensor B	P2	Loop
P3	Blank	P3	Flow sensor B	P3	Flow sensor HP
P4	Pressure sensor HP	P4	Solvent A Cross	P4	Waste
P5	Solvent 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		

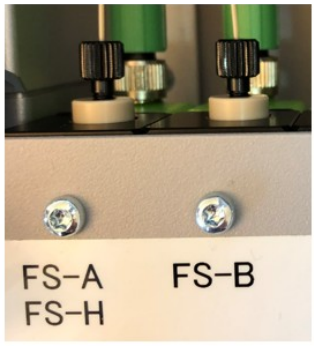
11.11 Replacing Tubing A, B Flow Sensor to Needle Tee Tubing

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 Evosep Support or contact support@evosep.com.



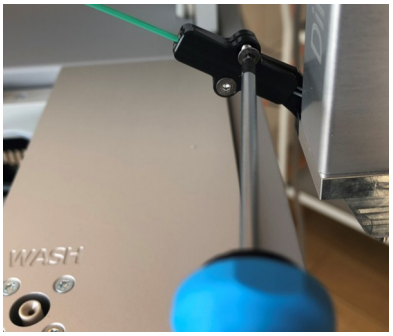
Flat bottom version

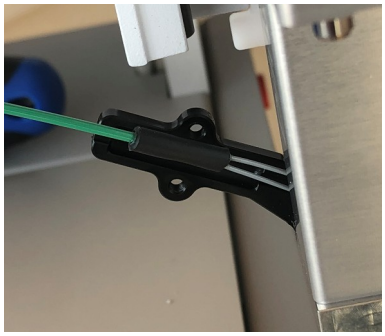


6-40 coned version

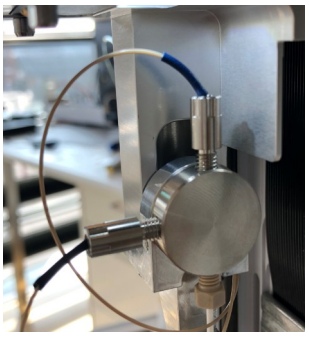
To remove the tubing:

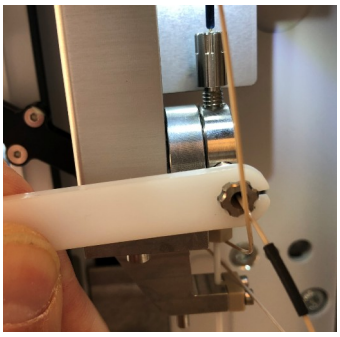
1. Remove the sample tray from the instrument.
2. From the PAL Terminal, select “RobotArmLeft”.
3. Select “Options” and “Change Syringe”.
4. Select “Move” to move the needle to the exchange position.
5. With a T6, remove the two Torx screws from the black tubing holder and remove the cover.



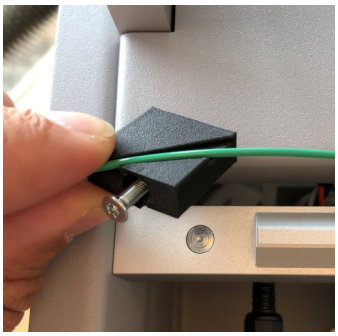


6. Disconnect tubing A and B from the stainless-steel Tee using the small torque wrench.
   - Note that tubing A is oriented vertical with a blue label and tubing B is oriented horizontal with a black label.

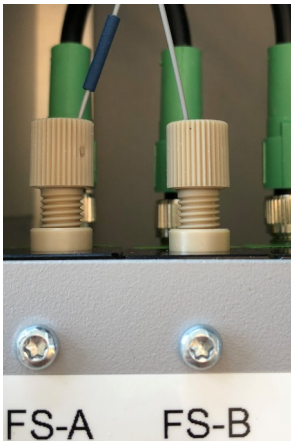




7. With a T10, loosen the screw from the tubing holder in the back-left corner of the cabinet and gently remove the holder.



