EVOSEP ENO

Advanced User Guide

UM-003A

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1 Preface

1.1 About this Advanced User Guide

This manual has been written for laboratory technicians who use the Evosep Eno system for execution of analytical runs. It is assumed that the user of this Advanced User Guide has appropriate laboratory technician training or more advanced laboratory training, Evosep user training, basic knowledge of how to use menu-driven computer software and that this person is familiar with standard laboratory and High Performance Liquid Chromatography (HPLC) terminology and practices.

This Advanced User Guide is designed as a companion to the User Manual, Quick Start Guides and Software Installation Guides to provide guidance on more advanced Evosep Eno functions, connections to specific Chromatography Data Systems (CDS), Mass Spectrometry (MS) ionization sources and troubleshooting above that included for general operation in the User Manual.

Disclaimer
The information contained in this Advanced User Guide is provided for general guidance and reference purposes only. While every effort has been made to ensure the accuracy and completeness of the information, Evosep assumes no responsibility for any errors or omissions. Evosep shall not be held liable for any direct, indirect, incidental, or consequential damages resulting from the use or misuse of Evosep Eno.

To ensure safety and proper functioning, Evosep Eno must be used strictly in accordance with the instructions in its User Manual and the Advanced User Guide. Any unauthorized modifications, alterations, or use outside the intended purpose will void warranties and may result in unsafe conditions.

This Guide may include references to, and instructions for, the use of third-party hardware and software. Such instructions are provided solely for the convenience of the user. Evosep does not assume responsibility for the performance, reliability, or safety of any third-party products, nor for any issues arising from changes or updates made to those third-party products after the time of writing. Use of third-party hardware or software is at the user's own risk.

In the event of any ambiguity or discrepancy in interpretation between User Manual and the Advanced User Guide, the English version of the User Manual shall be considered the original and shall prevail.

1.2 Safety and Special Notices

Make sure to follow the safety practices presented in the User Manual, this Advanced User Guide as well as those received from Evosep personnel.

All local laboratory safety regulations are to be followed.

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 the User Manual or Advanced User Guide 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 Manual and Advanced 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 with 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 Evosep

Support: support@evosep.com

Sales: sales@evosep.com

2 Instrument Control Software installation and updates

2.1 Software installation guides

The Evosep Eno can be controlled via several native Mass Spectrometry (MS) chromatographic data systems (CDS) drivers:

- Evosep Eno Chromeleon™ Driver (Thermo Scientific™ MS)
- Evosep Eno Compass® HyStar Driver (Bruker MS)
- Evosep Eno SCIEX OS Driver (SCIEX MS)
- Evosep Eno MassHunter Driver (Agilent MS)

In addition, the Evosep Eno instrument can be controlled through the Axel Semrau CHRONOS sample acquisition software via the Evosep Eno Chronos plugin. CHRONOS can control some of the common mass spectrometry vendors' chromatographic data systems (CDS) such as Xcalibur™ (Thermo Scientific), OpenLab ChemStation (Agilent) and Analyst (Sciex) - and hence start both the Evosep Eno and the mass spectrometer using one sample list.

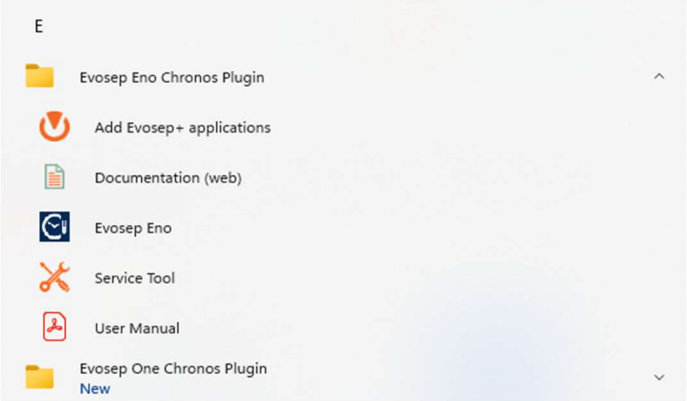
The Evosep Eno software drivers and plugins can be downloaded from:
https://www.evosep.com/support-zone/

The software installation guides for each of the configurations can be downloaded from:
https://www.evosep.com/support/documentation/

During the Evosep Eno installation, the Evosep engineer fully sets up the Evosep system and its control software with the specified Mass Spectrometer (MS). If the system is to be used with a different MS than set-up during installation the steps below can be followed by an Evosep trained user. Before installing the Evosep Eno control software, other LC systems must be removed from the instrument hardware configuration (see the relevant Evosep Eno software installation guide).

2.2 How to change hardware configuration from Evosep Eno and Evosep One configuration in CHRONOS

The Evosep Eno and Evosep One SW plugins can both be installed on a control PC. When choosing to run either Evosep Eno or Evosep One, open the program list and choose the appropriate Chronos shortcut.



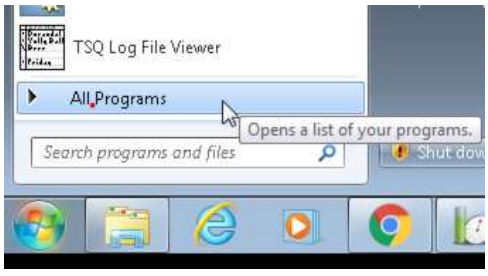
Please note, the PC can only be actively controlling one Evosep system at a time, either the Evosep Eno or the Evosep One.

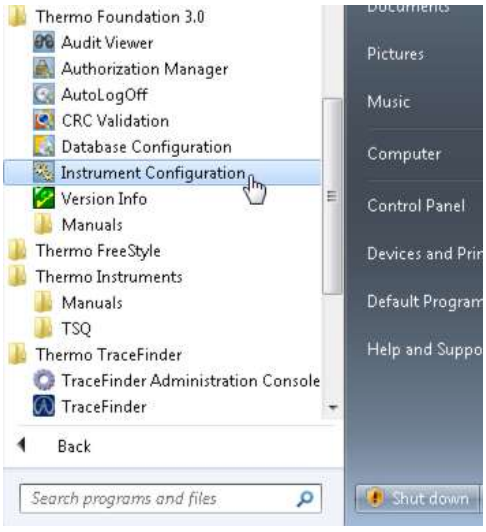
2.3 How to Remove Other LC Devices from MS System Configuration

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

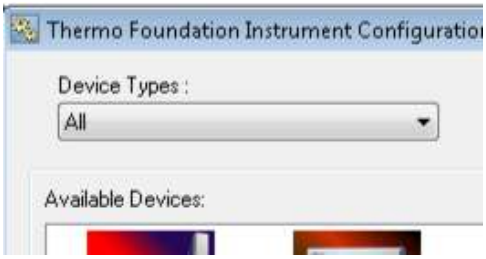
Please check for, and remove, other connected LC/Autosampler devices from the MS Instrument configuration before connecting the Evosep Eno. The example below is given for Xcalibur:

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.

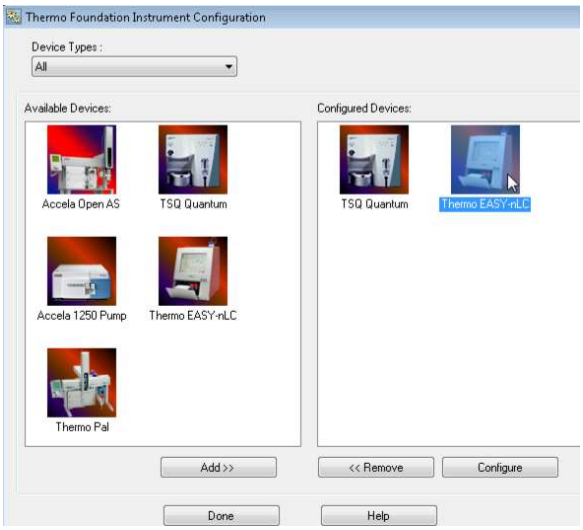




3. In the “Instrument Configuration” program, set “Device Types” to “All”.

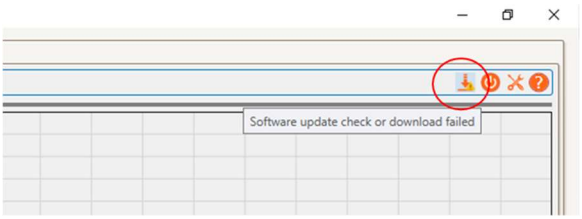


4. If any LC systems are visible in the “Configured Devices” window, select them and click “<< Remove” to remove them from the configuration (*note: do not remove the MS from the configuration*). Then click “Done” and re-open Xcalibur.

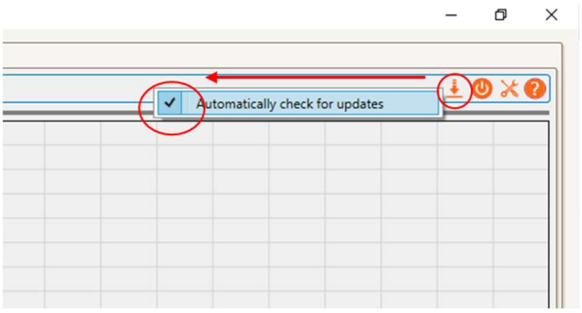


2.4 Automated Software Plugin Update

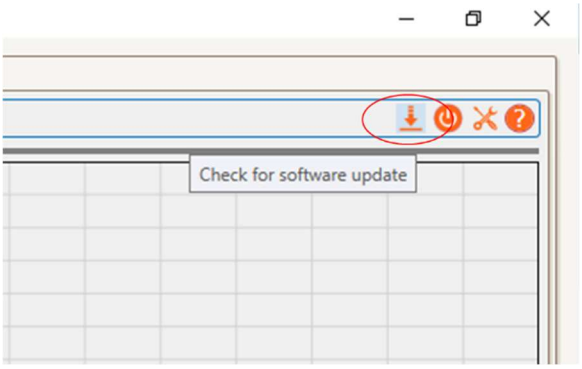
The plugin software will automatically detect if a newer version has been released and assist the user in the update process. For this feature to work, the PC must have access to evosep.com. If this page is blocked, a warning will be displayed on the software update button on the graphs page. Contact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



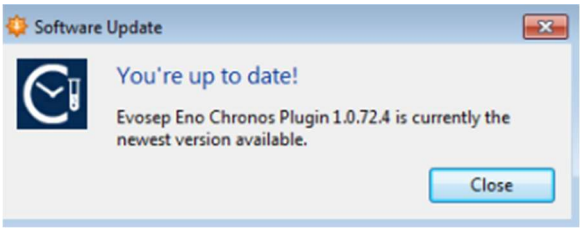
1. The software update process can be triggered in two ways:
   - Automatically during restart of Chronos or the native Evosep CDS driver. The automated feature can be turned on/off by right clicking “Check for software update” button and checking /unchecking the box.



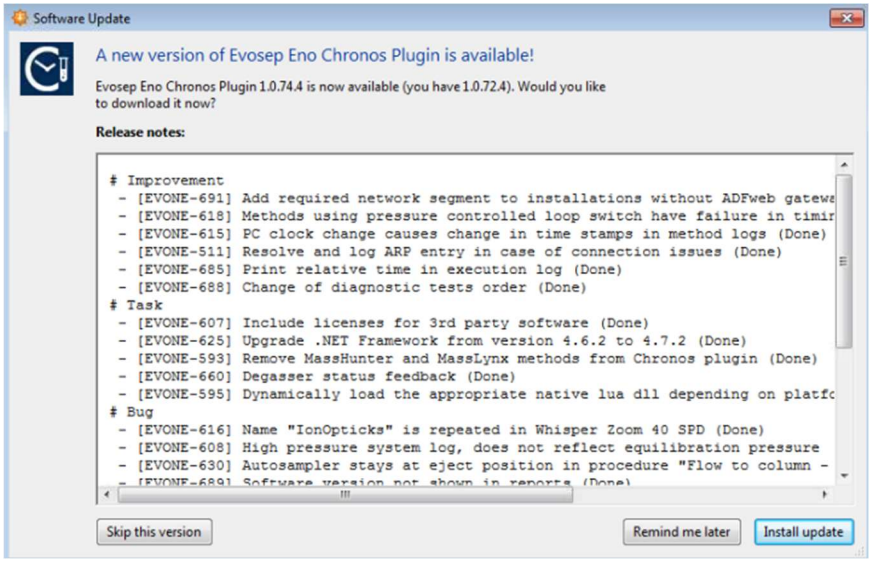
- Manually by pressing the “Check for software update” button on the graphs page.



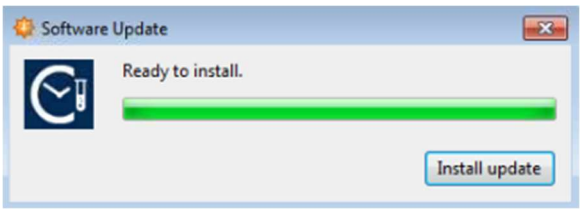
2. The software update window will open and show if the installed plugin is up to date.



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.



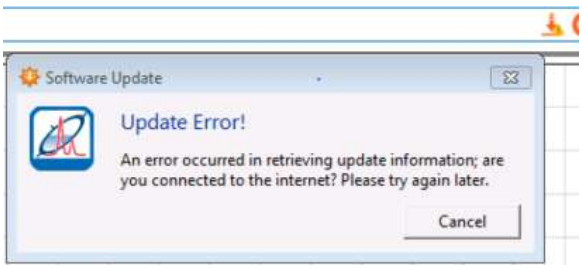
4. The new software plugin will be downloaded from evosep.com. Press “Install update” to open the software installer program.



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].



3 Instrument Control with CHRONOS

Please refer to the Evosep Eno User Manual Chapter 6 “Running Samples” to view all safety warnings, cautions, and concerns.

A quick start guide has been developed for each specific CDS with the Evosep Eno plugin. These can be found at the Evosep webpage:

https://www.evosep.com/support/documentation/

This advanced user guide covers most functionalities relevant for use of the Evosep Eno with Chronos software and the Evosep Eno plugin, including using Chronos to control mass spectrometry software such as Xcalibur.

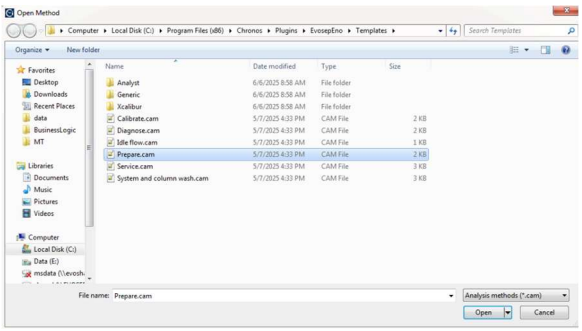
For all other CDS, we refer to the respective manuals for advanced functionalities that are beyond the scope of the quick start guides.

3.1 Chronos for Control of Thermo, Analyst (Sciex) and Agilent

The Evosep Eno instrument can be 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). This enables Chronos to start both the Evosep Eno and the mass spectrometer using one sample list.

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.



In this section, Evosep Eno-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).

3.1.1 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 to run
4. The schedule is executed

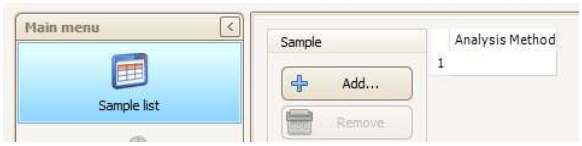
3.1.2 Methods

The Evosep Eno 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.

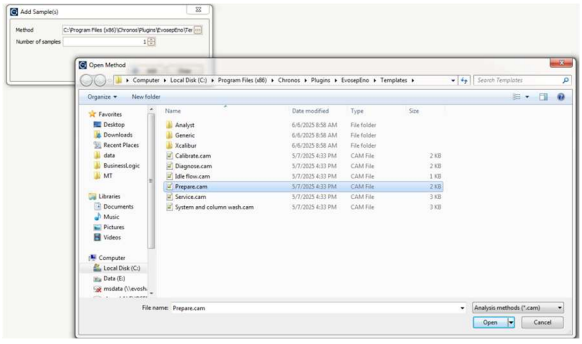
3.1.3 Sample Lists

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

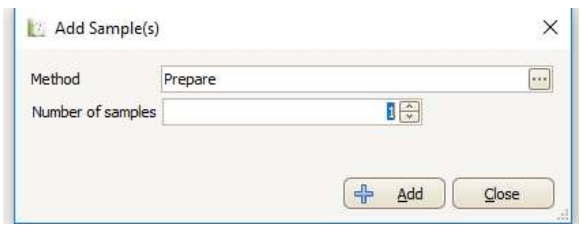
1. Click the “Add” button.



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

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

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

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

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

	Analysis Method	Pump HP	Pump A-D	HP system*	Injector*	Tip seal*	Restriction*
1	C:\Pro...se.cam	☐	☐	☐	☐	☐	☐

   - 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.

	Analysis Method	Flow sensor ABCD	Flow sensor HP
1	...\Calibrate.cam	☐	☐

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

	Analysis Method	Set valve 6	Syringe pumps	Drain pumps	Autosampler torque test	Loop flush*	Contact closure test*	Flow to column - 20% sol B*	Service diagnostics
1	C...\Service.cam	☐	none	☐	☐	☐	☐	☐	none

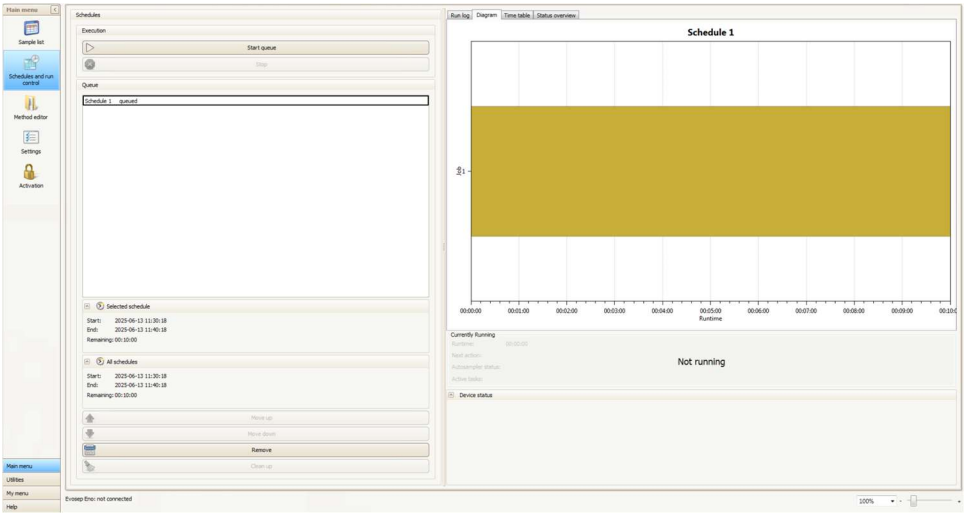
5. Additional methods and samples can now be added to the sample list.

