



v. 17

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1 Preface

1.1 About this manual

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

1.2 Safety and Special Notices

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

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

1.3 Contacting Us

Support: <a>support@evosep.com

Sales: sales@evosep.com

1.4 Declaration of Conformity

We:

Evosep Aps
Buchwaldsgade 35, 3rd floor
5000
Odense C
Denmark
+45 2566 2322
mba@evosep.com

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

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

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

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

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

lith

Michael Barrett Andersen, Head of Product Management

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July 4th, 2018,



2 Introduction

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

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

More uptime with improved reliability and robustness owing to:

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

Increased productivity with higher duty cycle utilization owing to:

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

Increased performance with better data quality owing to:

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

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

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



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

3 Installing the Evosep One hardware

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

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

3.1 Lifting instructions

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

Important Notes:

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

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

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

3.2 Table and trolley requirements

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

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



Evosep One footprint for installing on trolley:

Depth	440 mm	17.3 in
Width	420 mm	16.5 in

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

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

3.3 Power requirements

Line Voltage:

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

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

Grounding requirements:

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

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

3.4 Temperature and humidity requirements

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

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

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

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

Operating Humidity: 20-80%, non-condensing

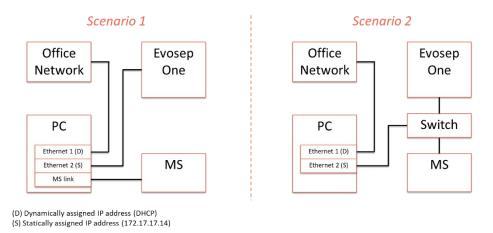
The maximum air conditioning load for the Evosep One is approximately 350 W



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

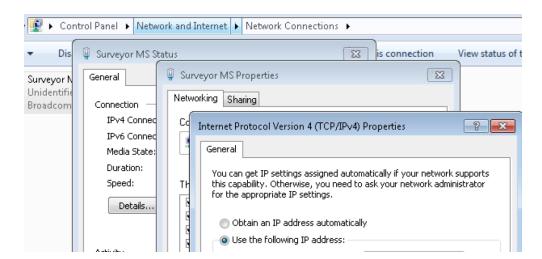
3.4.1 Connecting the ethernet communication cable and checking network adapter settings

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



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

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



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

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

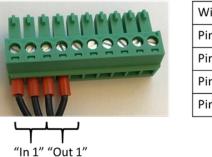
3.4.2 Connecting the contact closure cable

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

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



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



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

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

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

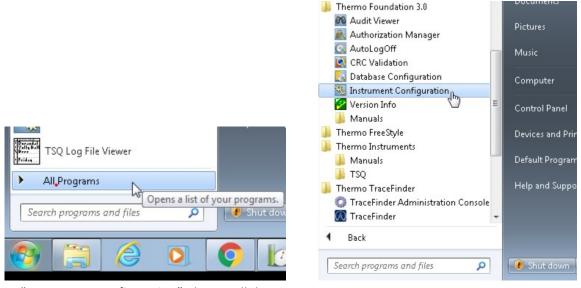
3.4.3 How to remove other LC devices from MS system configuration

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



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

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



3) In "Instrument Configuration" Choose all device types

-	Thermo Foundation Instrument Configuration
	Device Types :
	All
	Ausliele Deulesee
	Available Devices:

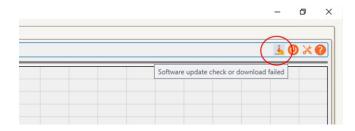
4) IF any LC systems are visible in the "Configured Devices" window then mark them and click remove, which will remove them from the configuration. (Do not remove the MS from the configuration). Then click "Done" and re-open Xcalibur.

5 995	5	
SQ Quantum	TSQ Quantum	Thermo EASY-nLC
rmo EASY-nEU		
	SQ Quantum	

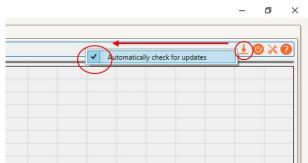
4 Installing control software

4.1 Automated software plugin update

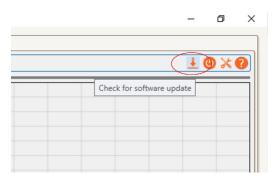
The plugin software will automatically detect if a new version is released and assist the user in the update process (available from plugin v1.4 for Chronos and v1.2 for HyStar). For this feature to work, the PC must have access to evosep.com. If this page is blocked, a warning will be displayed on the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



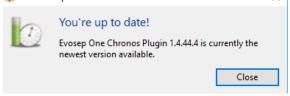
- 1. The software update process can be triggered in two ways:
 - a. Automatically during restart of Chronos/HyStar. The automated feature can be turned on/off by right clicking "Check for software update" button and checking /unchecking the box.



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



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





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.

🔅 Softwar	e Update	×
	A new version of Evosep One Chronos Plugin is available!	
	Evosep One Chronos Plugin 1.4.44.4 is now available (you have 1.4.43.4). Would you like to download it now?	
	Release notes:	
	# Improvement	^
	 - [EVONE-265] Flow to column (incl Idle flow) stability and flow rate optimization (Done) - [EVONE-207] Sample run overhead optimization (Done) 	
	 [EVONE-239] System and column wash improvement; needle gradient wash (Done) [EVONE-255] Automatically set HP flow sensor calibration defaults (Done) 	
	 - [EVONE-227] Verify autosampler configuration before running procedures (In Review) - [EVONE-250] Diagnose: pump A-D, Tip seal and HP system leak test; break pressure at the end of 	
	- [EVONE-226] Software shutdown function to position the autosampler in the "lock position", be - [EVONE-230] Sample file name improvements (Done)	
	- [EVONE-210] Disable the service script "LP system test*" (Done)	
	 - [EVONE-248] Service-purge loop opdatering; brug kun solventA til at purge loopet med (Done) - [EVONE-222] Allow HD6750xConfigurer to update target without knowing, or specifying, ip info: 	
	- [EVONE-208] Retention time reproducibility optimization #2, Gradient timetable precision (De	
	Skip this version Remind me later Install upda	te

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

🍄 Software	e Update	×
	Ready to install.	
		lastell on data
		Install update

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

n Setup	_		×
Welcome to the Evosep Plugin Setup Wizard	One Ch	ronos	
Back	lext	Cance	el
	Welcome to the Evosep Plugin Setup Wizard	Welcome to the Evosep One Ch Plugin Setup Wizard The Setup Wizard will install Evosep One Chror your computer. Click Next to continue or Cance Setup Wizard.	Welcome to the Evosep One Chronos Plugin Setup Wizard