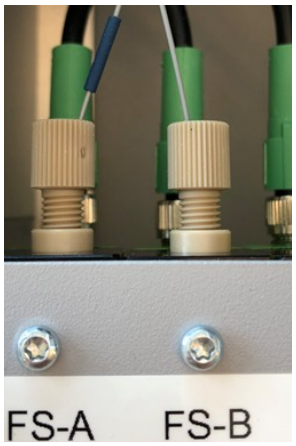
8. Disconnect the tubing from the exit side of flow sensor A and B.
   - Note that tubing A has a blue label.



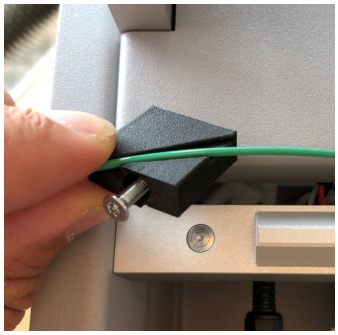
9. To remove tubing completely, slide the two metal fittings on tubing A and B down through the small opening where the tubing holder was sitting.

To connect new tubing:

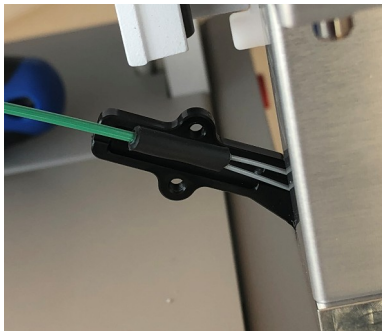
1. From the PAL Terminal, select “RobotArmLeft”.
2. Select “Options” and “Change Syringe”.
3. Select “Move” to move the needle to the exchange position.
4. Push the tubing with the two metal fittings up through the small opening. Do this from underneath in the up direction.
5. Route the other end of the tubing with the peek fittings underneath the tubing coming from the drain pump and connect the two peek fittings to flow sensors A and B.
    - Note that the tubing with the blue sleeve should be connected to flow sensor A.

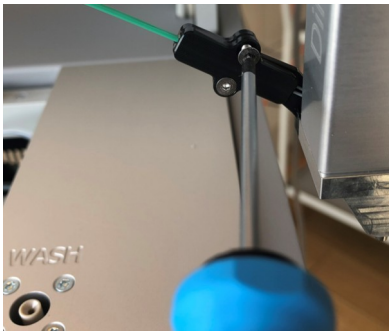


6. Put the green tubing in the groove of the tubing holder. Slide it into the small opening between the back top-cover and the metal frame. With a T10, tighten the screw in the tubing holder all the way in and verify the following:
    - The holder and screw are flush with the metal frame, and
    - leave as much length of the tubing as possible out of the instrument.

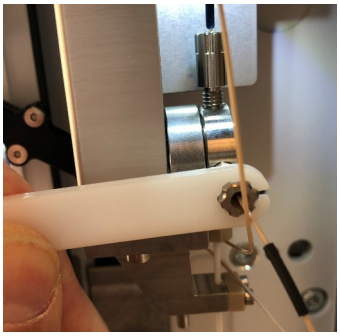


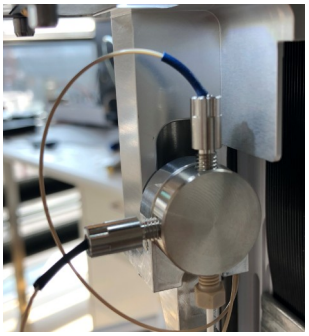
7. Put the black sleeve on the green tubing into the small groove on the black tubing holder. Route the tubing on the backside of the Tee holder and put the black cover back on the tubing holder using a T6 screwdriver.





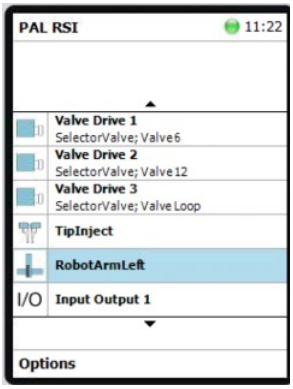
8. Connect tubing A and B from the stainless-steel Tee and tighten with the torque wrench.
   - Note that tubing A is oriented vertical with a blue label and tubing B is oriented horizontal with a black label.
   - Do not use any other tool besides the NanoConnect torque wrench for the NanoConnect fittings.