3.1.4 Creating a Schedule

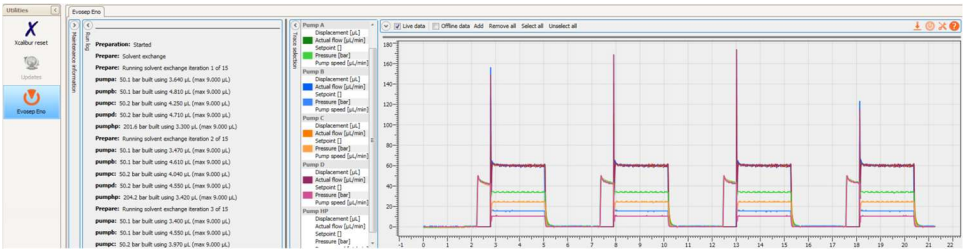
1. The user must create a schedule based on the sample list. Make sure that the “Overlapped” check box is cleared and press “Create”.

3.1.5 Running a Schedule

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

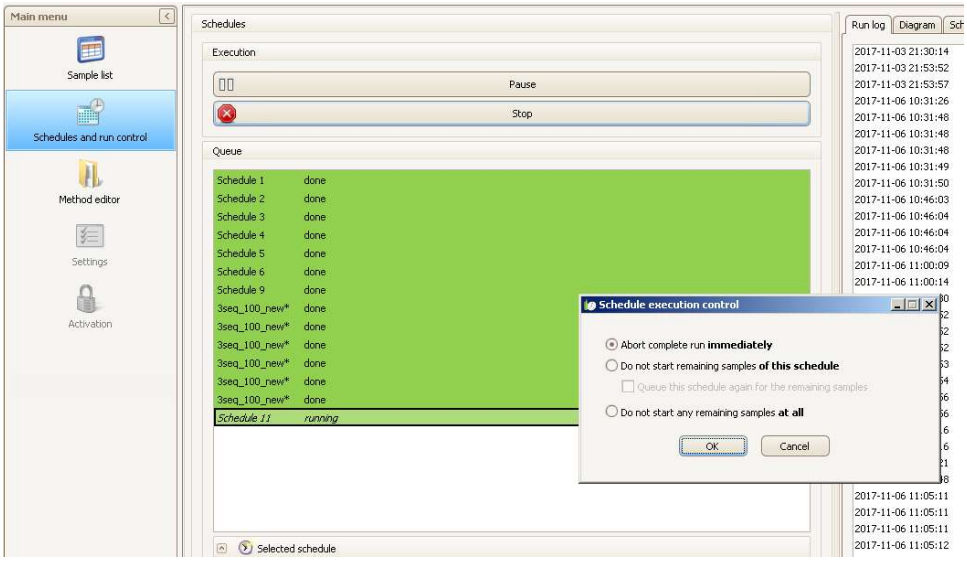


2. Start the sample queue by pressing the “Start queue” button.
3. Additional schedules can be entered in the sample queue. The execution order of the schedules can be shuffled up and down using the arrow buttons.
4. Information about current and previous analyses, such as diagnostic leak test, etc.,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

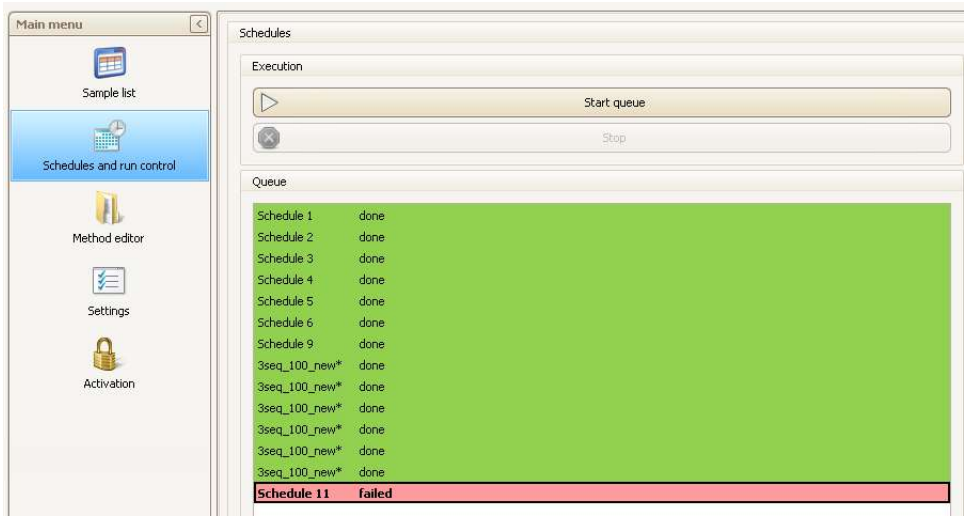


3.1.6 Aborting Samples

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.

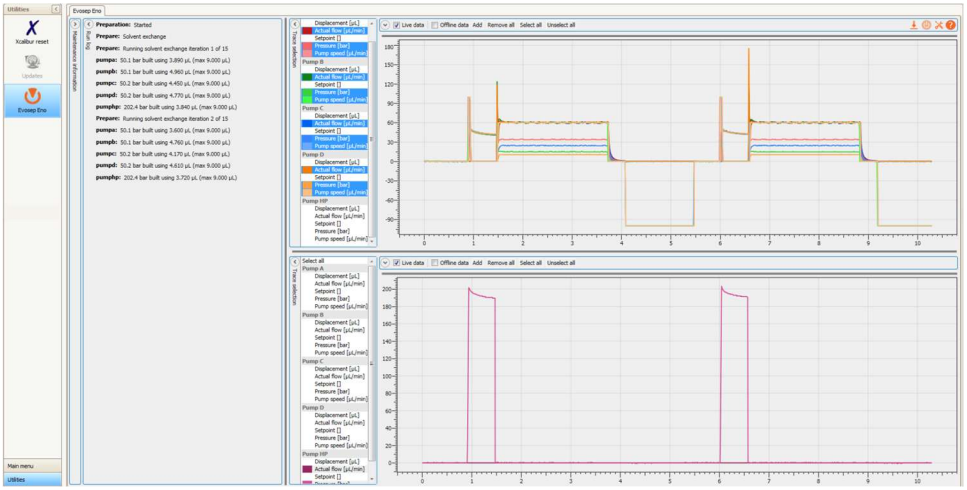
Important note:

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.

3.1.7 Looking at Graphs

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

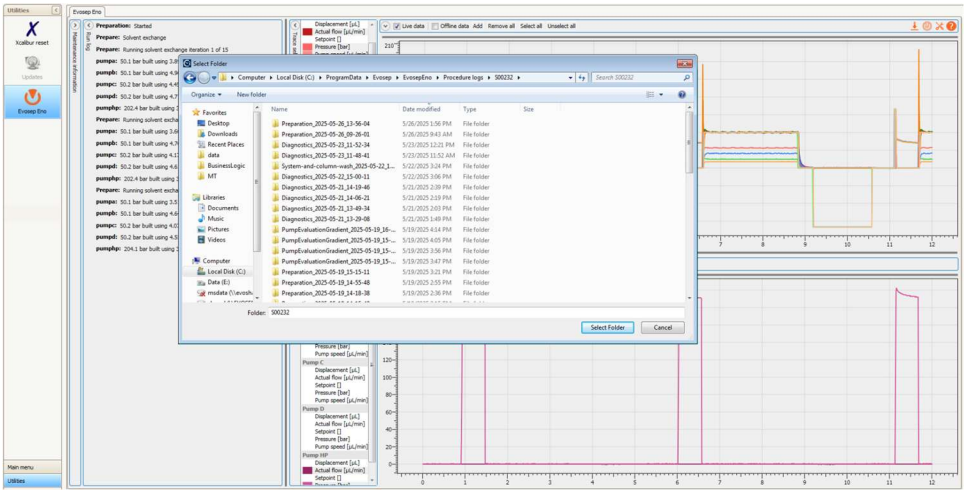


2. The following traces can be selected for each pump by a single left click on the side bar:

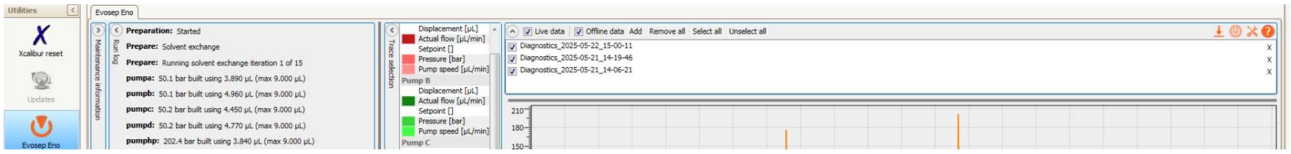
Name	Description
Displacement [µl]	Pump piston position. Pumps are full when Displacement is 0 µl and empty when displacement is 55 µl and 138 µl for Pump HP and Pump A/B/C/D, respectively.
Actual flow [µl/min]	The actual flow is measured in the liquid stream for each pump using an individual flow sensor 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.

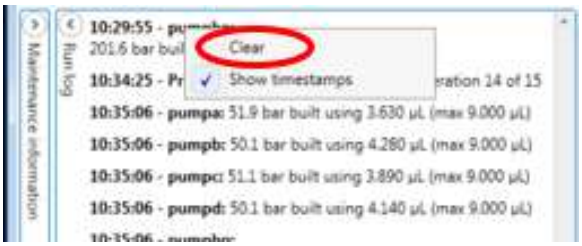


4. The opened sample folders are visible in the “Offline data” list. Individual data files can be selected/unselected using their respective checkboxes.

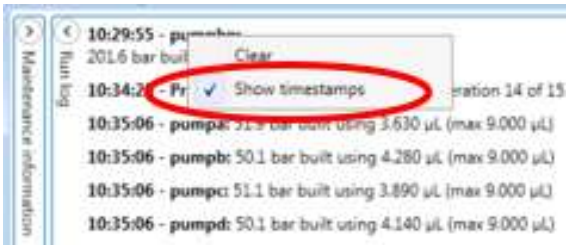


3.1.8 Run Log

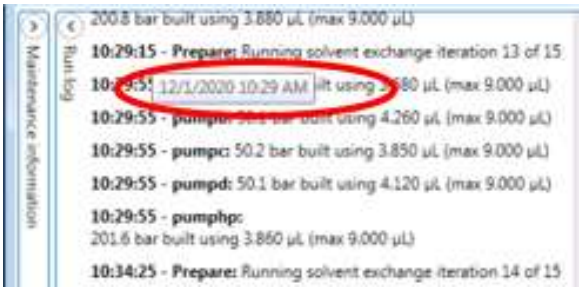
1. The run log displays information to the User regarding the Sample Methods, Calibration, Diagnostics, and Preparation procedures being executed. The log will show which, and when, each program was started and finalized, including pass/fail criteria.
2. The run log can be flushed by right clicking the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



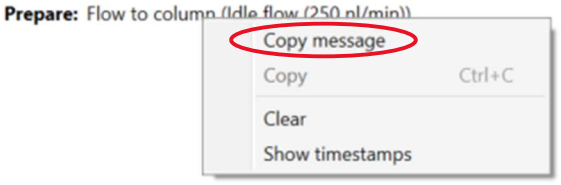
3. The User can choose to enable/disable timestamps in the log by right clicking the log and selecting/deselecting the “Show timestamps” option.



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



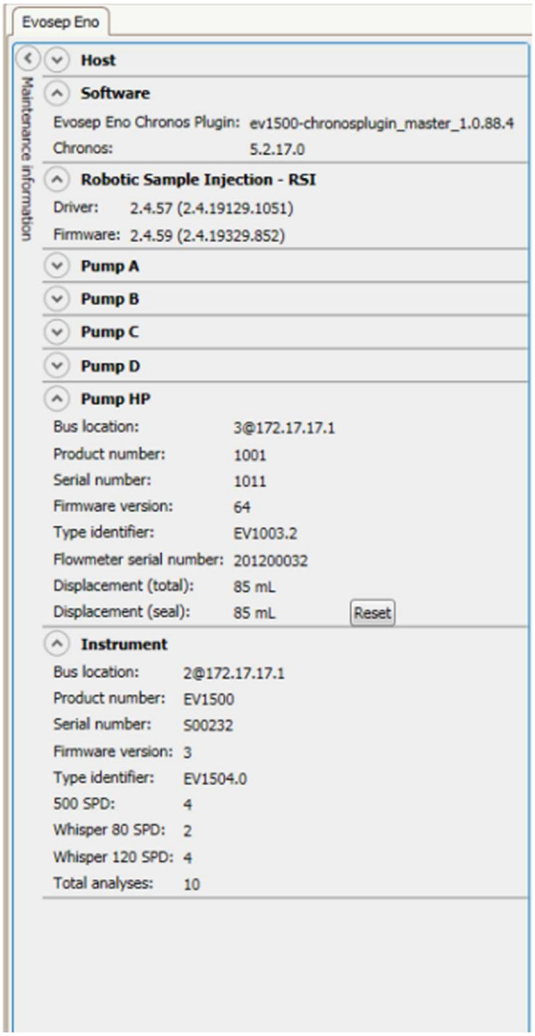
5. The User can also select and copy text from the Run log by right clicking and selecting “Copy message”.



3.1.9 Maintenance Information

The software version, autosampler, pump, and other instrument specific data is displayed in the “Evosep Eno” 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.

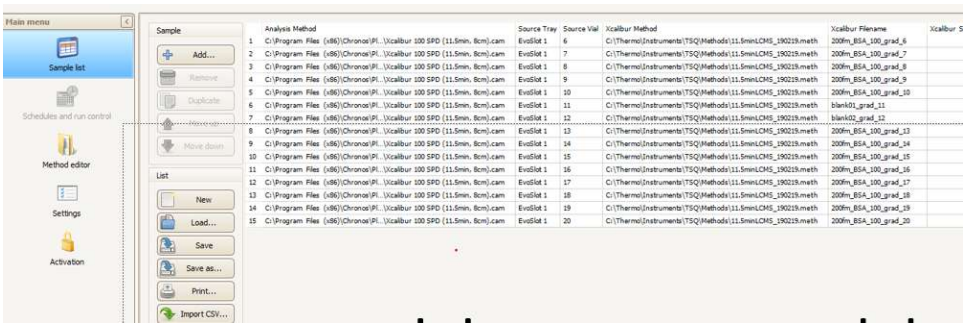
- Host
  - Computer name
- Software
  - Chronos and Plugin software version
- Robotic Sample Injection – RSI
  - Autosampler Driver and Firmware version
- Pump
  - Serial number
  - Firmware version
  - Displacement (total): Total pumped volume
  - Displacement (seal). Pumped volume since reset (if a pump seal was replaced)
- Instrument
  - Serial number
  - Firmware version
  - Analysis completed (by type)



3.1.10 How to Import CSV Files into Chronos

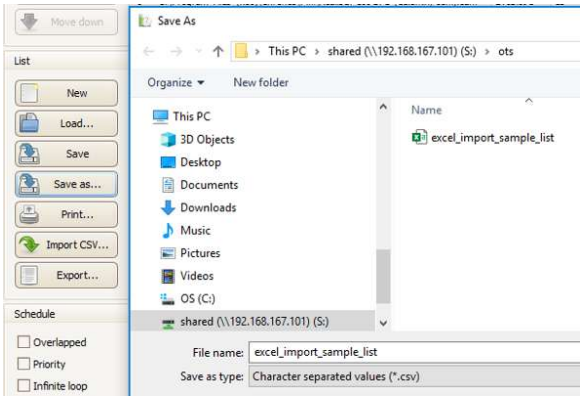
The general idea here is to use a mapping template to define the column matching between the CSV file you want to import and the Chronos sample list columns. This example uses a CSV file saved from Chronos, but you can use any CSV file as source if it contains the values to fill-in the columns described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

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

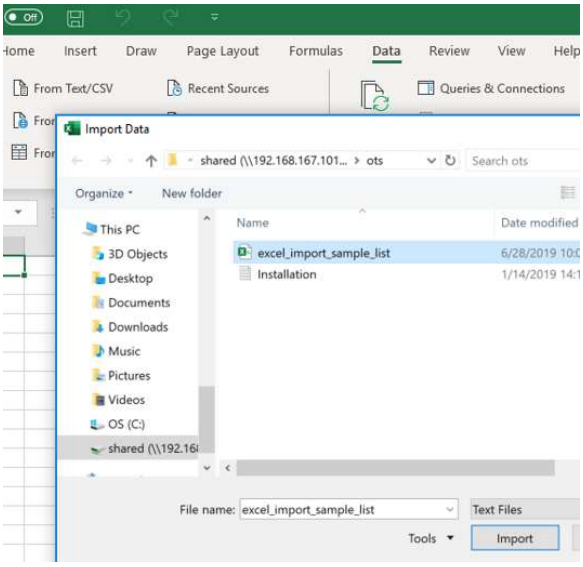


2. Click “Save as…” and choose “Character separated values” as type.

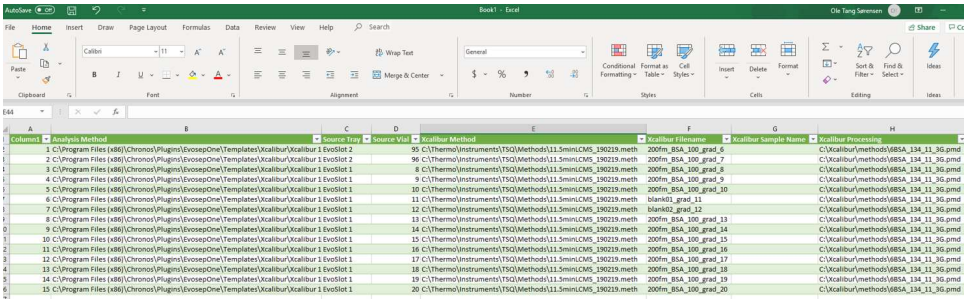
- The extension CSV typically (and by some standards) denotes “Comma Separated Values”. Depending on the Windows regional settings, another character may be used as separator, such as a semicolon or tab. Keep this in mind when you import the data later!