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

- Software	Update •) <u>L</u>	
	Update Error! An error occurred in retrieving update information; a you connected to the internet? Please try again later. Cancel	·	



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

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

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

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

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

Installing Chronos

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

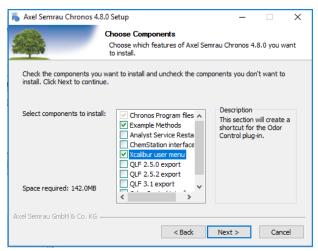
💫 Axel Semrau Chronos 4.8.0	etup	-		×
We sense good chemistry	Welcome to the Axel St Chronos 4.8.0 Setup W This wizard will guide you through the I Semau Chronos 4.8.0. It is recommended that you close all ot before starting Setup. This will make it relevant system files without having to computer. Click Next to continue.	rizard	on of Axel	
	Next	t>	Can	cel

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

1 ALLAN	License K To continu	ey e setup, you need a	license key.	
Please ente	er your license key			
8q6-9x8-7	7vg-93			



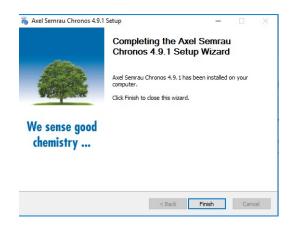
5. For installation on a Thermo MS data system please tick of the "Xcalibur user menu". For Analyst tick of "analyst"



6. Click "Install" to start the installation.

🐞 Axel Semrau Chronos 4	.9.1 Setup		-		×
1980a	Choose Start Menu Folder				
and the second second	Choose a Start Menu folder fo shortcuts.	r the Axel Se	mrau Ch	ronos 4.9).1
Select the Start Menu fold can also enter a name to	der in which you would like to creat create a new folder.	te the progran	n's short	cuts. You	1
Chronos					
Accessibility Accessories ADFweb.com Administrative Tools Chronos CutePDF Dell Dell Audio Dropbox HP Intel					*
Do not create shortcu					
Axel Semrau GmbH & Co, KG	< Back	Insta	11	Canc	el

7. Click "Finish".

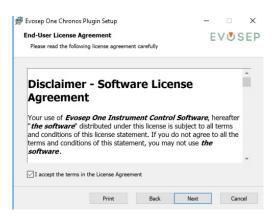


Evosep Chronos plugin installation

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

🖟 Evosep One Chronos Plug	in Setup — 🗆 X
	Welcome to the Evosep One Chronos Plugin Setup Wizard
EVOSEP	
	The Setup Wizard will install Evosep One Chronos Plugin on your computer. Click Next to continue or Cancel to exit the Setup Wizard.
	Back Next Cancel
	Back Next Cancel

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



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

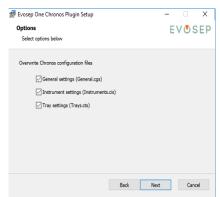


Select which Evosep+ applications you need their install state in the list below.	access to by setting
Evosep + application selection is also availab RC.Net Driver* folder in Windows Start.	le from a shortcut in the "Evosep One Whipper (BETA) methods are designed for highly sensitive, yet robust and reproductile analysis of low sample amounts.
Reset	Back Next Cance

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

륡 Evosep One Chronos Plugin Setup		_		×
Destination Folder			V 🛡 S	SEP
Click Next to install to the default folder or	click Change to o	hoose another.		
Install Evosep One Chronos Plugin to:				
C:\Program Files (x86)\Chronos\Plugins\				
Change				
	Back	Next	Cano	-1
	Dack	wext	Cano	.ei

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



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



🚽 Evosep One Chronos Plugin Se	etup	-		×
Ready to install Evosep On	e Chronos Plugin	E	V 🖲 S	SEF
Click Install to begin the installa installation settings. Click Cance		change any of y	our	
	Back	🖓 Install	Can	cel

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

Adapter	Subnets		Adapter Subnets		
User Network	IP: 192.168.167.206 /				
Surveyor MS	IP: 172.17.17.14 IP: 172.16.0.101	Mask: 255.255.255.240 Mask: 255.255.0.0			

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

Evosep One Network Gateway	→ ×
INIT : OK	Ver. 1.603
FIRMWARE : OK	
PROJECT : OK	

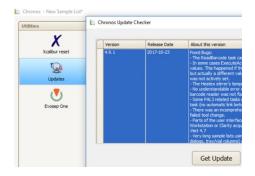
16. When the installation is complete, click "Finish", to exit the installer.

Evosep One Chronos Plugin	n Setup	_		\times
EVÖSEP	Completed the Evosep Plugin Setup Wizard Click the Finish button to exit the			
	Back	Finish	Cano	al

Please note the following, when updating Chronos:

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

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



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

Disease	iter your license key			
vmp-g		0 Setup	×	
	This licence key is in	nvalid or expire	d.	
		OK		

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



Main menu <	General Instruments Trays	Web Services Info	ormation	
Sample list	Show Report	Product version Version information		4.10.1
	Save snapshot	Path	File	
		3rdParty	3DTools.dll	
	Compare snapshot	3rdParty	3DViewerDesktop.exe	
Schedules and run control			AcquisitionServiceHost.exe	
	Gad ler diagriosocs	Plugins	AssertPlugin.dll	
		3rdParty	CarlosAg.ExcelXmlWriter.dll	
Method editor		3rdParty	Catel.Core.dll	
		3rdParty	Catel.MVVM.dll	
****		3rdParty\de	Catel.MVVM.resources.dll	
Settings			CCToCDFConv.exe	
Securigs			CCToCPConv.exe	
			Chronos.exe	
		Plugins\CSVImport	ChronosCSVImport.dll	
			ChronosUpdateChecker.exe	
		Plugins\EvosepOne	Common.Logging.Core.dll	
		Plugins\EvosepOne	Common.Logging.dll	
		Plugins\EvosepOne	Common.Logging.Log4Net.Unive	rsal.dll
			CSControlServerGUI.exe	
		3rdParty	DevExpress.BonusSkins.v13.1.dll	
		3rdParty	DevExpress.Charts.v13.1.Core.dll	
		3rdParty\de	DevExpress.Dashboard.v13.1.Cor	e.resources.dll
		3rdParty	DevExpress.Data.v13.1.dll	
		3rdParty\de	DevExpress.Data.v13.1.resources	.dll
		3rdParty\de	DevExpress.ExpressApp.AuditTra	il.v13.1.resource
		3rdParty\de	DevExpress.ExpressApp.Chart.v1	3.1.resources.dll
		3rdParty\de	DevExpress.ExpressApp.CloneOb	ject.v13.1.resour
		License information		
		License type:		AdvancedDor
		License includes updat	tes up to:	2018-10-07
		License key:		vmp-gpt-v43
Main menu				