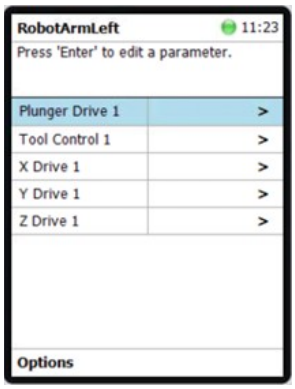




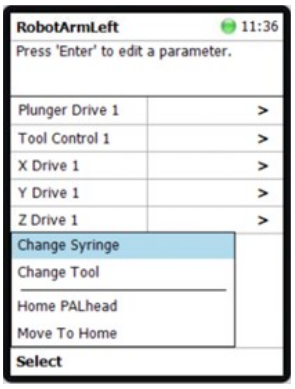
11.12 Replacing the Needle

1. From the PAL Terminal, select “RobotArmLeft”.

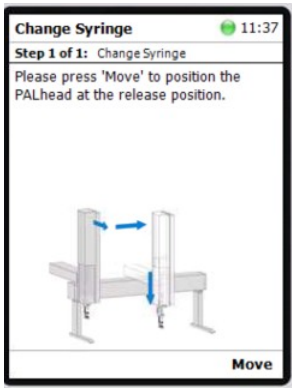




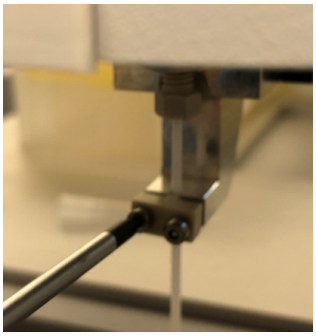
2. Select “Options” and “Change Syringe”.



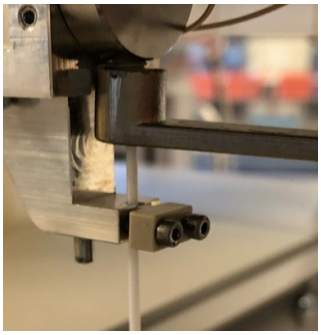
3. Select “Move” to move the needle to the exchange position.



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



5. Hold the needle with one hand and unscrew the peek nut holding the needle in the needle tee with the other hand.



6. With the peek nut fully unscrewed, remove the needle and peek nut.

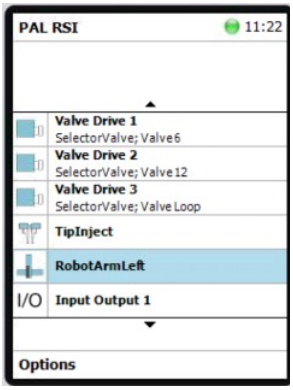
7. When inserting the new needle, work in reverse order by pushing the non-tapered end of the needle (EV1018) through the needle clamp, through the small PEEK nut, and into the bottom port of the needle tee.
8. Make sure that the needle is seated completely in the bottom of the port and finger-tighten the PEEK fitting. Use the ¼" socket wrench to tighten the PEEK nut a maximum ½ turn more. After that, tighten the needle clamp again.
9. When completed, press "Next" on terminal to move "RobotArmLeft" back to home position.
10. Press "Back" to go to the "PAL RSI" main terminal page.