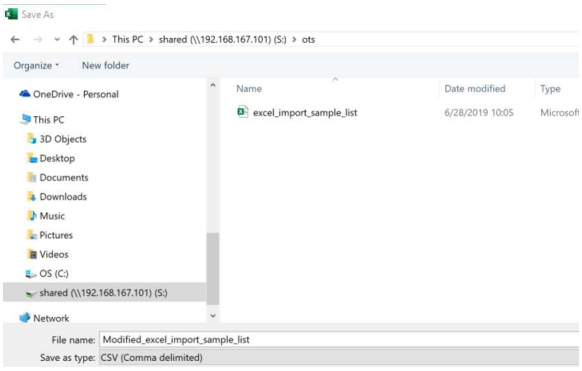
3. In Excel, click “Data” and choose to open “From Text/CSV”.



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

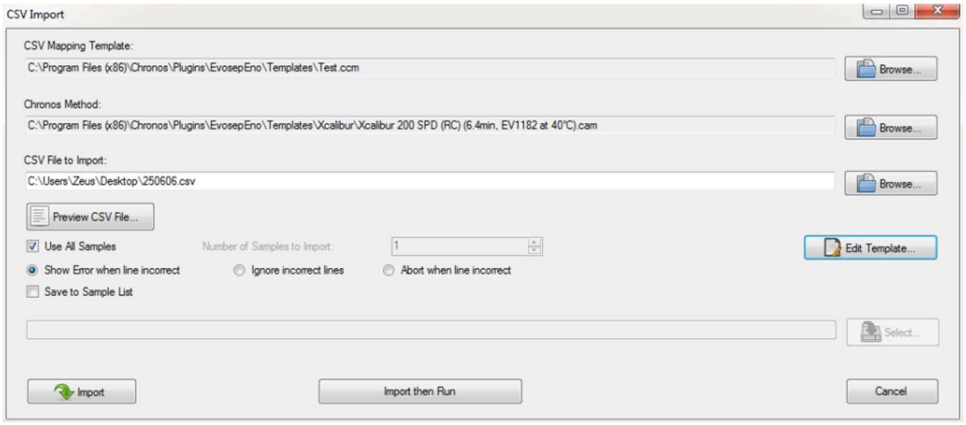


5. Click “Save As” to save the modified sample list and select type “CSV (comma delimited)”.

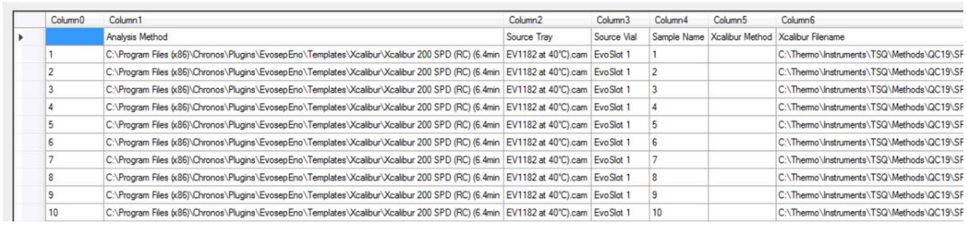


6. To import sample list into Chronos, click “Import CSV…”
   - The first time you perform this operation in Chronos, you will be taken directly to the following step.

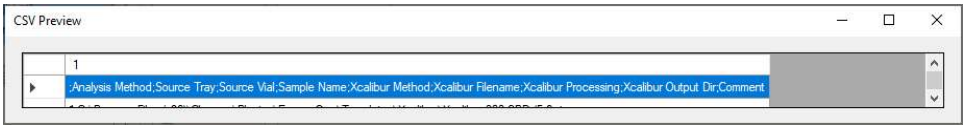
7. In the CSV import window, do the following.
   - Choose “Browse…” to select the desired “Chronos Method”. This ensures the correct format of the sample list columns (e.g. for Xcalibur, use one of the Evosep Xcalibur methods).



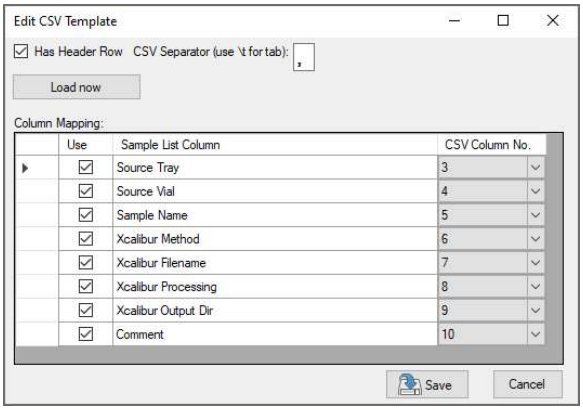
   - Choose “Browse…” to select the “CSV File to Import”.
   - Click “Preview CSV File…” to verify the correct number of columns in the sample list.



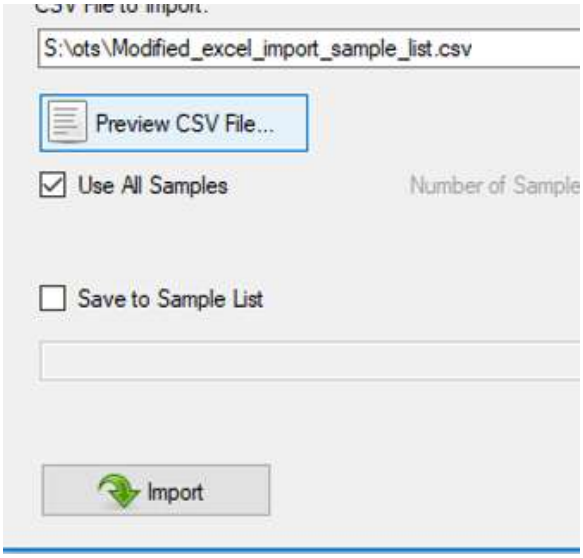
   - If all the columns shown are merged as a single column, you must adjust the CSV Separator, save the template, and preview the file again.



- 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.



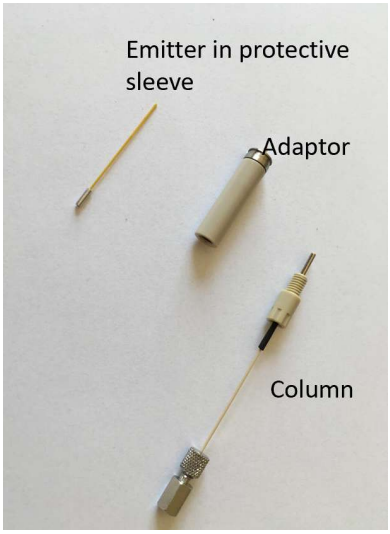
4 Configuration, Source, Column, and Emitter

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 Eno 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 Eno 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 Eno LC to various mass spectrometers and ion source configurations.

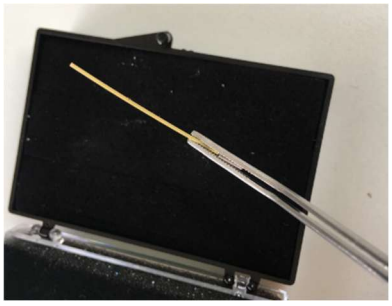
4.1 Thermo Scientific EASY-Spray Source

The column, Easyspray adaptor, emitter and a column heater (Evosep Pod) are used to run the Evosep Eno with an EASY-Spray source. Follow the procedure below to set up this connection.

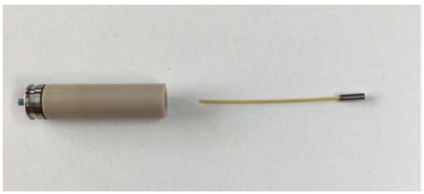


Item	Part number
Easyspray adapter	EV1072
Stainless steel emitters	EV1086
Evosep Pod	EV1187

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



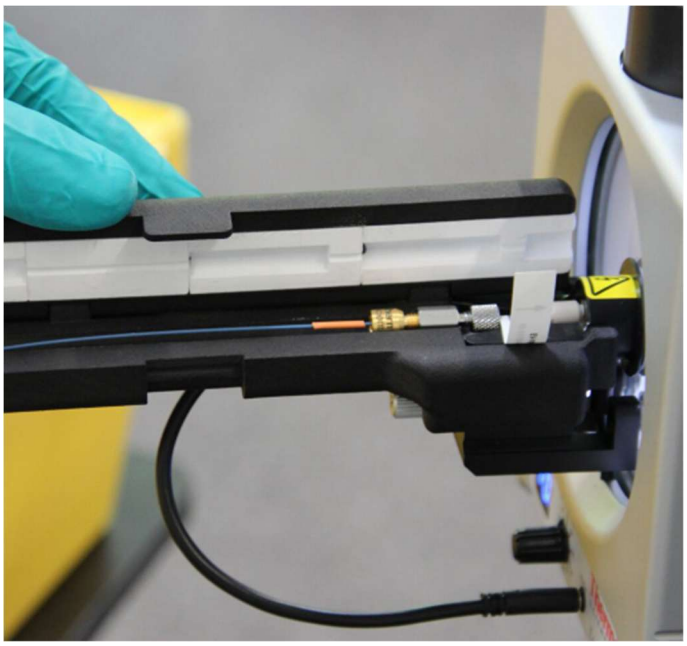
2. Slide the emitter with the sleeve into the spray adapter.




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

4. When the column is connected and the emitter is in position, the protective sleeve can be pulled off the emitter and the Evosep Eno transferline connected to the column.

5. After removing the sleeve from the emitter, the spray adapter can be gently positioned in the Evosep Pod and pushed into the Easy-Spray source. For more information see the Evosep Pod User manual.



6. 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.

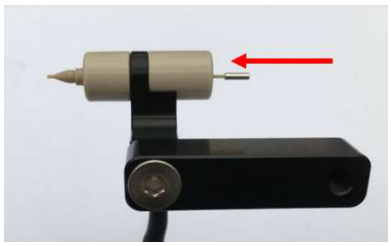
4.2 Thermo Scientific Flex Source

Item	Part number
Nanospray Flex adapter	EV1085
Stainless steel emitters	EV1086

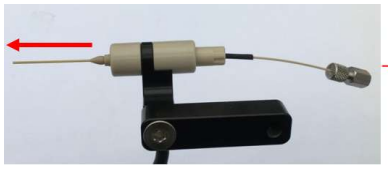
A source compatible column heater is required for Evosep Eno methods.

1. Carefully remove the 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.

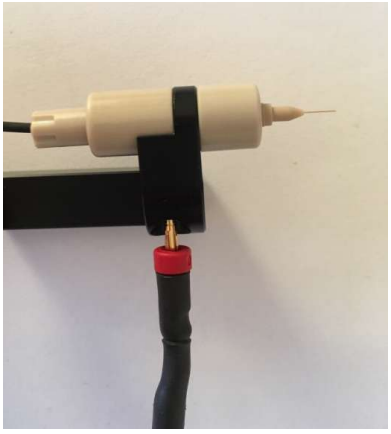


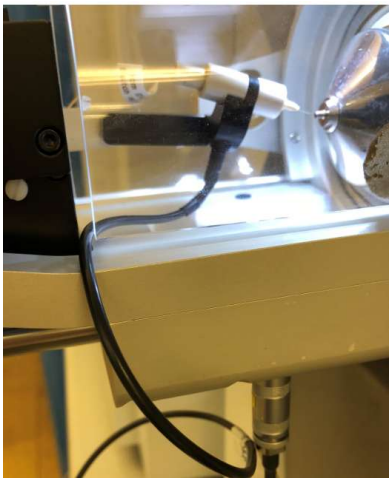
3. Connect the column to push the emitter into the correct position.



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. Use a compatible column oven to heat the column to the Evosep Eno standardized method required temperature.

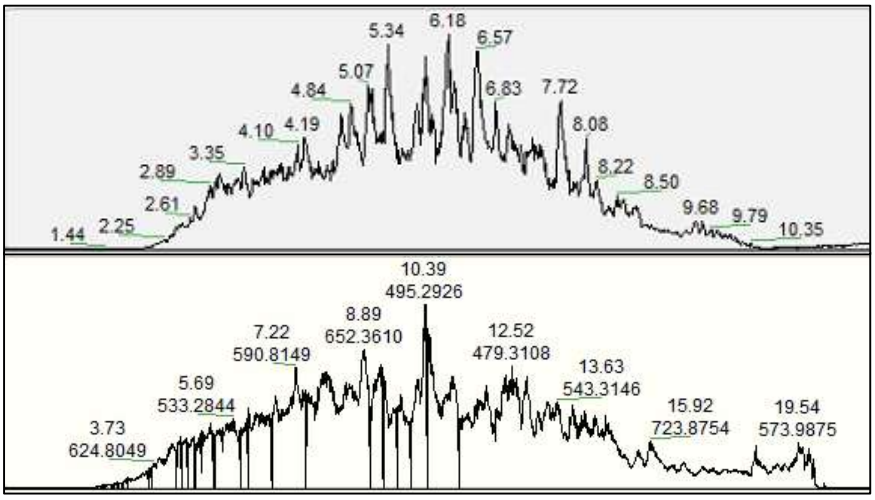




4.3 Thermo Scientific FAIMS Pro Interface

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 Eno 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.

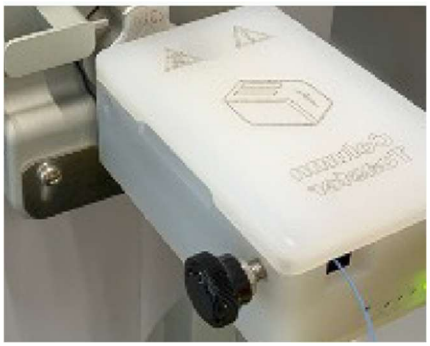
Before cleaning After cleaning

2. We recommend cleaning 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. An altered frequency of 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.

4.4 Bruker Daltonics CaptiveSpray Source



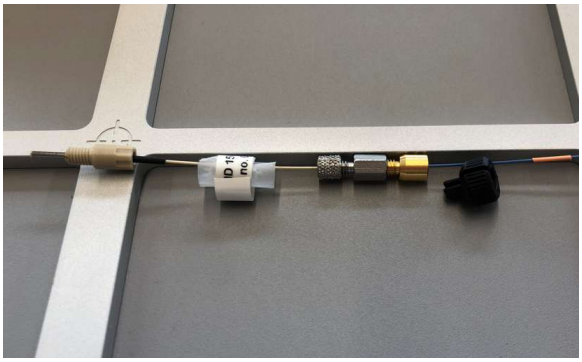
1. Connect the column to the CaptiveSpray source and connect the transfer line to the column.
2. Place the column in the Bruker column oven.

4.5 Agilent Nanospray Source

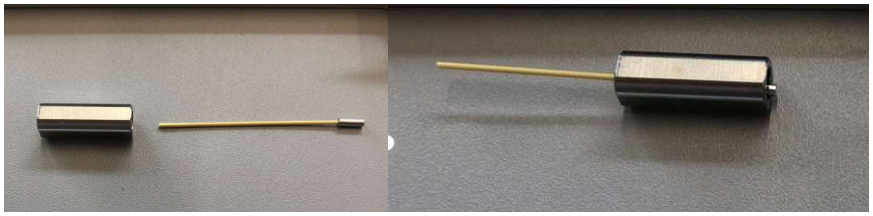
For connecting the Evosep Eno to the Agilent Nanospray source the following items are needed:

Item	Part number
Agilent Needle emitter(XL)	EV1117
Agilent sleeve adapter	EV1116

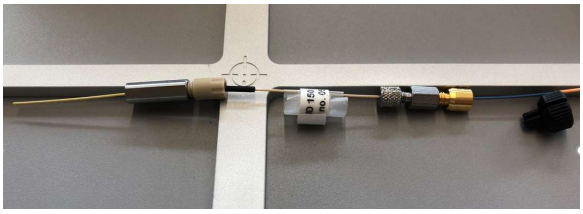
1. Connect the column to the Evosep Eno transfer line and remove the black knurl from the nanoViper fitting.



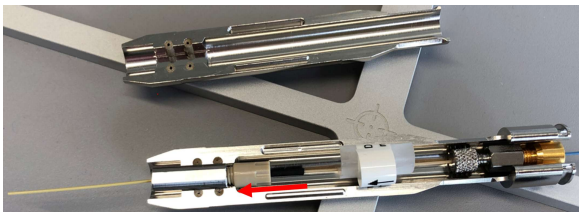
2. Slide the emitter with the sleeve through the sleeve adapter.



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



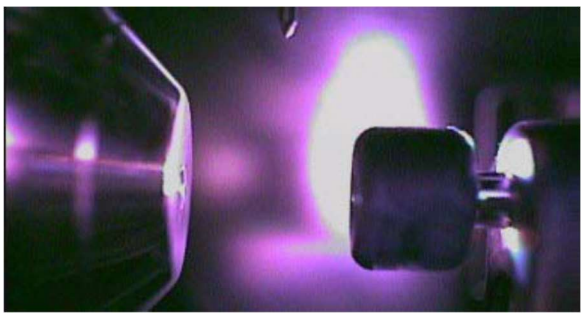
4. Insert the sleeve adapter with the column into the needle sleeve assembly. To ensure the correct position of the sleeve adapter and emitter, push the sleeve adapter towards the end of the needle sleeve assembly until the column peek nut is stopped by the small narrowing in the needle sleeve assembly,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



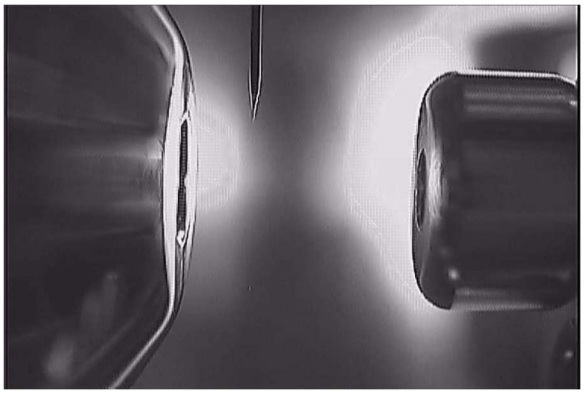
5. Now assemble the needle sleeve assembly and remove the protective sleeve from the emitter.