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

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

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



4.2.1 IP configuration

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

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

4.3 Evosep drivers for control of Bruker MS

4.3.1 Installing ICF for Bruker Compass HyStar

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

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

Plug-in for l	CF 5.6 & ICF 4.6
•	Install Release Notes for ICF 5.6 Release Notes for ICF 4.6 Contact
	 Exit

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



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

Network Services			\sim
Install Network Services		в	RUKE
Install BootP service			
InstallShield			

7. Now click "Install" to install the program.

🛱 Bruker Plug-In for ICF 4.6 - InstallShield Wizard	😼 Bruker Plug-In for ICF 4.6 - InstallShield Wizard 🛛 💼 🔳
Ready to Install the Program The witzerd is ready to begin installation.	Installing Bruker Plug-In for ICF 4.6 The program features you selected are being installed.
Click Install to begin the installation. If you want to review or change any of your installation settings, click Back. Click Cancel to exit the wizard.	Fieses wat while the InstallSheld Wizard installs Bruker Plug-In for ICF 4.6. This may take several minutes. Status:
InstallSheid	InstallShield < Back Next > Cancel

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

Bruker Plug-In for ICF	4.6 - InstallShield Wizard	Bill Road Waland providingson Colling and Control and Con	+ 4 P	x 2019	-
	InstallShield Wizard Completed	ins (bit Ver freeder lans Hap ∰ founds: ∰ to large ⊂		- 9 - 2 - 1	ger stro-
BRUKER		BRUKER INSTALLATION QUAL Product Broker Play-In for X29 4.6 Deter 6/02/018 59:558.4M Workstore MRION OF URU, Wakars 7 Professional Service Pash 1 (NL Bell 6.1.760, IE)	IFICATION		
	The InstallShield Wizard has successfully installed Bruker Plug-In for ICF 4.6. Click Finish to exit the wizard.	81 File(s) should 0 Hie(s) compt			
	_	Autoratio Check Succeeded	IQ failed		
		(circle appropriate)			
		Branks			
		(date, same)	(signature)		
	< Back Finish Cancel	FILE C/BDalSystemDaniPlySor/LePhysicClyFilesAgilant/CF8ystem.etg	EXPECTED CRC32: 816EA610 Size: 267	FOUND CBC32: 816EA610 Size: 267	RES Chec O.K.

9. Finally click "Exit" to close the CD start menu.





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

Make sure the ICF plugin for HyStar is already installed.

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

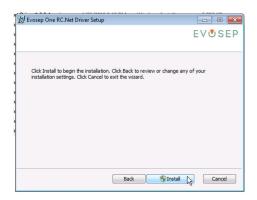
波 Evosep One RC.Net Driver	Setup 🗖 🗖 💌
	Welcome to the Evosep One RC.Net Driver Setup Wizard
EVUSEP	
	Back Next Cancel

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



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

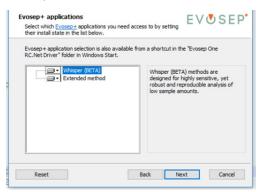




24. Click show details (if window not fully visible) then click "Yes" to allow the program to install the software.

🔡 Evosep One RC.Net Driver Setup		Evosep One RC.Net Driv	ver Setup	
Installing Evosep One RC.Net Driver	EV S EP	Installing Evosep Or	ne RC.Net Driver	EVÖSEP
Please wait while the Se Status: Status:		Please wait while the Se Status:	Softwar	want to allow the following program to install re on this computer? Program name: Evosep One Verified publisher: Evosep ApS Program location: Install 1.0.20.4 English (United States) Shoftware/15/starEvosep One RC.Net Driver 1.0.20.4.msi Show information about this publisher's certificate
Back Next	Cancel			Change when these notifications appear

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



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

Adapter	S	ubnets	
User Network	IP: 192.168.167.206	5 Mask: 255.255.255.0	
Surveyor MS	IP: 172.17.17.14 IP: 172.16.0.101	Mask: 255.255.255.240 Mask: 255.255.0.0	



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

INIT : OK	Ver. 1.603
FIRMWARE : OK	
PROJECT : OK	

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

Je Je	岁 Evosep One RC.Net Driver Setup	
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		t
	Badk Finish	Cancel

- 4.3.3 Create HyStar IFC configuration for Evosep One
- 1. Open Compass HyStar and click the gearwheel icon, to open the Configuration Editor.

Compass HyStar 4.1 - Navigator Acquisition			
Navigator Acquisition	View Options Compasi	Help	
Compass Meth	od Set 🔛 Sample Tabl	e 🚮 Acquisition	◆Quid: Data Viewer
Instruments			Open the Configuration Editor
HyStar	disconnected	Bruker OTOF MS	
disconnected		disconnected	
	Evosep One		
	Offine		
Connect all instruments	al	micr0T0F-Q III	
	+P Pressure bar +P Flow pL/min Method		

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

Configuration Editor *			
Displayed Configuration OTS_conf	a	Set to active	t. 🗶 Import.
Active Configuration OTS_conf	ig .		
General System Setup Colu	mns		
Agilent ICF System BDAL Demo Lc-System		Buker OTOF MS	Settings
BDAL Demo Lc-Pump BDAL Demo Lc-Autosampler Bruker OTOF MS Bruker Ion Trap MS Bruker FTICR MS Bruker Gag MS	🕂 add	micrOTOF-Q III SN 8228888.20359 time offset (sec.) 0	
MS simulation Bruker nanoElute BDall RS232 Event	down		
G	eate a new config	uration	
New 1	Open	Save As Report	Close



3. Mark "Agilent ICF System" and click "add".

Displayed Configuration Active Configuration OTS_c	onfig	Set to ad
General System Setup C Aglent ICF System BDAL Demo Lc-System BDAL Demo Lc-Pump BDAL Demo Lc-Autosampler Bruker TOF MS Bruker FICR MS Bruker FICR MS Bruker TARG MS Br	Add selected Plug-1	In module to configuration.