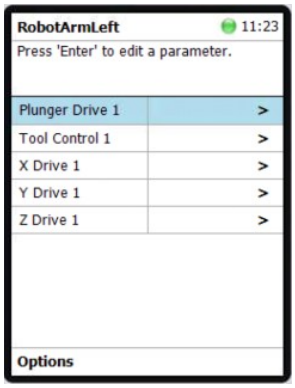
11.13 Replacing the Needle Tee

1. Remove the needle as described in Section 11.12 "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 reverse order.

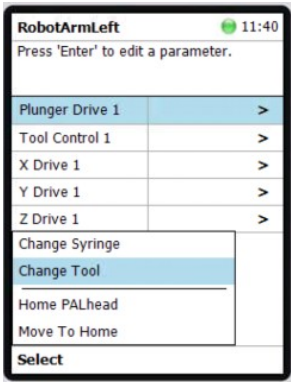
11.14 Replacing the Tool

1. From the PAL Terminal, select "RobotArmLeft".

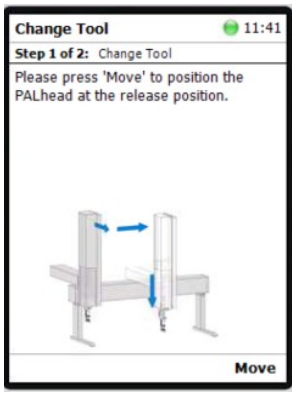




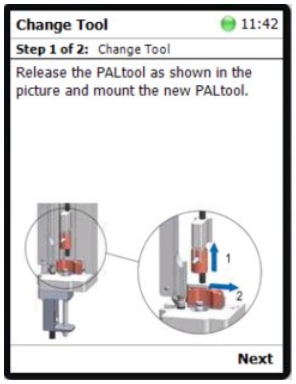
2. Press A to select "Options" and "Change Tool".



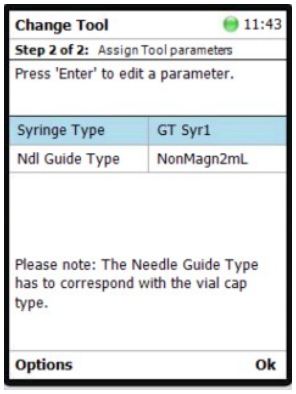
3. Select “Move” to move the tool to the exchange position.



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



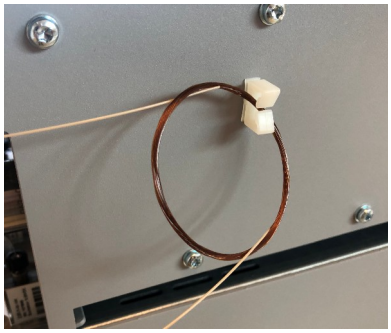
8. Install the new tool. Connect the tubing and needle.
9. Press “Next” on terminal.
10. Verify that the “Syringe Type” is “GT Syr1” and the “Ndl Guide Type” is “NonMagn2mL”.



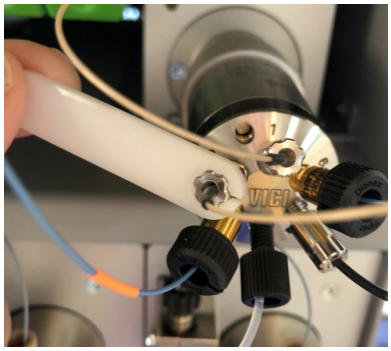
11. Press “Ok” to move robot arm to home position.

11.15 Replacing the Loop

1. Remove the left-hand side panel.
2. Carefully remove the storage loop from the holder.



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

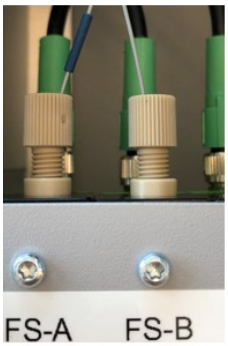


4. Install the new loop in reverse order using the small torque wrench to ensure proper tightening of the loop fittings.
5. After installation, run the Service – Loop flush script to flush the new loop with solvent.
6. Run the Diagnose – HP system script to verify that there are no leaks around the loop.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