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.
7. Install appropriate column oven.

8. 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.

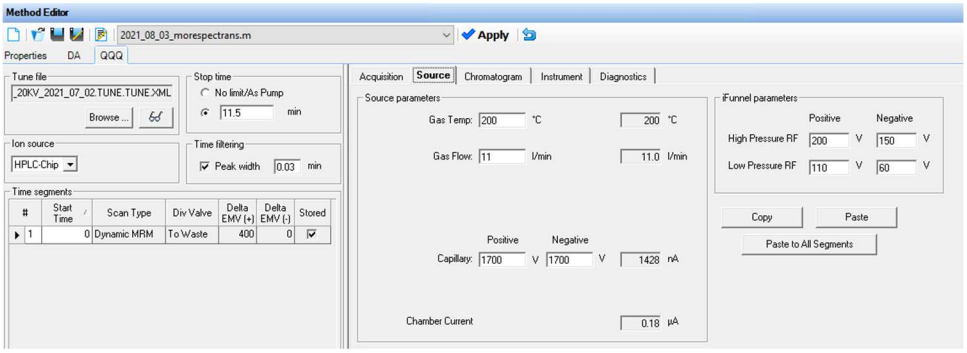


9. Use the adjustment knobs on the source to position the needle as in below picture.



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

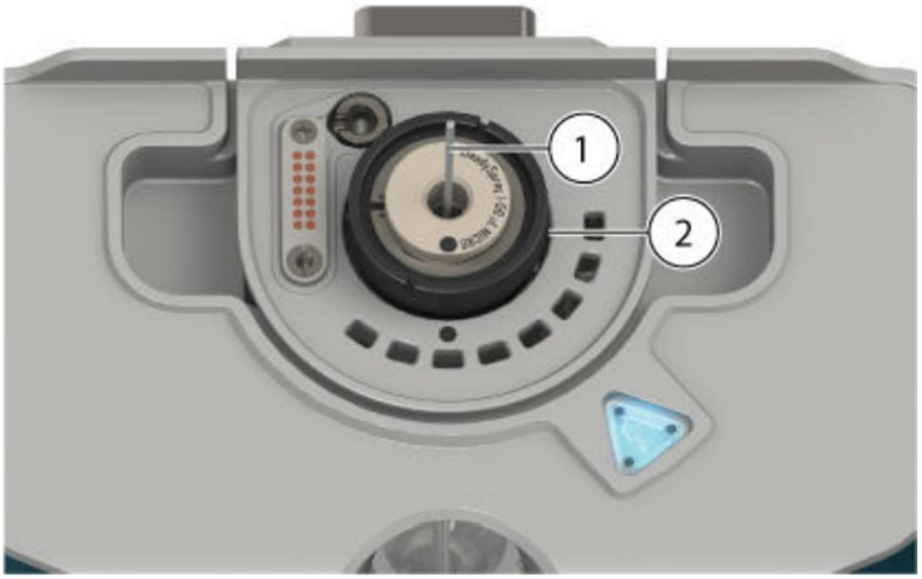
11. The recommended source conditions are shown below:
    - Gas Temp: 200 °C
    - Gas Flow: 11 L/min
    - Capillary: 1700 V



4.6 Sciex Optiflow Ion Source in Microflow Regime

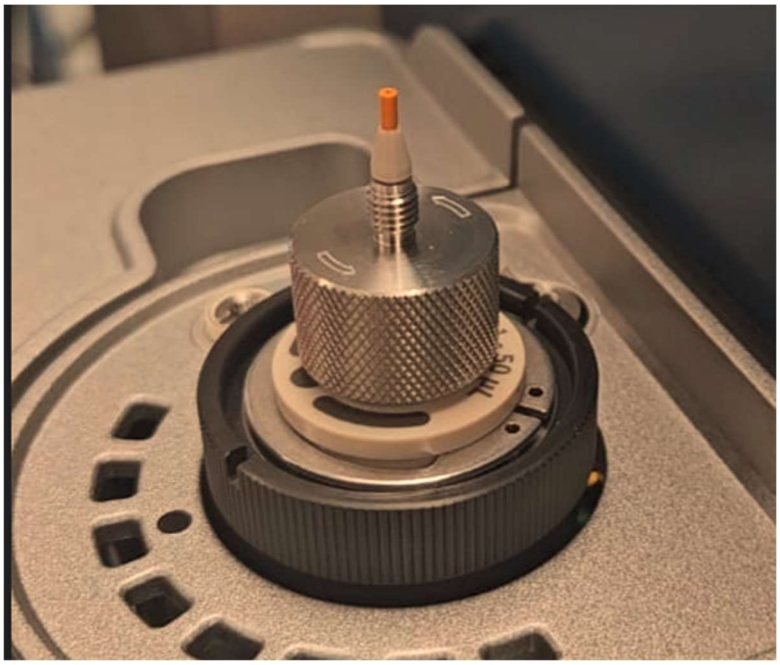
This section outlines how to run samples with the microflow probe on the Optiflow ion source and the Evosep Eno.

1. Install 1-10µL/min electrode (1) in the micro probe (2)

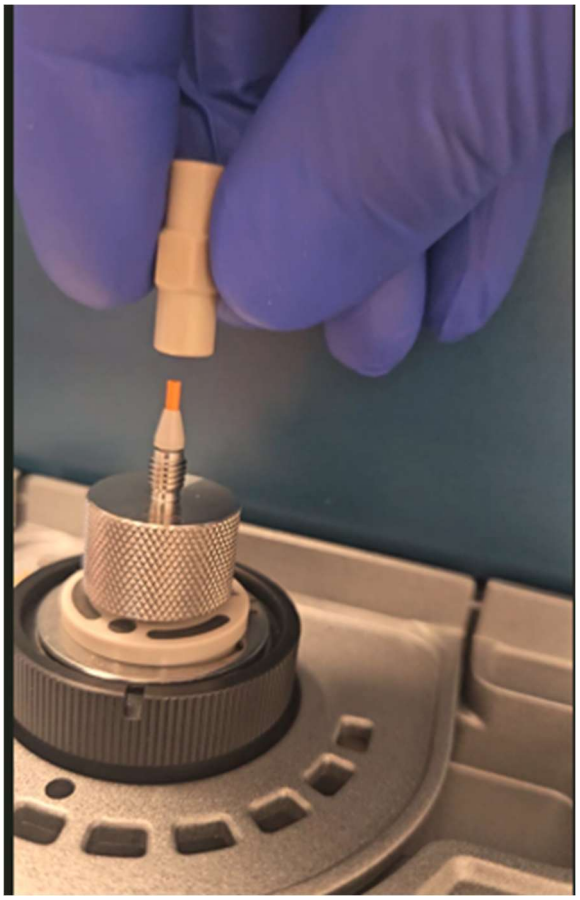


2. Install the bottom fitting (1) and tighten until finger tight to lock in the electrode

3. Install the peek ferrule (SCIEX p/n 5053325) to the metal top fitting, and install both over the electrode and bottom fitting



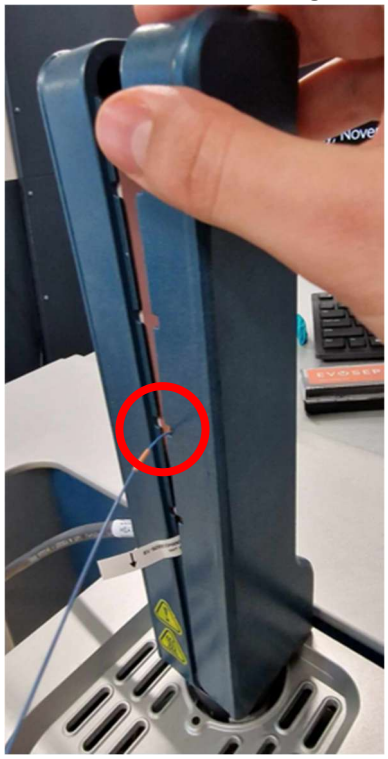
4. Install the PEEK union (IDEX p/n P-779), ensuring the electrode is fully bottomed out in the union to avoid dead volume. With the union fully installed on the electrode, the top fitting will need to be gently lifted so the thread can engage with the union. Take care not to lift the union and create dead volume. Finger tighten the top fitting to the union so that the union and top fitting cannot be pulled off the electrode.



5. Install the Evosep column to the other side of the union.

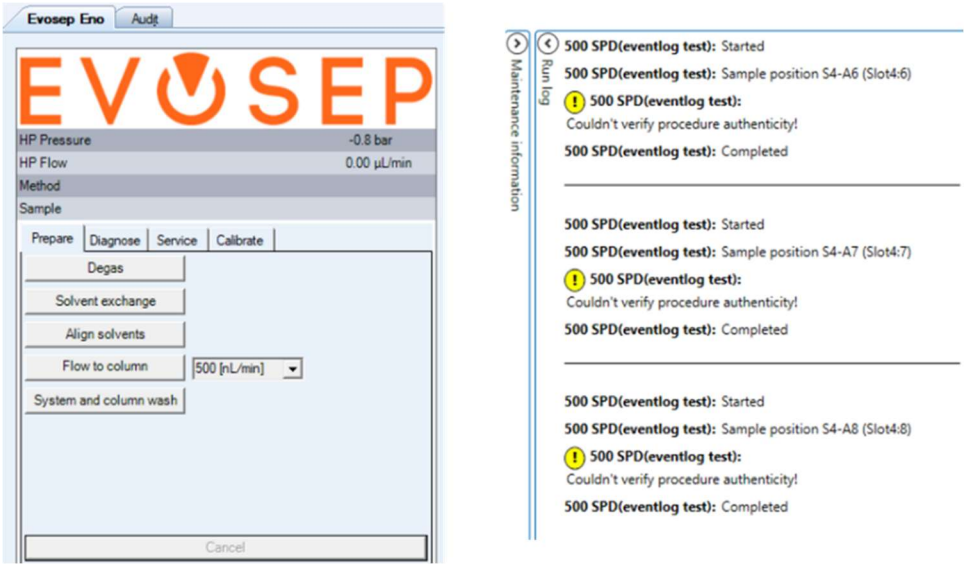
6. Install the column oven carefully around the Evosep column.

7. Attach the transfer line to the column. Be sure to route the transfer line through one of the tubing slots so the line does not get damaged.

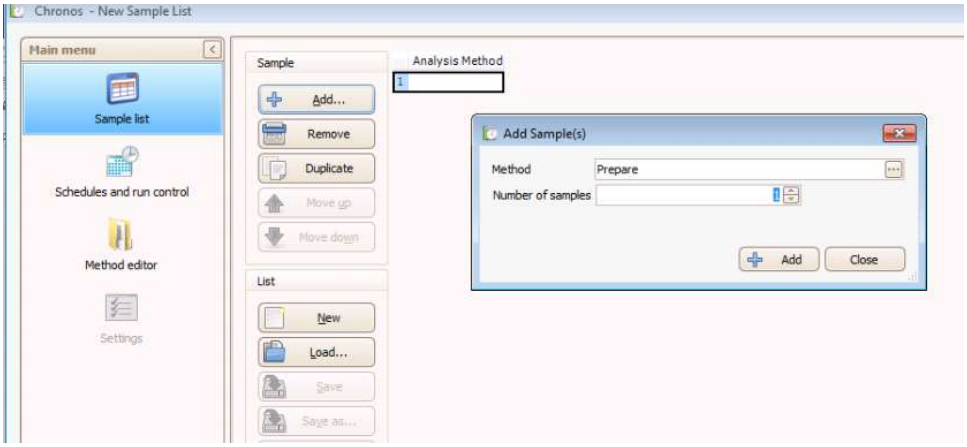


5 Preparing the Evosep Eno for Use

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



1. The Evosep Eno 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
2. The Evosep Eno 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
3. Additionally, all the preparation programs can be manually executed by adding a sample using the “Prepare” method (C:\Program Files (x86)\Chronos\Plugins\EvosepEno\Templates\Prepare.cam) in the “Sample list” panel.



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

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

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

5.1 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.

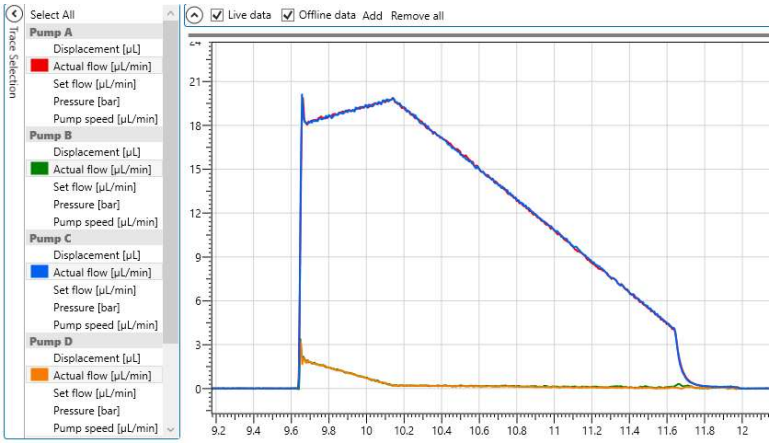
5.2 Solvent Exchange of Pumps

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

5.3 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.



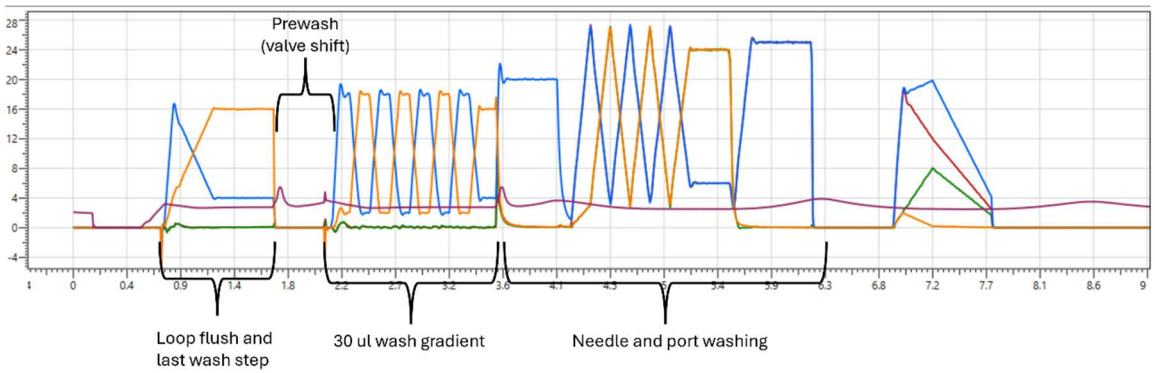
5.4 Flow to Column

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 0.1, 0.25, 0.5, 1, 2 or 4 µl/min for 10 minutes. Make sure that flow and pressure are stable before tuning the MS.

5.5 System and Column Wash

The “System and column wash” program can be used if the Evosep Eno autosampler, loop, or analytical column has been heavily contaminated from running a dirty sample. The program duration varies according to the backpressure of the connected analytical column (typically 10 – 25 minutes). The program includes 2 additional value shifts in comparison to a regular sample run, and hence induces slightly more wear and tear of the system

1. The program automatically creates a gradient of repeated increments of solvent B from 20-80%, 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 by repeated increments of solvent B from 20-80%.
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.

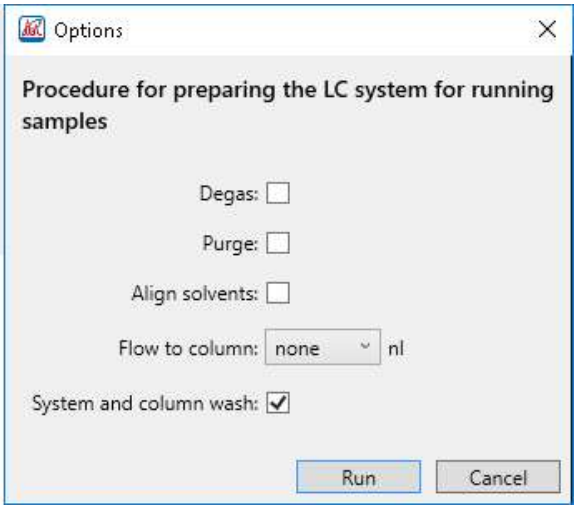


The “System and column wash” procedure can be started in two ways:

1. In Chronos and the native MS CDS drivers, 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\EvosepEno\Templates\System and column wash.cam	EvoSlot 1	1

2. In the native MS CDS drivers, the program can also be started by right clicking the Evosep Eno 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).



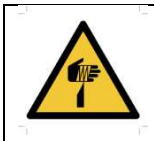
5.6 Idle Flow

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 idle 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\EvosepEno\Templates\Idle flow.cam).

6 Running Samples using Evosep Eno

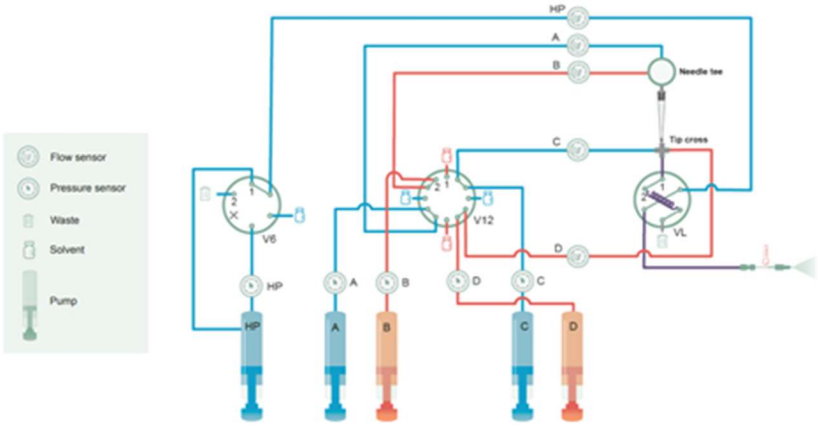
Warning/Caution	
	**Risk of danger: the use of proper PPE is essential to protect oneself from harm and to minimize contamination from entering the Evosep Eno instrument. Always exercise caution when dealing with potential health and safety risks.**
	**Electrical shock hazard: Risk of transfer of high voltage from MS to the Evosep Eno 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 Eno 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 health 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 Eno 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 Eno 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.