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

Configuration Editor*					23	
Displayed Configuration			Set to active	🟦 Export. 🛃	Import	
Active Configuration Ever						
General System Setup	Columns				1	
BDAL Demo Lc-System BDAL Demo Lc-Pump		Agient ICI	F System	😈 Sett	ings	
BDAL Demo Lo-Autosampler	+ add			1.2		
Hardware Configuration D	ialog					
 Aglert RC.Het SDK Seney CTC Analytics RC.Let SDK Seney Excesso Drivers Aglert 100/1200/1260/ CTC Analytics RAL at Aglert 100/1201/1260 CTC Analytics RAL at Aglert 1120/1220 LC Sys CTC Analytics GC Aglert 7100 CE 	1290 LC			3 × Auto Configure		
		4	Configure Evosep One Settings			
			Name	Evosep One		
			Pumps address Pal address	172.17.17.1		4
			Serial Number	S00016		en Configure Clear
f you have configured a 'Get Pump List" button a	system with mo and define the m	re than one sin pump fo	Auto Idle-flow	6		7
			Help		OK Canor	el OK Cancel

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

Displayed Configuration			Set to active	🟦 Export 🖳 Import
Active Configuration OTS_c	onfig			
General System Setup C	olumns			
Aglent ICF System BDAL Demo Lc-System		Aglent ICF System		Settings
BDAL Demo Lo-Pump BDAL Demo Lo-Autosampler	+ add	SAMPLER EVOSEP_C	INE-	
Bruker OTOF MS Bruker IonTrap MS Bruker FTICR MS	1 up	Bruker OTOF MS		Settings
Bruker (ap.) MS MS aimulation Bruker nano Bute BDel RS232 Event	remove			
New	Cpen	Save Save	As	Gose

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

Configuration Editor* Displayed Configuration Active Configuration Active Configuration OTS_config	Export.		X
General System State Colum	Setings	msControl detected Auto detect / connect to MS (press 'Auto detect' button to update the request instrument information) MS Instrument Information Name micr0T0F-Q III Ser. No. 8228888.20359 Type otof-series Station ID 1 Server Host localhost	be
New Doen Save Save As Report	Close	OK Cance	*I

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

Active Configuration OTS config		
Active Comparation 015_comp		
General System Setup Columns	12 Save Configuration	
Aglent ICF System BDAL Demo Lo-System	File name	Date modified
BDAL Demo Lo-Pump BDAL Demo Lo-Autosampler	add 270318TestConf	3/27/2018 3:01
Bruker OTOF MS	290318_Conf_with_Evosep_One	3/29/2018 2:11
Bruker IonTrap MS	up ARGON_MU1	9/18/2017 5:10
Bruker FTICR MS Bruker GoQ MS	CaptiveSpray_TOF_nanoEute	9/25/2015 9:20
MS simulation	CaptiveSpray_TOF_nanoEute_160307	7/21/2016 9:10
Bruker nanoEute BDal RS232 Event	down CaptiveSpray_TOF_nanoEute150928	9/28/2015 9:50
	CaptiveSpray_TOF_nanoEute150928_HT	10/5/2015 10:3
	remove CaptiveSpray_TOF_nanoEute150928_LT	10/5/2015 11:5
	CaptiveSpray_TOF_nanoEute150928_Toff	10/5/2015 11:4
	CaptiveSpray_TOF_nanoEute151217	12/17/2015 11:
	CaptiveSpray_TOF_nanoEute160120	1/20/2016 10:3
	configuration	3/19/2018 2:18
	CTC_ICF	11/21/2017 9:1
	· [
	Selected Name	
	Evosep One micrOTOF	
	L	ОК Са

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

Displayed Configuration Evosep Active Configuration OTS_cc		Set to ective	Export	
General System Setup Co	-			
Agtent ICF System BDAL Demo Lo-System		Aglent ICF System	Settings	
BDAL Demo Lo-Pump BDAL Demo Lo-Autosampler	+ add	SAMPLER EVOSEP_ONE-		
Bruker OTOF MS Bruker Ion Trap MS Bruker FTICB MS	tup 🛔	Bruker OTOF MS	Settings	Compass HyStar
Bruker FTICH MS Bruker QqQ MS MS simulation		micrOTOF-Q III SN:8228888.20359 time of	offset (sec.) 0	
Bruker nano-Bute BDal RS232 Event	remove			The configuration has been changed. In order to update the 'Active Configuration' HyStar will restart now.
				ок
New New	Open	Save As Report	Close	

🖉 Configuration Editor Make the displayed configuration active.



- 9. If upgrading from an earlier ICF plugin, carefully check all the HyStar hardware profiles (using ICF) and recreate if needed.
- 4.3.4 Create Evosep separation methods
- 1. Create Evosep One separation methods by clicking "Method Set", then set Injection method to "Standard" and then click the small pencil to edit the separation method.

			Method Set Editor. Method Set:	New		
🔣 Compass HyStar 4.1			Separation Method			
Navigator Acquisition	View Options Compa	ss H			U	
Compass Met	hod Set 📰 Sample Tab	ole	Injection Method	Standard	•	/ Edit
Instruments	Open the Method Set I	ditor	MS Method			= ×
HyStar	disconnected	Bru	Scheduled Precursor List (SPL)		1	×
disconnected		d)				
	Evosep One		Processing Method			= ×
Connect all instruments	Offline					

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

Separation Method	ICF System Method Dialog
Separation Method Name:	ICF Method Auxiliary Traces
Hardware Modules	Name 100 samples per day 🗸
Total Runtime 5.00 min Available Modules	Description 11.5 min acquisition time
Agilent ICF System	Missing method data Runtime 11.5 min
Acquisition Time	
Start time 0.00 min	

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

Throughput	Gradient Length	
Samples/day	Minutes	Separation Method
300	3.2	Separation Method Name
200	5.6	Hardware Modul
100	11.5	Total Runtime 11.50 min Available Mod Agilent ICF S
60	21	Edit acquisition time Acquisition Time
30	44	Start time 0.00 min Acquisition time range 5.00 min

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

paration Method			
eparation Method	Name: 100 samples	per day	
		Hardware Modules	
Total Runtin	ne 11.50 r	nin Available Modules	
		Agilent ICF System	Edit Method
Edit acquisiti	on time		
Acquisition Time			
Start time	0.00 r	nin	
Acquisition time range	11.50 r	nin	
	11.50 r	nin	

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

🖉 Method Set Editor. Method Set:	New	- • •
Separation Method	100 samples per day	= 🗾
Injection Method	Standard	•
MS Method		
Scheduled Precursor List (SPL)		= ×
Processing Method		
Comment:		*
		*
,	New Gpen Save As Proport	OK Cancel
Intens.		Save and close