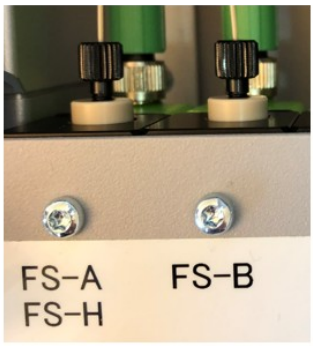
11.16 Replacing a Flow Sensor

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

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



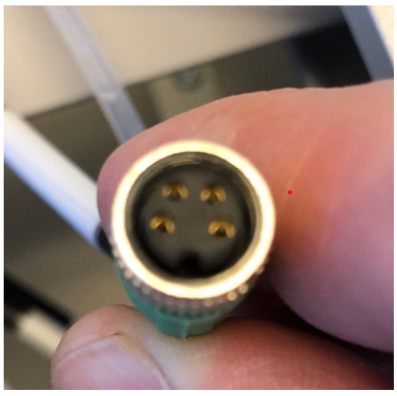
Flat bottom version



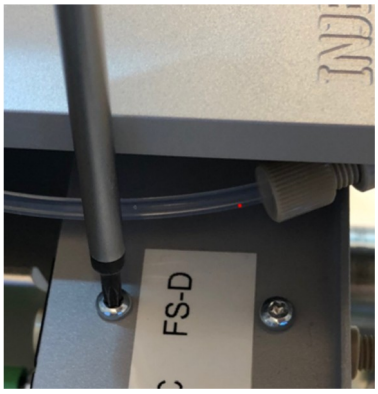
6-40 coned version

The example shown is for a low-pressure flow sensor, but the procedure is the same for high-pressure flow sensor.

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

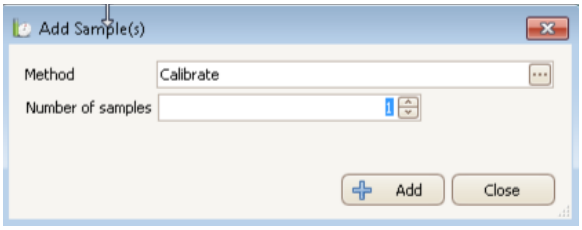


4. With a T10 screwdriver, remove the two flow sensor screws and carefully remove the flow sensor from the instrument. For the HP flow sensor, the screws are located horizontal on the front side of the instrument behind the door.



5. Install the new flow 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 tightened securely to create a good seal. For the nanoViper connections on the HP flow sensor, they should not be overtightened.
7. When the new flow 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 – Flow sensor ABCD or Flow sensor HP to calibrate the new flow sensor.



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

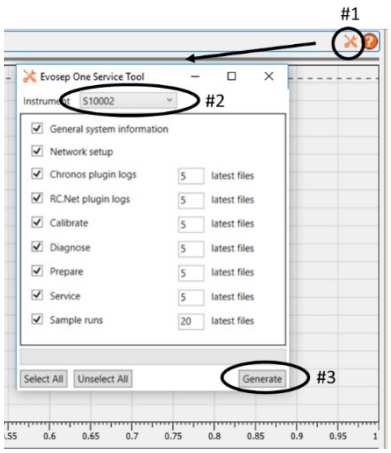
9. If a low-pressure sensor (FS-A-D) has been replaced, schedule and run the Diagnose – Tip seal script to verify that the tubing connections around the flow sensor is leak free. If the high-pressure sensor (FS-HP) has been replaced, schedule and run the Diagnose – HP system script.

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:

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

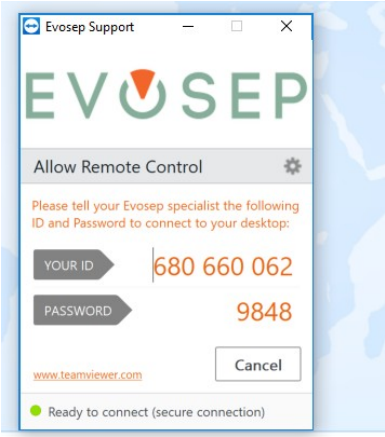


- For remote support please supply TeamViewer ID and Password as described below.

An Evosep support specialist will get back to you with a case number for tracking the problem. In addition, expect further questions and recommended tests to determine the probable cause and 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. The service engineer will carry smaller wear parts to 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, whichever date 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 due to normal use or manufacturing defects.

The warranty does not cover defects or failures of the Evosep One system caused by accidents, neglect, misuse or abuse.

In most cases, instrument wear parts are parts which are in contact with solvents and/or sample and are not covered by the warranty period. Example wear parts include tubing, fittings, rotors, stators, seals, needle, etc.