6.1 Separation Principle

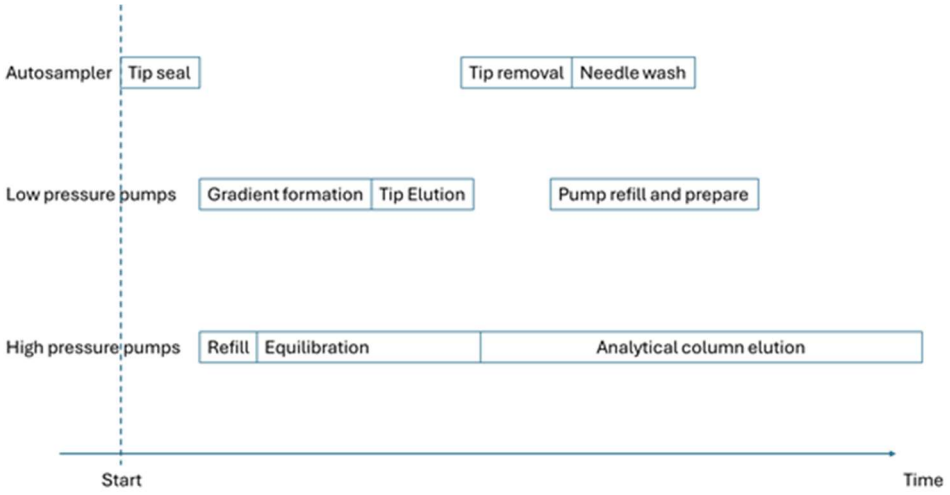
The central element of the Evosep Eno technology is the Evotip, which allows for the integration of sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with the C18 stationary phase fastened inside as a small plug. The evotips are used to de-salt and clean up the samples prior to LC-MS analysis. The traditional subsequent steps of eluting, drying down, and resuspending the samples from the tips are not necessary with the Evotips and are therefore omitted. The approach to sample handling results in a significantly simplified workflow which in turn leads to significantly less sample loss and a more robust protocol. The Evosep Eno sample tray accommodates up to 6 racks of 96 tips, i.e. 576 rinsed samples may be lined up for fast analysis. See figure below for the Evosep Eno plumbing diagram.



Evosep Eno plumbing diagram. H: High-pressure Pump, A/B/C/D: Low-Pressure pumps, V6: 6 port high-pressure solvent valve, V12: 12 port low-pressure solvent valve, VL: Loop Valve.

The sample analysis starts with the autosampler picking up the loaded Evotip. The Evotip is placed in-line with the low-pressure pumps and the sample loop. When the Evotip is sealed in the injection port, it is included as part of the flow path for the low-pressure pumps, A and B. The gradient is then being formed and pushed into the sample loop using the low-pressure pumps, C and D, while the column is being equilibrated using the high pressure pump. Once the gradient is formed, the sample is eluted from the tip, diluted to a proper concentration, and pushed into the loop using the low pressure pumps. When the sample is placed in the loop, the loop valve switches the loop in-line with the high pressure system and the column. The sample and gradient are pushed out through the transfer line into the column. The dilution and gradients are

optimized to enable the best possible refocusing of each analyte as well as the best possible separation and peak shape, greatly enhancing the chromatographic performance.



High level sample acquisition process diagram.

While the high pressure pump is eluting the sample from the chromatographic column, the autosampler removes the current Evotip from the injection port and prepares for the next sample. Meanwhile, the low pressure pumps are refilling and preparing the flow path for the next sample.

The last section of the gradient includes a column wash step with a high concentration of organic solvent, to minimize the risk of carryover and remove any highly organic contaminants that might be eluted from the Evotip during tip elution.

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 Eno Methods

Throughput	Cycle time	Gradient length	Flow rate	Temperature	Column Part Number/Details
Samples/day	Minutes	Minutes	µl/min	°C	Evosep P/N
500	2.9	2.3	4.0	40	EV1182
300	4.8	4.0	4.0	40	EV1182
200	7.2	6.4	2.0	40	EV1182
100	14.4	12.7	1.5	40	EV1109
60	24.0	22.1	1.0	40	EV1109
30	48.0	45.4	0.5	40	EV1137
W120	12.0	10.3	0.2	50	IonOpticks Aurora Rapid 5 x 75 µm
W80	18.0	16.3	0.2	50	IonOpticks Aurora Rapid 5 x 75 µm
W40	36.0	32.5	0.2	50	IonOpticks Aurora Elite 15 x 75 µm
W20	72.0	68.0	0.2	50	IonOpticks Aurora Elite 15 x 75 µm

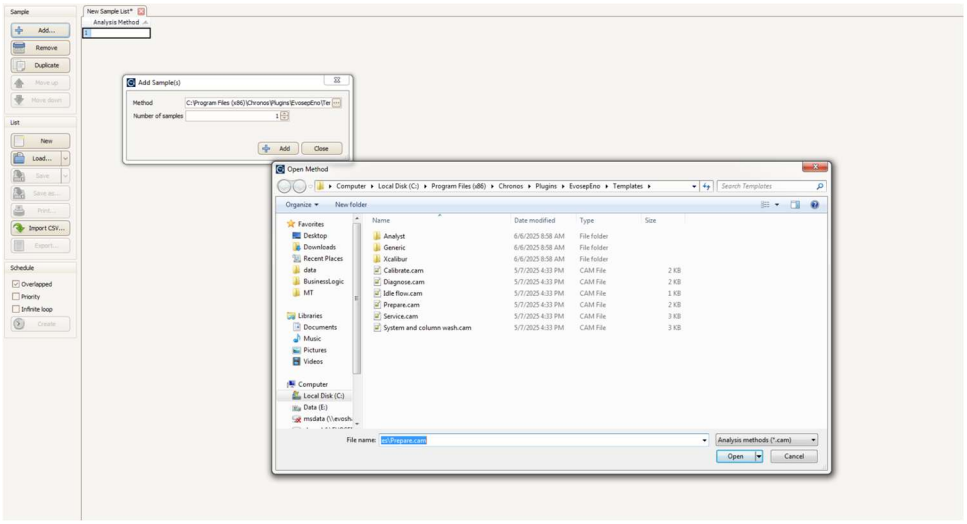
6.2 Sample Acquisition

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

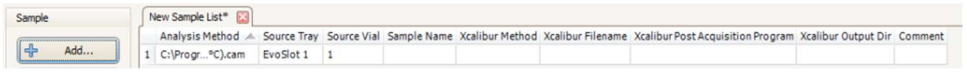
The sample acquisition methods are executed using Chronos software, as described in the SW section. The instrument can be operated in two distinct fashions.

1. Integrated LC-MS mode: Chronos controls both the Evosep Eno 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 Eno (in Chronos) and one for the mass spectrometer (in the MS CDS).

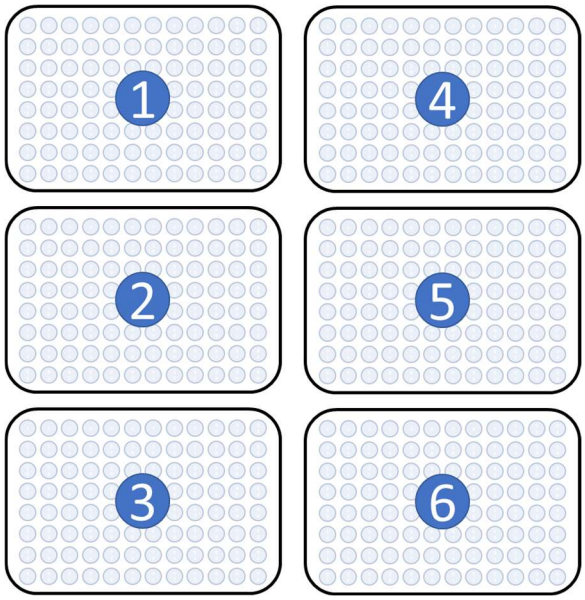
The sample methods for both modes are stored in the "C:\Program Files(x86)\Chronos\Plugins\EvosepEno\Templates" folder. Standalone mode methods are stored in the "Generic" folder, whereas LC-MS integrated methods are stored in a MS CDS specific folder, e.g. methods for Thermo mass spectrometers are saved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



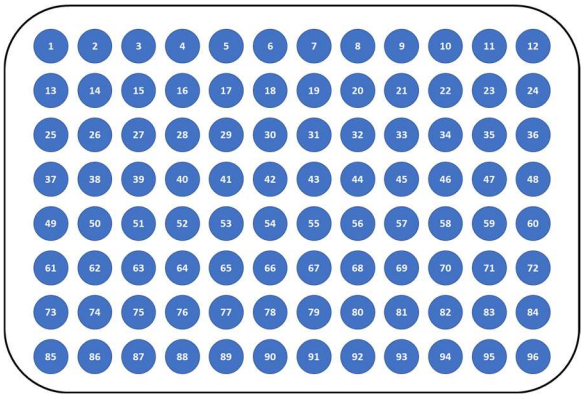
1. Integrated LC-MS mode:
    - The appropriate sample method is chosen based upon the MS CDS vendor and the required sample/day throughput (Table 1).



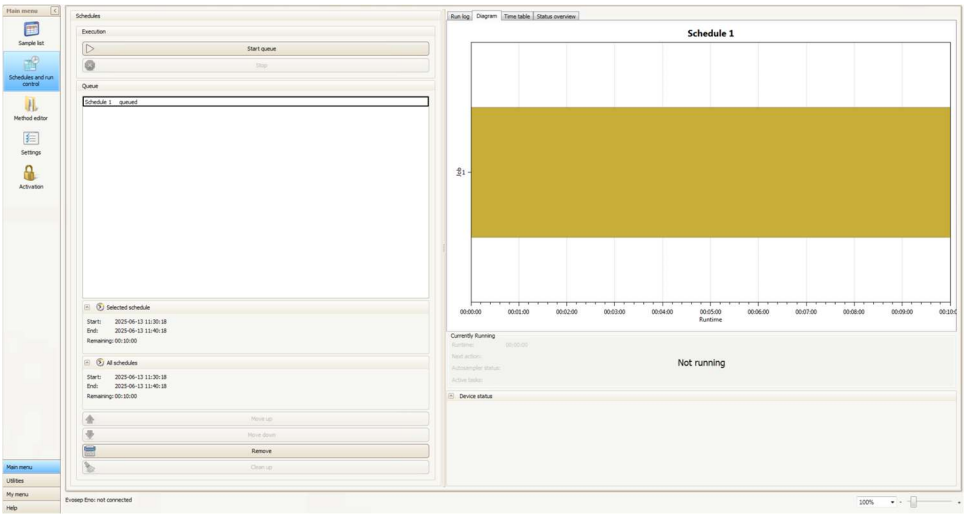
- Source Tray (Slot 1-6) must be specified using the dropdown menu.



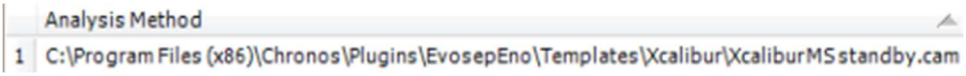
- Source vial position (1-96) must be specified using the dropdown menu.



- 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 Eno sample separation will start.



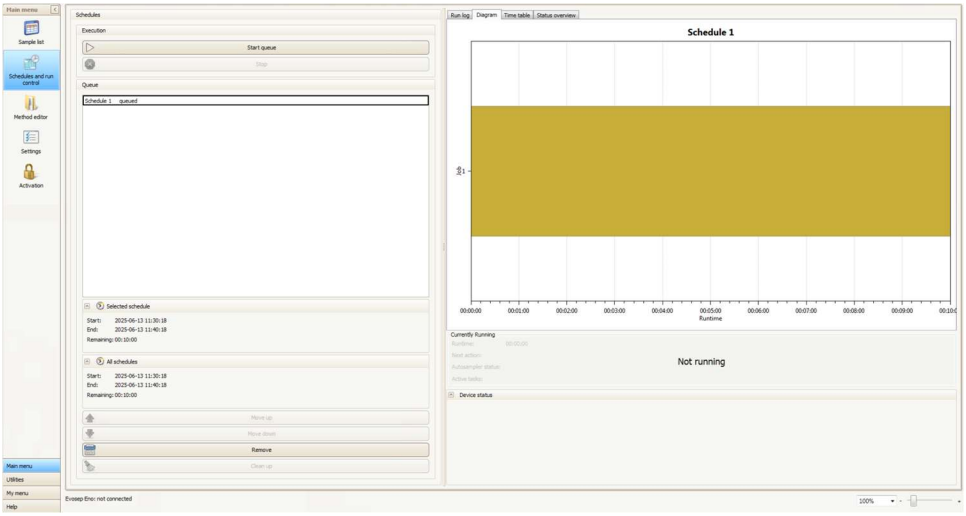
- For Thermo MS: Running the Xcalibur MS standby program will set the MS in standby, even if time remains in the chosen MS method. Typically, this method is chosen as the last sample in a batch.



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 Eno schedule.

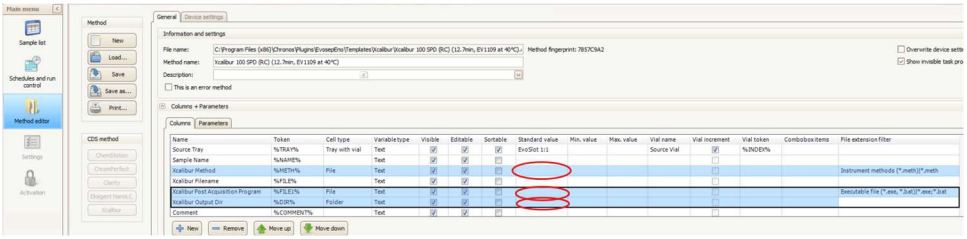
   - For MS CDS, create a synchronous sample list, queue the sample list for acquisition, and wait for the MS to be in "waiting for contact closure" mode.
   - Start the Evosep Eno schedule queue.



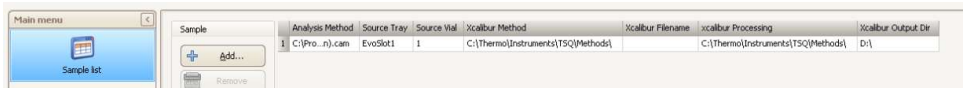
   - The Evosep Eno starts the sample separation procedure and sends a contact closure signal to the MS CDS when the sample elution begins.

Tips and tricks:

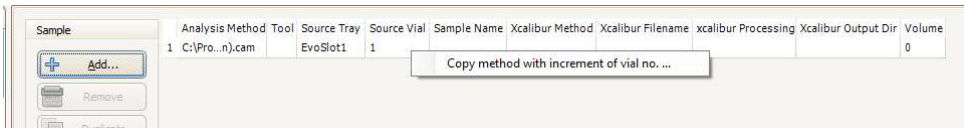
- When running integrated LC-MS methods, e.g. for Thermo mass spectrometers, the user can set default values for:
  - "Xcalibur Method": folder or file name
  - "Xcalibur Post Acquisition Program": folder or file name
  - "Xcalibur Output Dir": folder
  - These values are set in the "Method editor" section.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

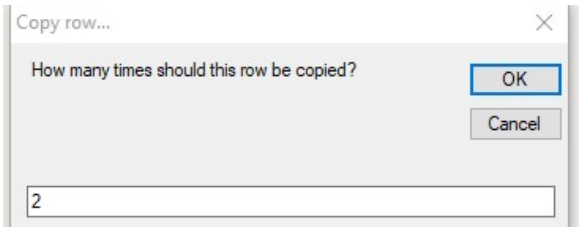


- Save the method with the same name.
- When submitting new samples with the updated method, the user can now browse from the specified default directory.

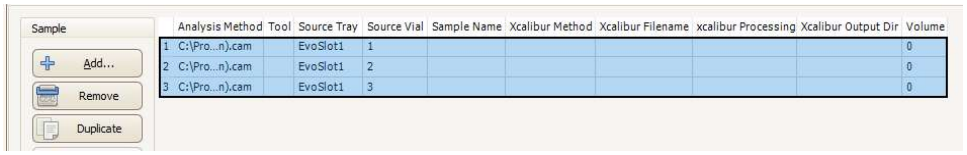


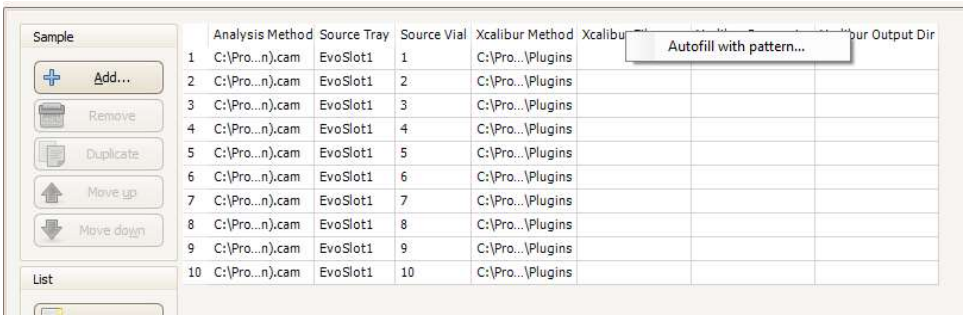
- If several samples in consecutive autosampler positions are to be processed using the method, right-clicking the “Source Vial” column will open a “Copy method row…” dialog. Define how many samples must be added to the sample list and press “OK”.