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

osep 100 smo per day			
	3/27/2018 2:34 PM	File folder	
Bruker-100_270318.m	3/27/2018 3:14 PM	File folder	
100 Samples Evosep One.m	3/29/2018 2:15 PM	File folder	
P.m	4/6/2018 3:11 PM	File folder	
	100 Samples Evosep One.m	IP.m 4/6/2018 3.31 PM Image: 100 Samples Evosep One.m 3/29/2018 2.15 PM Image: 100 Samples Evosep One.m 3/29/2018 2.15 PM Image: 100 Samples Evosep One.m 3/27/2018 3.14 PM Image: 100 Samples Evosep One.m 3/27/2018 2.34 PM	IP.m 4/6/2018 3:31 PM File folder International Distribution 3/29/2018 2:15 PM File folder Bruker-100_270318.m 3/27/2018 3:31 PM File folder GeometryFiles 3/27/2018 2:34 PM File folder

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



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

🔣 Compass HyStar 4.1 -	[Compass]		
Navigator Acquisition	View Options Comp	ass Help	
😭 Compass 📄 Meth	nod Set 🔤 Sample Ta	able 🗛 Acquisition	👁 Quick Da
Instruments	Ō	pen the Sample Table B	ditor (Offline)
HyStar	disconnected	Bruker OTOF MS	
disconnected		disconnected	
	Evosep One		
	Offline		
Connect all instruments	1	micrOTOF-Q III	
	HP Pressure bar	I	

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

(Tray Config	guration	- • •	Tray	Configuration	- • ×
		Evosep	-	Tray type	e: Evosep	•
	/	Unknown Agilent 100 Vial Holder Agilent Multisampler Holder		 Slot 1:	96Evotip 👻	
		Aglier Maubashipel Houle Agliert Val Holder CTC-PAL3 Petter Stack CTC-PAL3 Tray Holder CTC-PALXT Cooled Tray CTC-PALXT MR V3800 CTC-PALXT MR V3800 CTC-PALXT MT Holder		 Slot 2:	96Evotp none 96Evotp	
	0	CTC-PALXT Peltier Stack		Slot 3:	96Evotip 👻	
		EVOSEP		Slot 4:	96Evotp 🔹	
				Slot 5:	96Evotip 💌	
				Slot 6:	96Evotip 🔹	
		0	K Cancel			OK Cancel

- 3. In the sample table line 1 set following:
 - a. Vial: S1-A1
 - b. Sample ID: test
 - c. Method Set: Click the small arrow and check mark "Use individual Methods"
 - d. Separation Method: choose Evosep 100 samples per day

⊙ S'	Ľ.				O S2				O 53					O 54	
Î N	ew 🕶	📥 Open	💥 Delete	Save	Save .	As	Report -	Import/Export •	Op	tions •					
	Line	Vial	Status	Sample ID	Inj.	Volume [µl]		Data Path		Met	hod Set	Separation Meth	nod	Injection Met	MS Method
•	✓1	S1-A1	*	test	1	0	D:\Data\		~			100 samples per day		-	
			•			0			-	0	pen		-	•	
										✓ U:	e Individu	al Methods			
										Ev	osep 60 sn	np per day.m			
												mp per day.m			

- 4. Now click Save As... and set name as "Evosep One Sample table" and then click ok to save the sample table.
- 5. Click Close to close the Sample Table Editor window.



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

4.4 Evosep One driver for SCIEX OS

4.4.1 Installation

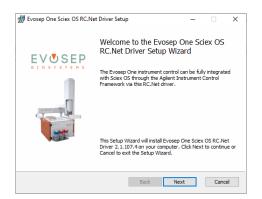
Prerequisites:

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

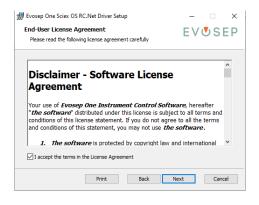
Services (Local)							
Clearcore2 Service	Name	Status	Startup Type	Description			
	Clearcore2 Service	Running	Manual	Required for Mass Spectrometer acquisition.			
Stop the service Restart the service	🔍 Client License Servic		Manual (Trigger Start)	Provides infrastructure support for the Microso			
Restart the service	CNC K	n	M	The ONO loss inclusion and in the based in the l			

Installation procedure:

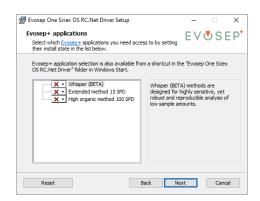
- 1. Connect the Evosep One instrument to the computer via ethernet cable, and make sure that the instrument is switched on.
- 2. Run the Evosep One SCIEX OS RC.Net Driver 2.x.x.x Windows installer.
- 3. Click "Next".



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



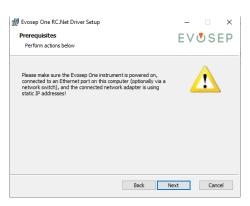
5. In the Evosep+ applications window, click "Next".



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

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

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





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



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

B Evosep One RC.Net Driver Setup		Evosep One RC.Net Driver Setup
Installing Evosep One RC.Net Driver	EVঊSEP	Installing Evosep One RC.Net Driver
Please wat while the Se Status: Statu		
Back Next	Cancel	Hide details Yrg No Change when these notifications appear

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

Evosep One Network Gateway	→ ×
INIT : OK	Ver. 1.603
FIRMWARE : OK	
PROJECT : OK	

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



🛃 Evosep One Sciex OS RC.Ne	t Driver Setup	-		×
EVÖSEP	Completed the Evosep RC.Net Driver Setup Wi Click the Finish button to exit the S	izard		
	Back	inish	Cano	el

4.4.2 Create SCIEX OS hardware configuration for Evosep One

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



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



- 4. Click the "Add" button on the toolbar.
- 5. On the "Device" dialog, select "Integrated System" and "Agilent Integrated System" and click "Settings...".