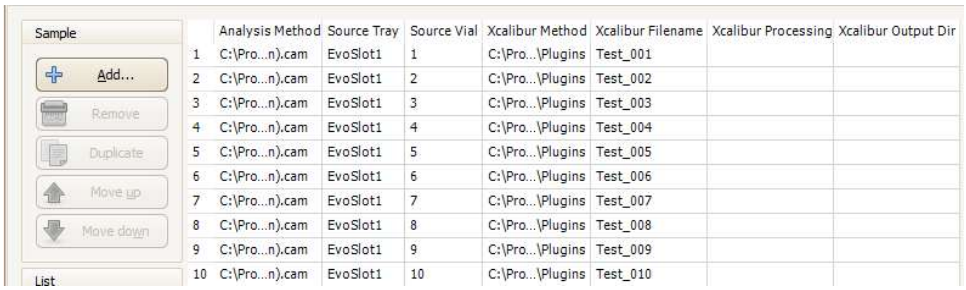




- If the sample name of several samples only vary by a suffix, right clicking the “MS Filename” will open a “Autofill with pattern…” dialog. Fill in the sample “base name” followed by one or more asterixes (*). When you press “OK” the sample names will be filled down in the sample list with the starting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







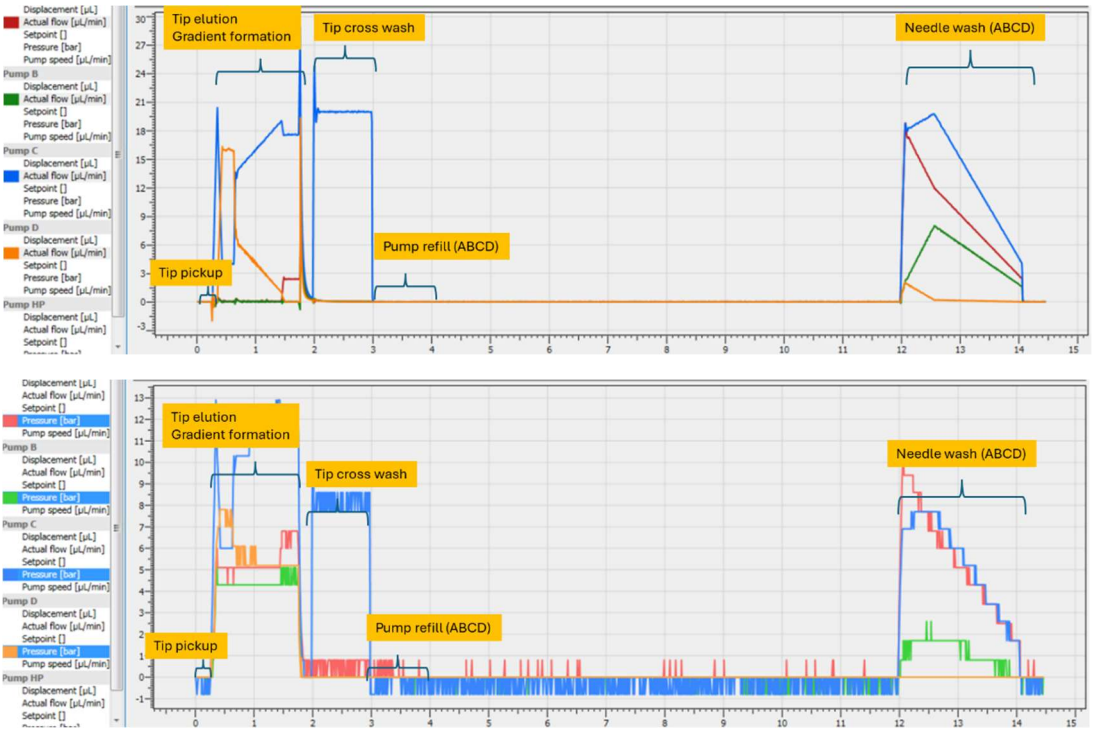
	Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur Processing	Xcalibur Output Dir
1	C:\Pro...n).cam	EvoSlot1	1	C:\Pro...\Plugins	Test_001		
2	C:\Pro...n).cam	EvoSlot1	2	C:\Pro...\Plugins	Test_002		
3	C:\Pro...n).cam	EvoSlot1	3	C:\Pro...\Plugins	Test_003		
4	C:\Pro...n).cam	EvoSlot1	4	C:\Pro...\Plugins	Test_004		
5	C:\Pro...n).cam	EvoSlot1	5	C:\Pro...\Plugins	Test_005		
6	C:\Pro...n).cam	EvoSlot1	6	C:\Pro...\Plugins	Test_006		
7	C:\Pro...n).cam	EvoSlot1	7	C:\Pro...\Plugins	Test_007		
8	C:\Pro...n).cam	EvoSlot1	8	C:\Pro...\Plugins	Test_008		
9	C:\Pro...n).cam	EvoSlot1	9	C:\Pro...\Plugins	Test_009		
10	C:\Pro...n).cam	EvoSlot1	10	C:\Pro...\Plugins	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.

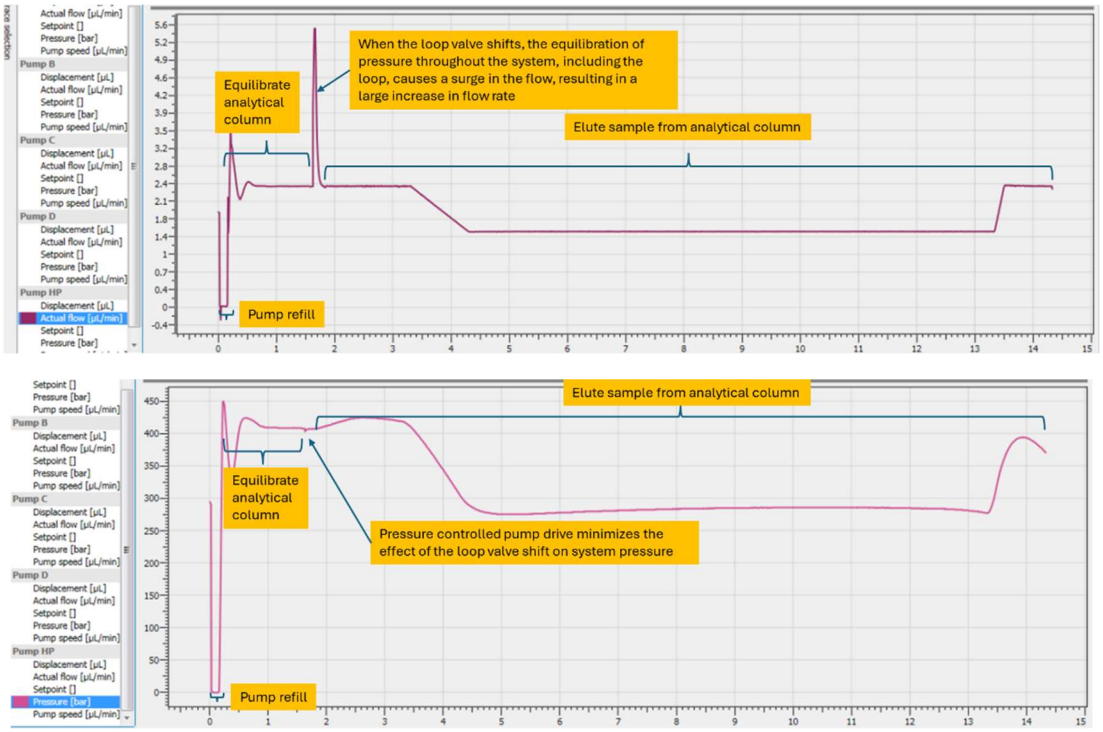
6.3 Example Pump Data

1. For information purposes, the pump data is shown of a typical chromatographic method, in this case 100 SPD on an Evosep Eno system using the EV1137 Performance column.

   - 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



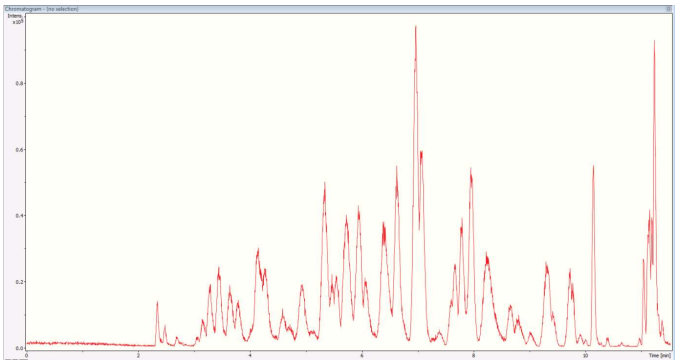
7 Troubleshooting

Please refer to Chapter 6 “Running Samples Using Evosep Eno” to view all safety warnings, cautions, and concerns.

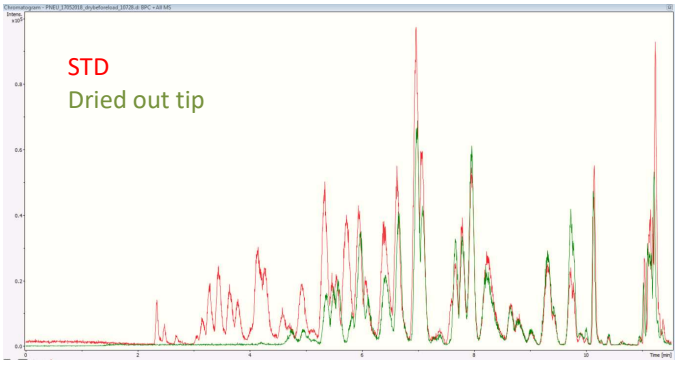
7.1 Evotip Troubleshooting

If the Sample loading onto the Evotip Pure 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.

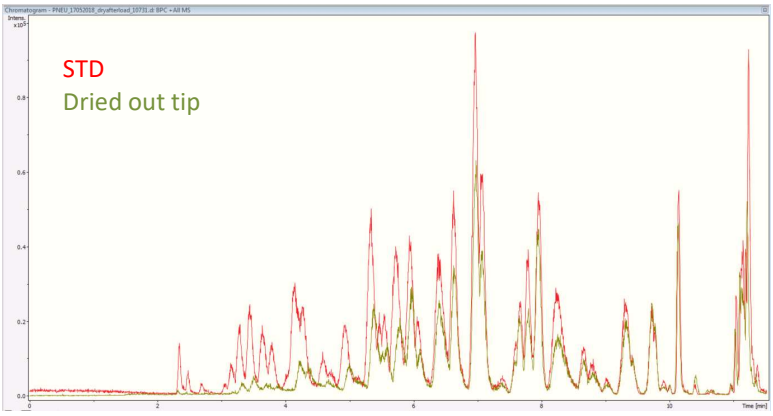
1. BSA correctly loaded on the Evotip according to the SOP.



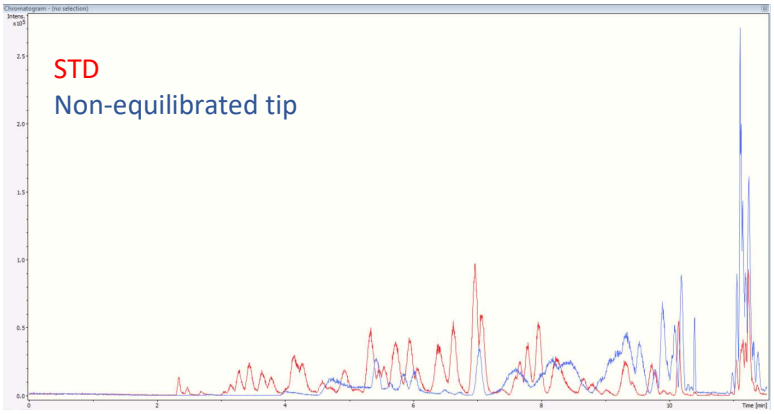
2. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out before loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



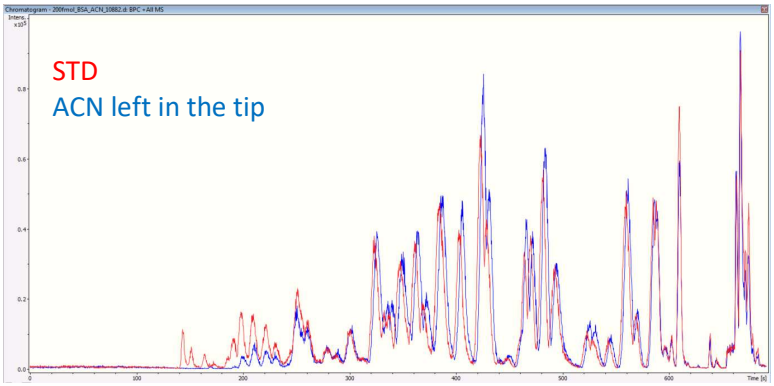
3. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out after loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



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).



7.2 How to Troubleshoot Connection Problems Between PC and Evosep Eno

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

7.2.1 Check that Both the Pump Box and Autosampler are Powered On

1. Pump box

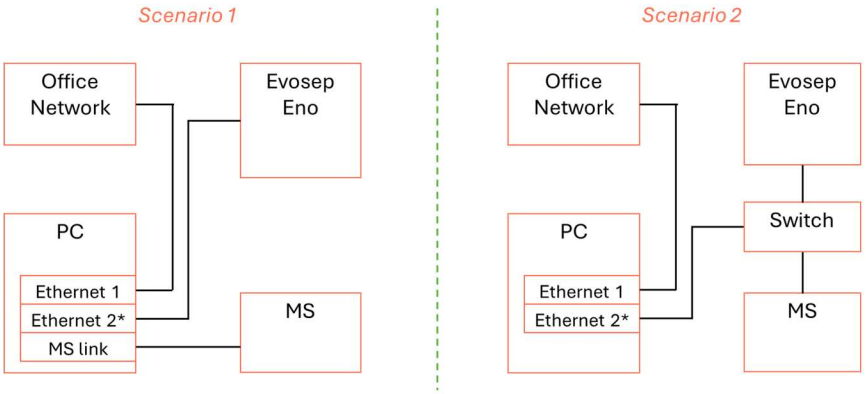
   Open the door on the pump box and verify that the LEDs on the five pumps are on. The LEDs could be either orange or green depending on the status of the instrument.

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 Eno, and that the power switch on the autosampler power supply is switched on.

7.2.2 Check LAN Connection

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



*Statically assigned IP address (172.17.17.14)

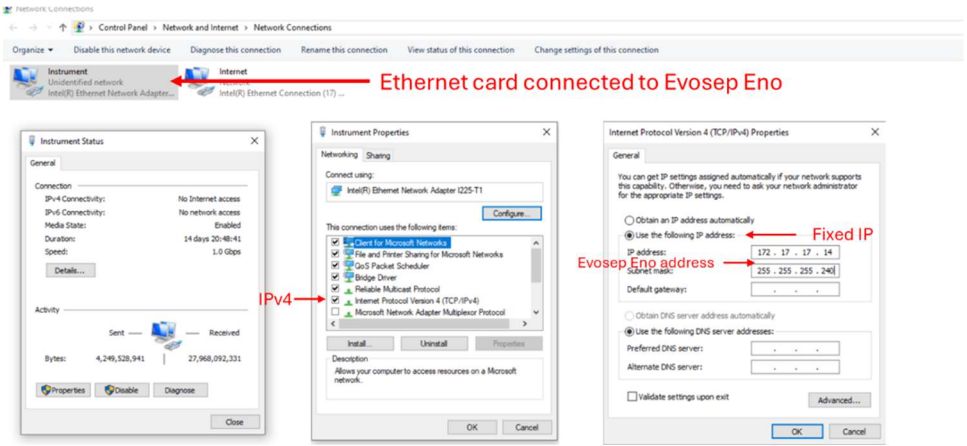
Please verify that the following:

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

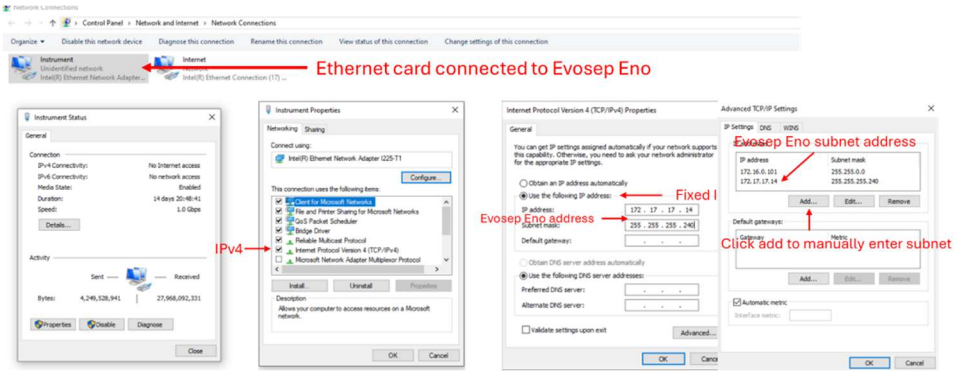
7.2.3 Check Network Adapter Set Up

When the Evosep Eno 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 Eno 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



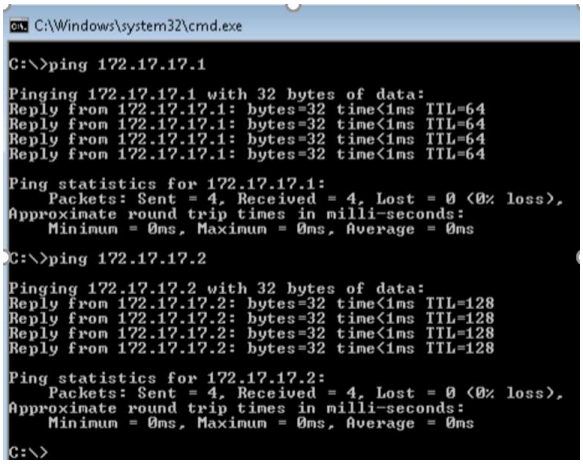
3. If the adapter is set up to use another fixed IP address, but an Evosep Eno subnet has not been created in the Advanced TCP/IP Settings, try to uninstall the plugin and reinstall it. Make sure the Evosep Eno is powered on and connected to the PC through ethernet cable. If reinstalling does not set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



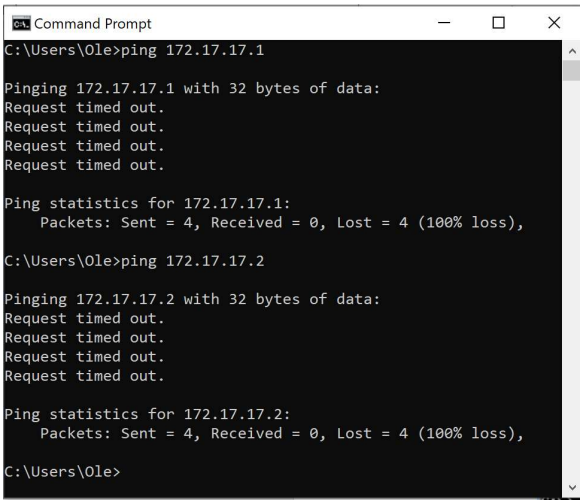
7.2.4 Ping Hardware Units

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.

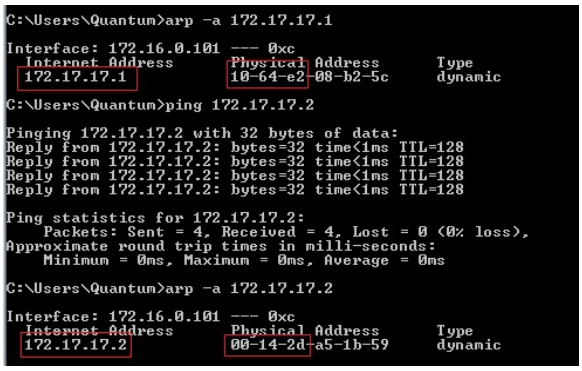
1. From Windows Start menu, open the “Command Prompt” and type “ping” followed by the IP address of the hardware unit.
   - Pump box: “ping 172.17.17.1”
   - Autosampler: “ping 172.17.17.2”



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.

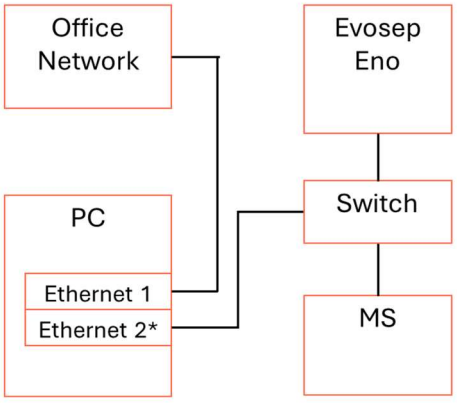


4. To verify that the Ping reply is coming from the Evosep Eno hardware and not another hardware unit with the same IP address, perform the following commands:
   - Pump box: “arp -a 172.17.17.1”
     - Verify the physical address has prefix “10-64-e2” or “00-90-e8”
   - Autosampler: “arp -a 172.17.17.2”
     - 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 Eno, then please try and bypass the switch by connecting the Evosep Eno 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 Eno). 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 Eno 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.

7.3 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.

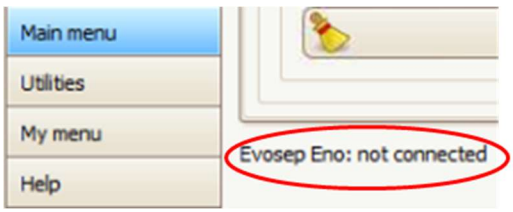
Please note that IP addresses stated in the table are the default. For a non-default installation, IP addresses may differ but possible cause and action will be the same.

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 Eno 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 authenticity
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

7.4 Error Messages Regarding Communication Issues With Chronos and Evosep Plugin

Please note that the status of the Evosep Eno 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 defective
   - 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 Eno”.

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 Eno 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 Eno 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 Eno, 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 Eno instrument software package to obtain compatibility with the connected hardware!
   - Possible cause:
       - Mismatch between firmware version of Evosep Eno and the Evosep Eno software plugin installed on the PC. Please note that this only refers to the Evosep Eno software and not Chronos.
   - Action:
       - Update the Evosep Eno software on the PC to the latest version and connect to the Evosep Eno 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.

7.5 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.

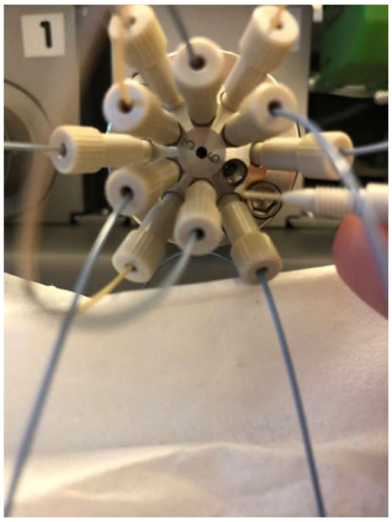
Error Message:

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 Eno 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 9.11) 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 EvoSlot 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.

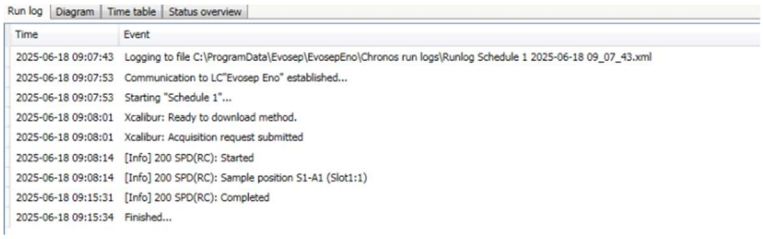
- Action:
  - Verify the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile).
  - Check that the system is level and free of vibration.
  - If both above are correct, then perform a Prepare – Pump preparation – Solvent exchange and run a new sample.
  - Run Diagnose – Tip seal and HP system scripts to check for leaks.

7.6 Schedule / Sample Not Starting / Contact Closure Problems

7.6.1 Troubleshooting Tips for Xcalibur Set Up

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

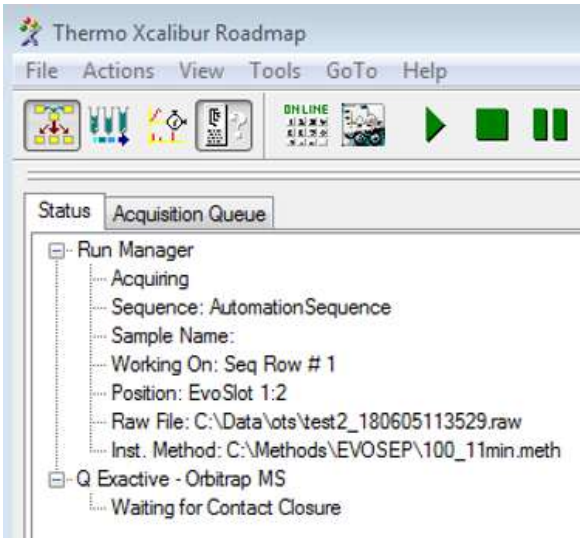
The run log for a typical schedule will look like this:


Run log | Diagram | Time table | Status overview

Time	Event
2025-06-18 09:07:43	Logging to file C:\ProgramData\Evosep\EvosepEno\Chronos run logs\Runlog Schedule 1 2025-06-18 09_07_43.xml
2025-06-18 09:07:53	Communication to LC"Evosep Eno" established...
2025-06-18 09:07:53	Starting "Schedule 1"...
2025-06-18 09:08:01	Xcalibur: Ready to download method.
2025-06-18 09:08:01	Xcalibur: Acquisition request submitted
2025-06-18 09:08:14	[Info] 200 SPD(RC): Started
2025-06-18 09:08:14	[Info] 200 SPD(RC): Sample position S1-A1 (Slot1:1)
2025-06-18 09:15:31	[Info] 200 SPD(RC): Completed
2025-06-18 09:15:34	Finished...

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 Eno 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 Eno 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 Eno.
- 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. Evosep Eno 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 Eno 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.

7.7 Hardware Troubleshooting

The Evosep Eno 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.

Go to www.evosep.com/support/troubleshooting/

For extensive troubleshooting guides on how to solve minor instrument issues

8 Advanced Maintenance

Please refer to Evosep Eno User Manual Chapter 5 “Running Samples” to view all safety warnings, cautions, and concerns and Chapter 6 for Routine Maintenance.

8.1.1 Prepare Instrument for Storage

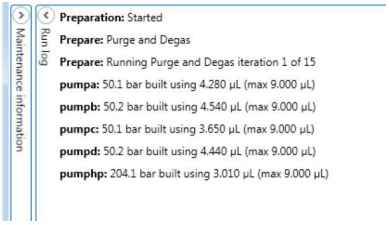
If the instrument is not going to be used for >2 weeks, please perform the following tasks.

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.

9 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 Eno 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 Eno 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 Eno 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 health 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 Eno 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.**

9.1 Recommended Wear Part List

Evosep recommends having the 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
- EV1018 – needle
- EV1058 – HP piston seal
- EV1060 – LP piston seal
- EV1034 – Transferline

After replacing a wear part, please see the table below 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	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.

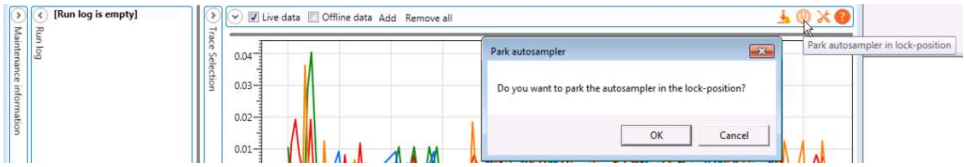
Visit the Evosep webshop: Spare parts and consumables for Evosep Eno

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

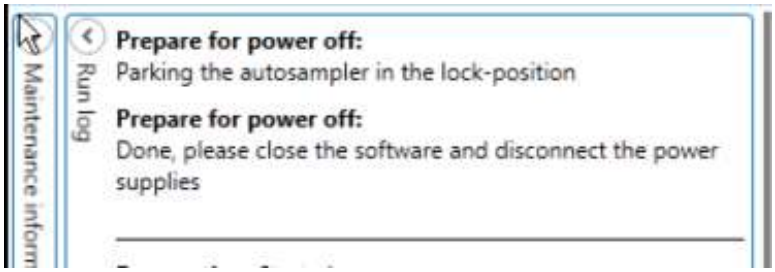
9.2 Power Off the Instrument for Repair and Maintenance

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 the 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.

9.3 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 with the solvent A tubing put into the solvent B bottle to ensure the new pump is fully degassed.
10. Put the solvent A tubing back into the solvent A bottle.

11. Run Preparation – Pump preparation – Solvent exchange to ensure the new pump is fully purged and degassed.
12. Run the Diagnose – Pump HP to ensure that no leaks are present after the replacement of the pump cassette.

9.4 Replacing the LP Pump Cassette

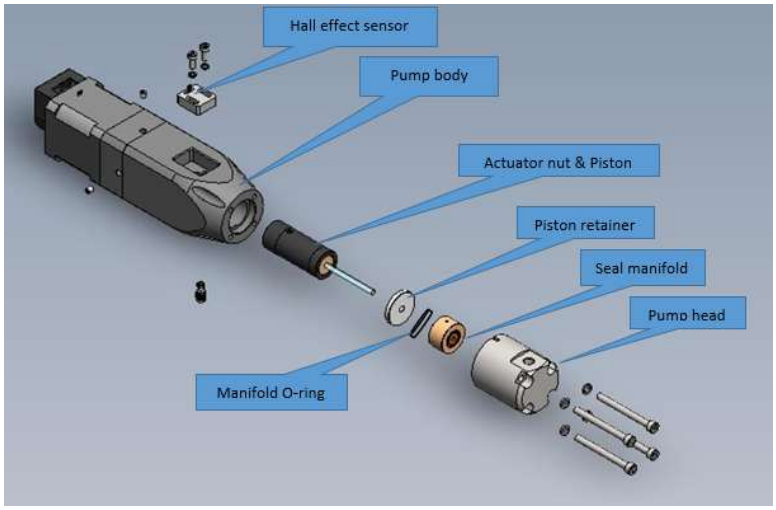
1. Power off the instrument.
2. Disconnect the peek tubing from the bottom port on the LP pressure sensor.
3. Loosen the knurled nut in the lower right corner of the pump cassette.

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. If pump A or C were replaced, please continue with point 7. if pump B or C were replaced then point 7. and 8. can be skipped.
7. Run Preparation – Pump preparation – Solvent exchange with the solvent A tubing put into the solvent B bottle to ensure the new pump is fully degassed.
8. Put the solvent A tubing back into the solvent A bottle
9. Run Preparation – Pump preparation – Solvent exchange to ensure the new pump is fully purged and degassed.
10. Run the Diagnose – Pump A-D to ensure that no leaks are present after the replacement of the pump cassette.

9.5 Replacing HP/LP Pressure Sensor

1. Remove the pump cassette from the instrument as described in Sections 9.3 and/or 9.4.
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.

9.6 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.

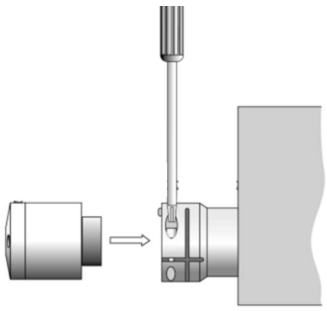
Upon setup or when servicing the seals, wetting the seal and pump head piston bore with acetonitrile will help reduce air bubbles and will allow faster flow stabilization. Use an adjustable pipette with a plastic tip to avoid damaging the sealing surfaces. Flush the spring cavity of the pressure seal 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. If piston seal in pump A or C were replaced, please continue with point 6. if piston seal in pump B or C were replaced then point 6. and 7. can be skipped.

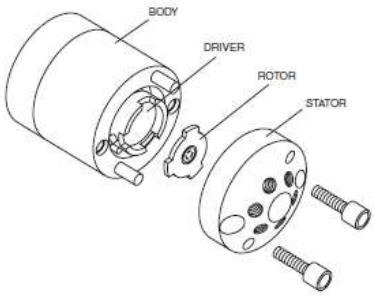
6. Run Preparation – Pump preparation – Solvent exchange with the solvent A tubing put into the solvent B bottle to ensure the new pump is fully degassed.
7. Put the solvent A tubing back into the solvent A bottle
8. Run Preparation – Pump preparation – Solvent exchange to ensure the new pump is fully purged and degassed.
9.
10. Run the degas script to remove air from the pump.
11. Run leak test for that pump to ensure that the new seal is sealing correctly.

9.7 Replacing a Valve Stack



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.

9.8 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 the 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.

9.9 Tubing and Fittings

The following paragraph contains a description of the various kinds of tubing and fittings used on the Evosep Eno 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:

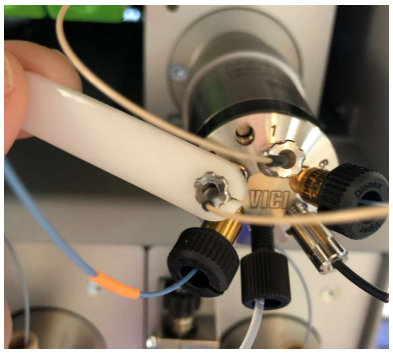
Viper and nanoViper tubing are finger tight fitting systems that require little torque to seal. It is essential to follow the guidelines below to avoid damage by over-tightening:

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.

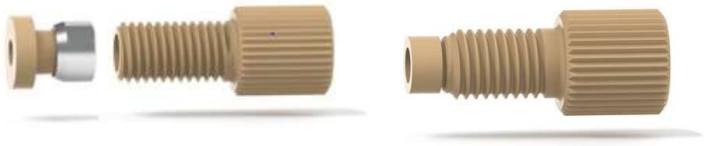
1. Insert the NanoConnect fitting into the receiving port and finger-tighten the nut securely.
2. For male NanoConnect fittings, use the NanoConnect torque wrench.



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.



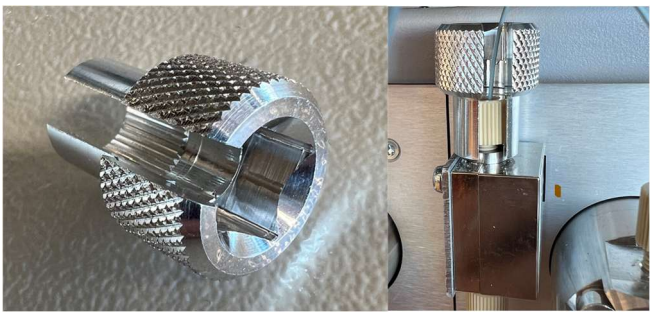
1. Check that the tubing is either level or sticking slightly out of ferrule.
2. Insert into the receiving port and finger-tighten the nut securely.

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

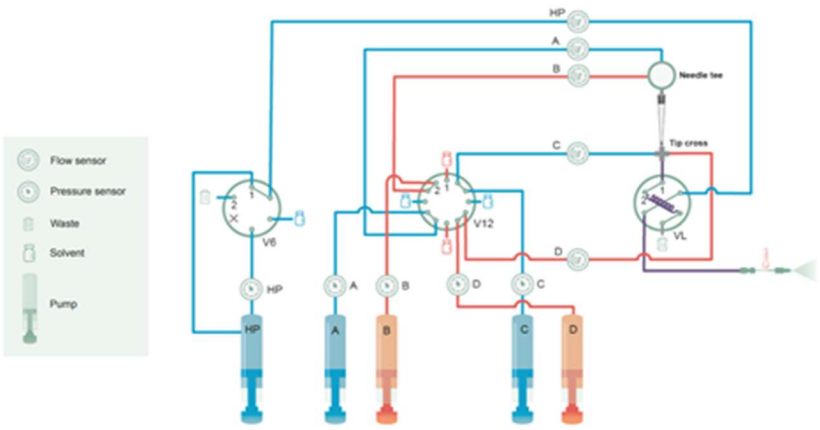
1. Slide the peek nut onto the tubing.
2. Insert the nut and tubing into the receiving port.
3. Finger-tighten the nut securely while making sure that the tubing is bottomed out in the port.
4. When tightened, gently pull the tubing to verify it is seated correctly.