Add

Devic	ce 🔀
Select th	ne device and then adjust the communication settings to test the device.
Туре	Integrated System 👻
Model	Agilent Integrated System 💙 Settings
Test Dev	vice
	Save <u>C</u> ancel

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

Settings			X
Device Dr	iver		
Name:	Agilent Integrated System		
Version:	1.0.0.0		
Manufacturer:	Agilent		
Simulate Dev			
Agilent Settin	igs		
Communication			
IP Address	172 • 17 •	17 . 1	Auto Configure
Configuration -			
Available Devices	5		Selected Devices
Iso. Pump		*	Sampler 1: Evosep One[EVOSEP_ONE:n/a]
Bin. Pump Quat. Pump			
Low Flow Pump)		
Prep. Pump Prep. Pump Clu	ster		
Column Comp. Sampler		Add >	•
Low Flow Samp	ler	Add	
HiP Sampler Low Flow HiP S	omplor	< Remove	
Prep. Sampler	ampier	Configure	
DAD			·
Restore Defaults			Test Device <u>C</u> ancel

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

Configuration		_		×
Settings				
Name	Evosep One			
Pumps address	172.17.17.1			
Pal address	172.17.17.2			
Auto idle-flow				
Simulation mode				
	<u>о</u> к	<u>C</u>	ancel	

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

Test Device

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

Device			×
Adjust the con	nmunication parameters and	then test the device	».
Type Integ	rated System	×	
Model Agile	nt Integrated System	V Settings.	
Test Device	The test was successful.		
Device Display	Names		
Integrated Syste	m Evosep One		
: Autosamp	ler - Evosep One		
Ê			
		Save	Cancel

- 12. Click "Save".
- 13. Click "Activate Devices".

Activate Devices

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

K 🖉 Stopped	? - 🗗 X
Projects	
Default	✓ (+)
In Root: C:\SCIEX OS Data	
Queue	
Acquisition samples waiting: Acquisition sample time remaining: Acquisition queue time remaining:	0 0d 0h 0m 0s 0d 0h 0m 0s
Devices	
Agilent ICF	🕕 🙂
Evosep One	٧
ZenoTOF™ 7600 System (simulation)	👬 😃
Calibrant Delivery System	ம
MS Check	+-
Direct Control	
Standby	O Equilibrate



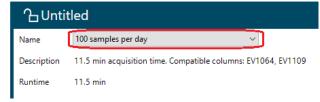
- 4.4.3 Create SCIEX OS LC methods for Evosep One
- 1. In the top-left dropdown menu, click "LC Method"



2. Click "New"

New

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



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



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



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

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

4.5 Evosep One driver for Thermo Chromeleon / Xcalibur

4.5.1 Chromeleon vs SII for Xcalibur

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

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

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

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

4.5.2 Compatible software:

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

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

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

4.5.3 Prerequisites:

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



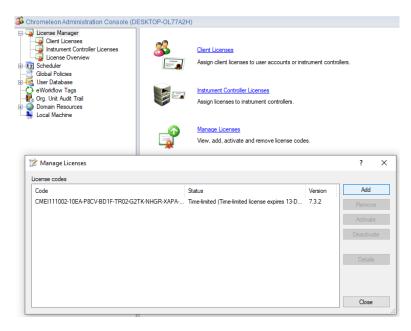
- b. Thermo SII for Xcalibur 1.7 or newer is installed.
- E. Close Chromeleon and/or Xcalibur, if running.
- F. Open Chromeleon Services Manager and click "Stop Instrument Controller", if running.

🎭 Chromeleon Services Manager	? ×
Instrument Controller Service	Stop Instrument Controller Saft service on system start Configure instruments
Other Chromeleon services All other Chromeleon services are running.	

4.5.4 Chromeleon/SII for Xcalibur license

License can be added through the Chromeleon Administration Console.

Click "Manage Licenses" and "Add" the license key:



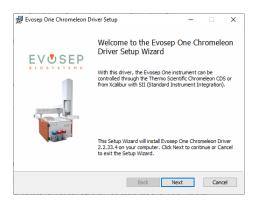
Under "Instrument Controller Licenses", make sure that at least one Class 3 instrument is allowed:

Giense Manager Gient Licenses Instrument Controller Licenses		Assign licenses to instrumer		nses			
License Overview		Drag a column header here to	group by that col	lumn.			
Global Policies		Instrument Controller	Status	Instrument Class 1	Instrument Class 2	Instrument Class 3	A
📲 💀 🖓 🖓 🖓 🖓		DESKTOP-OL77A2H	🗸 ОК	0 🗸	0 🗸	1 🗸	
Oomain Resources Local Machine	-	*		•	-	-	



4.5.5 Installation procedure:

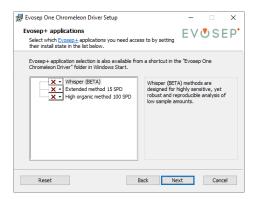
- 29. Connect the Evosep One instrument to the computer via ethernet cable, and make sure that the instrument is switched on.
- 30. Run the Evosep One Chromeleon Driver 2.x.x.x Windows installer.
- 31. Click "Next".



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



33. In the Evosep+ applications window, click "Next".

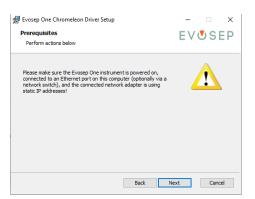




The Evosep+ applications are not installed during a standard installation, because Evosep prefers that the customer actively chooses the Evosep+ applications they need.

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

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



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



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

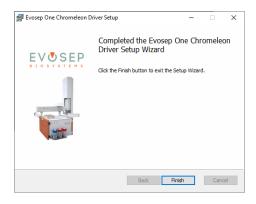
18 Evosep One RC.Net Driver Setup		Evosep One RC.Net Driver Setup	
Installing Evosep One RC.Net Driver	EVঊSEP	Installing Evosep One RC.Net Driver	EVॵSEP
Please wait while the Se Please wait wait wait wait wait wait wait wait		software on this	allow the following program to install computer?
Status: Program name: Verified publisher: Show det/ ¹ / ₂ s		e Reference Refe	me: Evosep One Distance Twosep App Status The State State State State State State State State State State State State State State Dires 12.02 Amis mation about this publisher's certificate
		Hide details	Yrs No
Back Next	Cancel		Change when these notifications appear



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

Ver. 1.004

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



4.5.6 Checking driver installation

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

4.5.7 Create Chromeleon hardware configuration for Evosep One10. From the Chromeleon Services Manager, click "Start Instrument Controller".

🎭 Chromeleon Services Manager	? ×
Instrument Controller Service	Start Instrument Controller Start Instrument Controller Start service on system start Configure instruments
Other Chromeleon services All other Chromeleon services are running.	

11. Click "Configure instruments"



🎭 Chromeleon Services Manager	? ×
Instrument Controller Service	Stop Instrument Controller
	Start service on system start Configure instruments
Other Chromeleon services	
 All other Chromeleon services are running. 	