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:

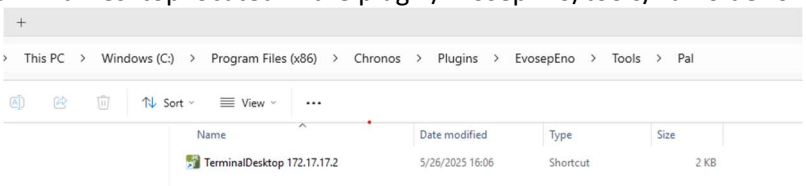


Valve Drive 1 Valve 6 tubing position		Valve Drive 2 Valve 12 tubing position		Valve Drive 3 Valve Loop tubing position	
P1	Pump HP	P1	Solvent B Tee	P1	TipCross
P2	Waste	P2	Pressure sensor B	P2	Loop
P3	Blank	P3	Flow sensor B	P3	Transferline
P4	Pressure sensor HP	P4	Solvent A Cross	P4	Waste
P5	Solvent A Cross	P5	Pressure sensor A	P5	Loop
P6	Flow sensor HP	P6	Flow sensor A	P6	Flow sensor HP
		P7	Solvent Tee		
		P8	Pressure sensor D		
		P9	Flow sensor D		
		P10	Solvent C Cross		
		P11	Pressure sensor C		
		P12	Flow sensor C		

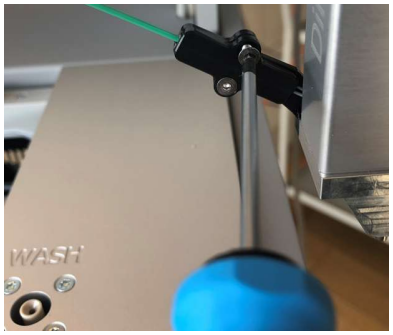
9.10 Replacing Tubing A, B Flow Sensor to Needle Tee Tubing

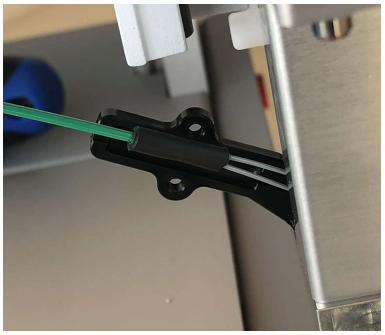
To remove the tubing:

1. Remove the sample tray from the instrument.
2. Open the PAL TerminalDesktop located in the plugin/EvosepEno/tools/Pal folder on the control PC.

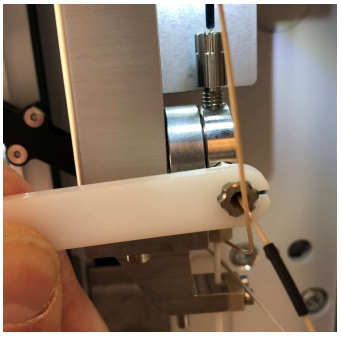


3. From the PAL TerminalDesktop, select “RobotArmLeft”.
4. Select “Options” and “Change Syringe”.
5. Select “Move” to move the needle to the exchange position.
6. With a T6, remove the two Torx screws from the black tubing holder and remove the cover.

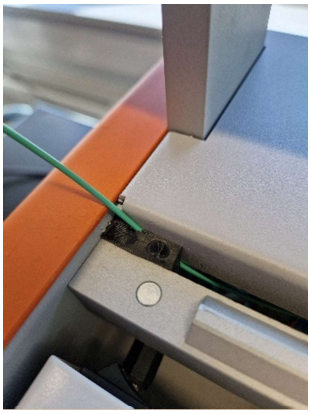




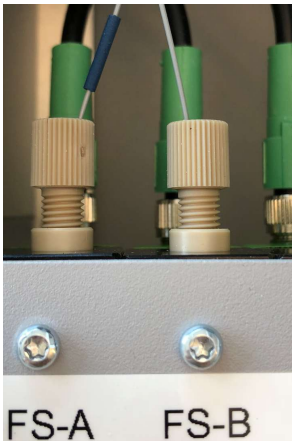
7. Disconnect tubing A and B from the stainless-steel Tee using the small torque wrench.
    - Note that tubing A is oriented vertical with a blue label and tubing B is oriented horizontal with a black label.



8. Use an appropriate tool to carefully push the black tubing holder in the back-left corner of the cabinet out from the metal frame. Ensure the holder does not drop into the instrument.



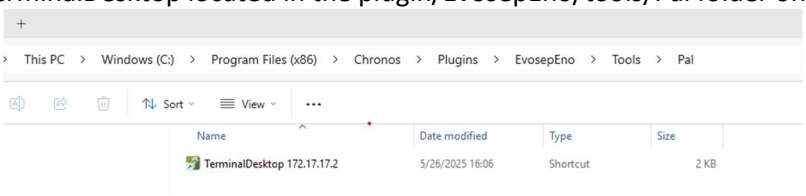
9. Disconnect the tubing from the exit side of flow sensor A and B.
    - Note that tubing A has a blue label.



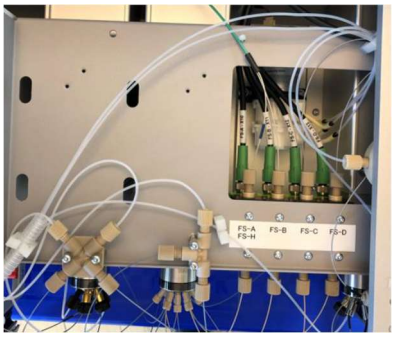
10. To remove tubing completely, slide the two metal fittings on tubing A and B down through the small opening where the tubing holder was sitting.

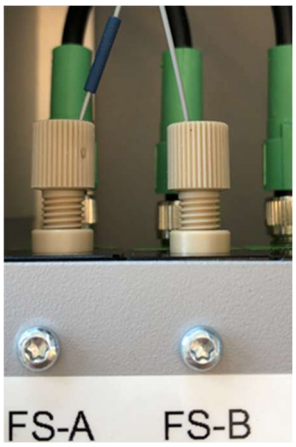
To connect new tubing:

1. Open the PAL TerminalDesktop located in the plugin/EvosepEno/tools/Pal folder on the control PC.

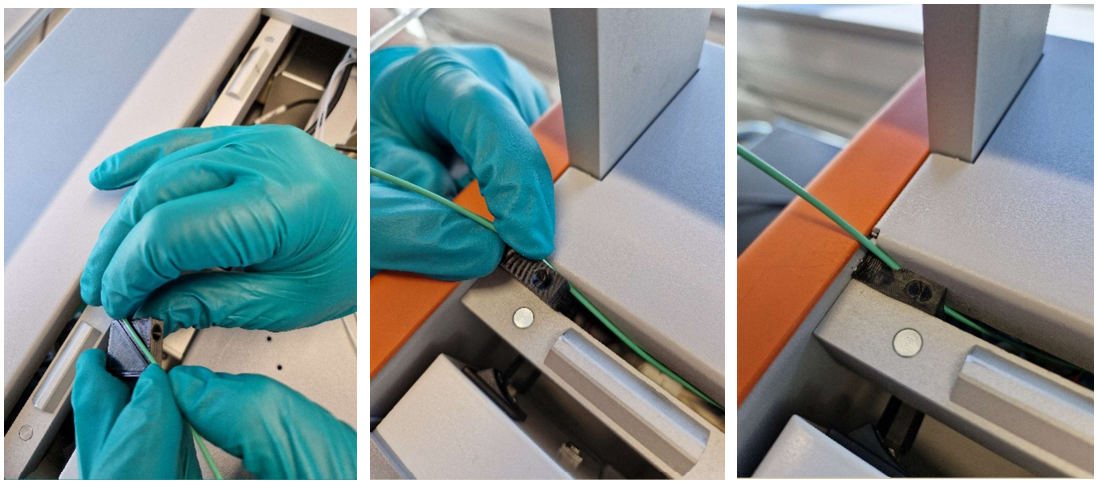


2. From the PAL TerminalDesktop, select “RobotArmLeft”.
3. Select “Options” and “Change Syringe”.
4. Select “Move” to move the needle to the exchange position.
5. Push the tubing with the two metal fittings up through the small opening. Do this from underneath in the up direction.
6. Route the other 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.





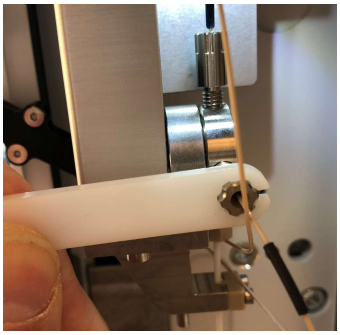
7. Put the green tubing in the groove of the tubing holder. Slide it into the small opening between the back top-cover and the metal frame. Maneuver the holder into the top left corner of the metal frame using your hands making sure not to pinch the green tubing and verify the following:
   - The holder is flush with the metal frame
   - leave as much length of the tubing as possible out of the instrument.



8. 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.

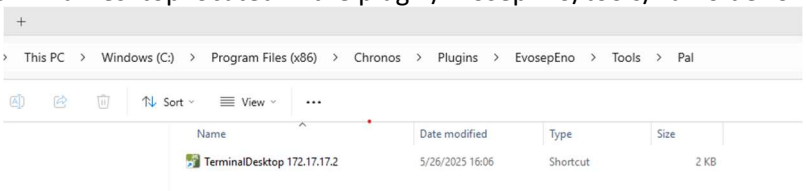
9. Connect tubing A and B from the stainless-steel Tee and tighten with the torque wrench.

- Note that tubing A is oriented vertically with a blue label and tubing B is oriented horizontally with a black label.
- Do not use any other tool besides the NanoConnect torque wrench for the NanoConnect fittings.

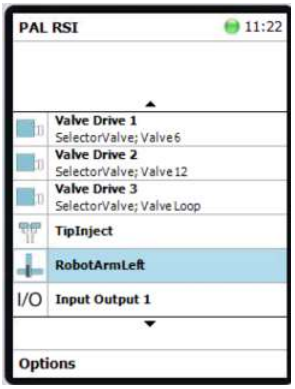


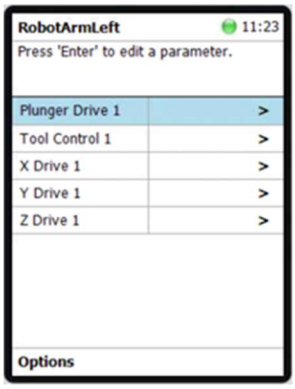
9.11 Replacing the Needle

1. Open the PAL TerminalDesktop located in the plugin/EvosepEno/tools/Pal folder on the control PC.

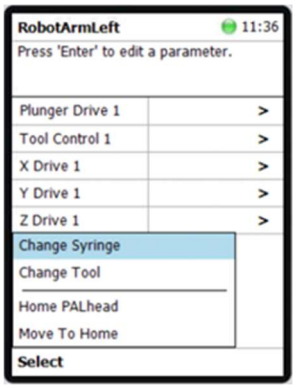


2. From the PAL TerminalDesktop, select “RobotArmLeft”.

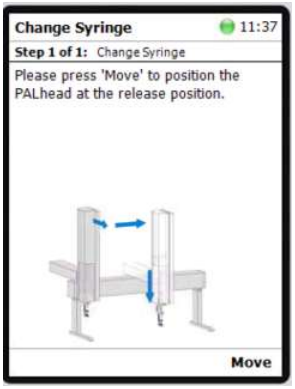




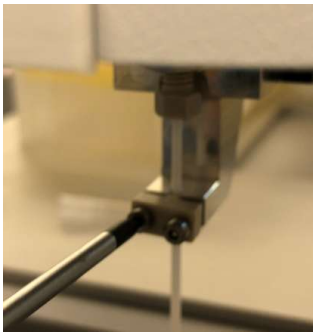
3. Select “Options” and “Change Syringe”.



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



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



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

7. With the peek nut fully unscrewed, remove the needle and peek nut.
8. 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.
9. 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.
10. When completed, press "Next" on terminal to move "RobotArmLeft" back to home position.
11. Press "Back" to go to the "PAL RSI" main terminal page.

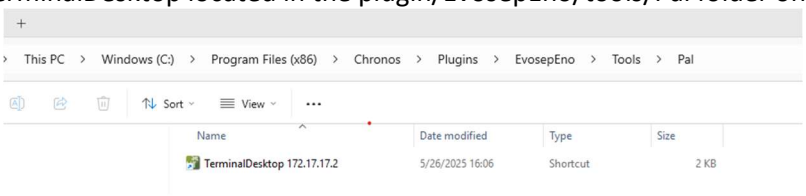
9.12 Replacing the Needle Tee

1. Remove the needle as described in Section 11.12 "Replacing the Needle".

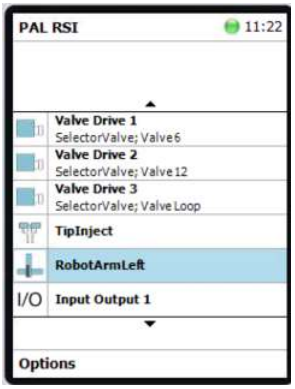
2. Disconnect the A and B NanoConnect tubing from the needle tee. Be careful not to kink or stress the tubing.
3. Loosen the lock screw from the needle tee holder and remove the needle tee.
4. Install needle tee in reverse order.

9.13 Replacing the Tool

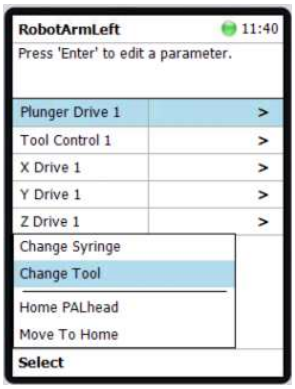
1. Open the PAL TerminalDesktop located in the plugin/EvosepEno/tools/Pal folder on the control PC.



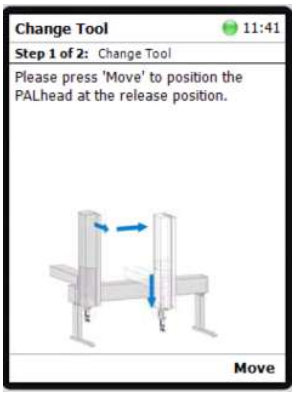
2. From the PAL TerminalDesktop, select “RobotArmLeft”.
3.



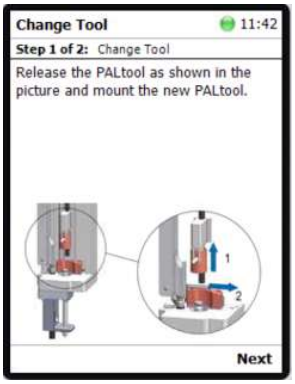
4. Press A to select “Options” and “Change Tool”.



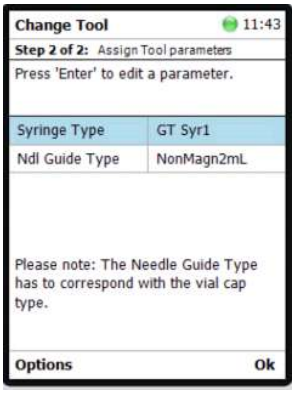
5. Select “Move” to move the tool to the exchange position.



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



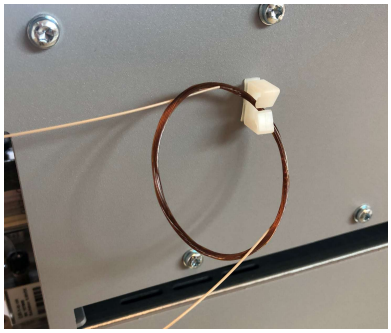
10. Install the new tool. Connect the tubing and needle.
11. Press “Next” on terminal.
12. Verify that the “Syringe Type” is “GT Syr1” and the “Ndl Guide Type” is “NonMagn2mL”.



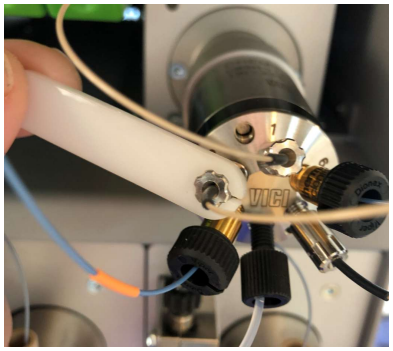
13. Press “Ok” to move robot arm to home position.

9.14 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.

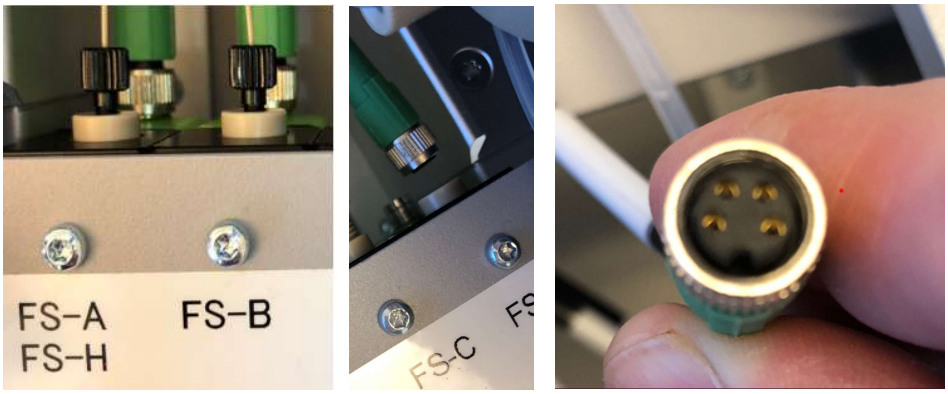


4. Install the new loop in reverse order using the small torque wrench to ensure proper tightening of the loop fittings.
5. After installation, run the Service – Loop flush script to flush the new loop with solvent.
6. Run the Diagnose – HP system script to verify that there are no leaks around the loop.

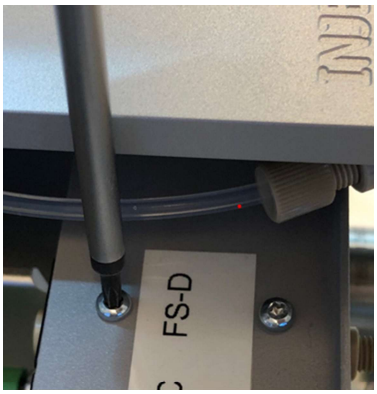
9.15 Replacing a Flow Sensor

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.