12. Right-click the controller instance and click "Add Instrument...".

🌯 Instru	umen	t Configuration - Chromeleon Instrument Configu	ration Man
File Edi	it Vi	ew Controller Help	
🗃 🖪	χı	da 🖻 📍 🗏 🧏 🖋 🗽 🗸 🕸	>
		How to	
		Undo	Ctrl+Z
		Cut	Ctrl+X
		Сору	Ctrl+C
		Paste	Ctrl+V
		Delete	
		Disconnect	
		Add Instrument	
		Add Sharable Interface	
		Add Module	
		Show Instrument Configuration Audit Trail	
		Rename	F2
		Properties	Enter

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

Ø	How to	
	Undo	Ctrl+Z
	Cut	Ctrl+X
	Сору	Ctrl+C
	Paste	Ctrl+V
	Delete	
	Disconnect	
	Add Instrument	
	Add Sharable Interface	
	Add Module	
	Show Instrument Configuration Audit Trail	
	Rename	F2
	Properties	Enter

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

🎒 Add module to instrument	×
Instrument JPLAPTOP_1	
Manufacturers:	Modules:
Extere Blogstems PAte Giton PAte Giton HP Ison Kontron Matel Matel Matel Romr Polymer Laboratories Ramin Recogne Simodou Sinodex SoftA (Matel) TGE cuppation Valoo Valion	Evosep One
	Cancel

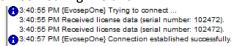


16. On the Evosep One Driver Configuration dialog, you can set the basic instrument settings:

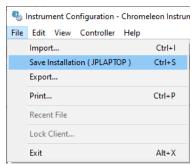
Device Configuration	
bottoo oottigaaaton	
Device Name: EvosepOne	
Pumps Address 172.17.17.1	
Pal Address 172.17.17.2	
Auto Idle-flow	
Simulation mode	
OK Cancel	

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

The message window should show something like this:



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



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

4.5.8 Configuring Thermo Scientific SII for Xcalibur

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

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





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

· ·	
🎇 Thermo Foundation Instrument Configuration	×
Device Types : All	
Available Devices:	Configured Devices:
Themo Scientific SII for Xcalbur	Themo Scientific SII for Xealibur
Add >>	<< Remove Configure
Done	Help

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

SII for Xcalibur (Configuration	×
	<u>C</u> onfigure Device	
	Selected instrument system:	
	JPLAPTOP_1 Controlled by <u>e</u> xternal autosamp	bler
ОК	Cancel	Help

10. Close the window that opened, then click "OK".

(This may seem unnecessary, but if this step is not performed, the configuration will not be initialized correctly!)

11. Back in the "Thermo Foundation Instrument Configuration" window, click "Done".

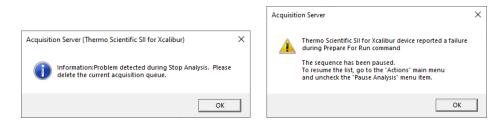
4.5.9 Important - When upgrading from earlier driver version

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

🏩 Services						- 🗆	×
File Action View	Help						
⊨ → 🗖 🖨 🧕) 🔒 🛛 🖬 🕨 🔳 💵 🕨						
🧟 Services (Local)	Services (Local)						
	Thermo Foundation Acquisition	Name	Description	Status	Startup Type	Log On As	1
	Service	Carteria Foundation Acquisition Service	Manages acquisition queue and performs sample ac	Running	Automatic	.\Xcalibur_System	
	Stop the service	🆏 Thermo Foundation Acquisition Service Monitor	Monitors the acquisition service operations	Running	Automatic	.\Xcalibur_System	÷.,
	Pause the service	🆏 Thermo Foundation Auto Log Off Service	Manages and implements autologoff configuration	Running	Automatic	Local System	
	Restart the service	🆏 Thermo Foundation Database Service	Manages and implements database configuration a	Running	Automatic	Local System	
		🌼 Thermo Foundation Device Message Dispatcher	Thermo Device Message Dispatcher	Running	Automatic	Local System	
	Description:	🆏 Thermo Foundation Enterprise Service	Manages VI Extensions and Enterprise Data Acquisiti	Running	Automatic	.\Xcalibur_System	
	Manages acquisition gueue and	🆏 Thermo Foundation Message Listener Service	Manges centralized logging and dispatch	Running	Automatic	Local System	
	performs sample acquisitions.	🎑 Thermo Foundation Security Service	Manages and implements security configuration an	Running	Automatic	Local System	



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



4.5.10 Create LC methods for Evosep One

This section describes how to create LC methods through Xcalibur.

1. In Xcalibur, click "Instrument Setup".



2. Click "Next"

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

	io Xcalibur Instrument Setup –		×
File SII Xcalibur			
	<u>X</u> ?		
Thermo Scientific SII for	General Settings for System.	200000000000000000000000000000000000000	6
	Select diagnostic channels to be used: No Channel Select all channels		
	Deselect all channels		
Ready	< Back Net > Cancel	Help	

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

	11.5 min Channel Pump_A_Displacement Pump_A_Actual_flow Pump_A_Setpoint Pump_A_Pressure Pump_A	atible columns: EV10	964, EV1109	
• •	Channel Pump_A_Displacement Pump_A_Actual_flow Pump_A_Pressure			
	Pump_A_Actual_flow Pump_A_Setpoint Pump_A_Pressure			
שושושו	Pump_A_Setpoint Pump_A_Pressure			
	Pump_A_Pressure			
5				
	Pump_A_Pump_speed			
	Pump_B_Displacement			
	Pump_B_Actual_flow Pump_B_Setpoint			
	Pump_B_Setpoint			
	Pump_B_Pressure			
	Pump_C_Displacement			
	Pump_C_Actual_flow			
	Pump_C_Setpoint			
	Pump_C_Pressure			
	Pump_C_Pump_speed			
	Pump D Displacement			
	Pump D Actual flow			
	Pump D Setpoint			
	Pump D Pressure			
	Pump D Pump speed			
	Pump HP Displacement			
	Pump_HP_Actual_flow			
	Pump_HP_Setpoint			
	Pump HP Pressure			
	Pump_HP_Pump_speed			

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

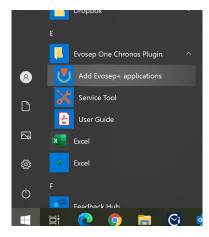
4.6 Adding specialized applications to the Evosep One

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

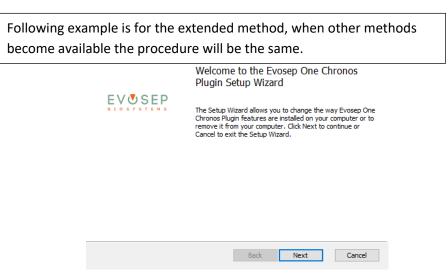
- 1. Close the Evosep One control software (Chronos/HyStar)
 - 2. Depending on what software is being used on the Evosep One, choose the appropriate folder from

the Windows Start menu. I will be one of:

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



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



5. Click the Extended method icon.

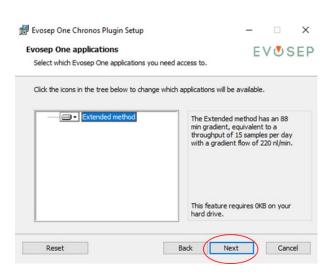


🖶 Evosep One Chronos Plugin Setup	- 🗆 X
Evosep One applications	EVÖSEP
Select which Evosep One applications you r	leed access to.
Click the icons in the tree below to change	which applications will be available.
Extended method	The Extended method has an 88 min gradient, equivalent to a throughput of 15 samples per day
	with a gradient flow of 220 nl/min.
	This feature frees up 32KB on your hard drive.
Reset	Back Next Cancel

6. Choose "Will be installed on local hard drive".

🛃 Evosep	One Chronos Plugin Setup	– 🗆 X
	One applications hich Evosep One applications you need access to.	EVঊSEP
Click the	icons in the tree below to change which applications will b	be available.
	X - Extended method The Extended	d method has an 88
	Will be installed on local hard drive	to a
	Entire feature will be installed on local hard d	rive 20 nl/min.
	× Entire feature will be unavailable	
	This feature hard drive,	frees up 32KB on your
Res	et Back Ne	ext Cancel

7. Click Next.



8. Click Change.



	igin Setup	- 0
Ready to change Evos	ep One Chronos Plugin	EVঊS
Click Change to begin the installation settings. Click (installation. Click Back to review or Cancel to exit the wizard.	change any of your
	Back	hange Cance
😸 Evosep One Chronos Plu	gin Setup	
伊 Evosep One Chronos Plu	^{gin Setup} Completed the Evose Plugin Setup Wizard	
Evosep One Chronos Plu	Completed the Evos	ep One Chronos
	Completed the Evos Plugin Setup Wizard	ep One Chronos
	Completed the Evos Plugin Setup Wizard	ep One Chronos
	Completed the Evos Plugin Setup Wizard	ep One Chronos
	Completed the Evos Plugin Setup Wizard	ep One Chronos

- 10. Allow the installation procedure to finalize.
- 11. Start the Evosep One control software.

9. Click Finish.

12. The Extended method is now available in the method directory in Chronos.

· → • ↑ 📙·	« Evosep(One > Templates > Generic	~	5	Search Generic		م
Organize 🔻 New	folder						
🗦 Dropbox	^ N	ame	Da	te mo	odified	Туре	
OneDrive	1	30 SPD (44min, 15cm)	202	20-03	-26 08:43	Chrono	s Analysis
- OneDrive	1	0 SPD (21min, 8cm)	202	20-03	-26 08:43	Chrono	s Analysis
This PC	1	100 SPD (11.5min, 8cm)	202	20-03	-26 08:43	Chrono	s Analysis
3D Objects	1	200 SPD (5.6min, 4cm)	202	20-03	-26 08:43	Chrono	s Analysis
Desktop	1	300 SPD (3.2min, 4cm)	202	20-03	-26 08:43	Chrono	s Analysis
Documents		Extended method (88min, 15cm, 15SPD)	202	20-04	-30 05:57	Chrono	s Analysis
Downloads							
Music							
E Pictures							
Videos							
🛀 OS (C:)	11. see					_	
	~ <						
	File name:	Prepare		~	Analysis meth	ods (*.can	r) ~

13. For Compass HyStar go to the previous section of the User guide "Create evosep separation methods"

and follow the description for creating a separation method for the 88 min Extended method.

🖉 Method Set E	ditor. Method	Set: Nev	N	
Separation M	ethod			
Separation Method				
Total Runtim		min	Hardware Modules Available Modules Agilent ICF System Edit Method	
Acquisition Time	0.00	min	ICF System Method Dialog	
Acquisition time range Stop time	88.00 88.00	min] min	Name Extended method v Description 88 min acquisition time, 15 cm column, 15 samples per day Runtime 88.0 min	
		_		



5 Instrument Software Control

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

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

🕐 Open Method			×
← → 🔺 🕇 😽 er Plu	igins » EvosepOne » Templates	✓ ♂ Search Temp	olates 🔎
Organize 🔻 New folde	er		::: • 💷 ?
Templates	Name	Date modified	Туре
User Guide	Analyst	2020-04-28 14:16	File folder
OneDrive	Generic	2020-04-28 14:16	File folder
_		2020-04-28 14:16	File folder
This PC	Xcalibur	2020-04-28 14:16	File folder
3D Objects	Calibrate	2020-03-26 08:43	Chronos Analysis M
Desktop	Diagnose	2020-03-26 08:43	Chronos Analysis M
Documents	Prepare	2020-03-26 08:43	Chronos Analysis M
🖶 Downloads	Service	2020-03-26 08:43	Chronos Analysis M
Music	System and column wash	2020-03-26 08:43	Chronos Analysis M
Pictures			
Videos			
🛀 OS (C:) 🗸	<		>
File na	ame: Prepare	V Analysis me	thods (*.cam)
		Open	Cancel

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

5.1.1 Running samples

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

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



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

5.1.2 Methods

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

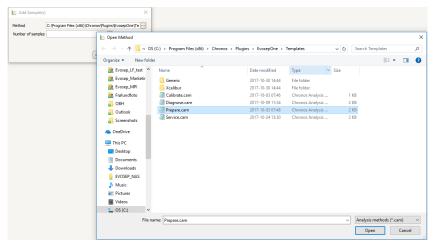
5.1.3 Sample lists

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

1. Click the Add button

Main menu	Sample	Analysis Method
	유 Add	1
Sample list		
<i>(</i>)	Remove	

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



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

🙋 Add Sample(s)	×
Method Number of samples	Prepare
	<u>A</u> dd <u>C</u> lose

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

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

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

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

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

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

Analysis Method 🔍	Source Tray	Source Vial
eq:c:Program Files (x86) Chronos Plugins Evosep One Templates System and column wash.cam	EvoSlot 1	1

For Diagnose methods, select subsystem test, e.g. "Pump HP" and "HP system" method by using

the checkboxes .						
Analysis Method	P	ump HP	Pump A-D	Restriction*	Tip seal*	HP system*
C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Diagnose.ca	m					

For Calibrate method; select "Flow sensor" using the check box

	Analysis Method	Flow sensor ABCD	Flow sensor HP	Loop volume*
1	C:\Prote.cam			

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



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

For Service methods, select one of the options.

Analysis Method	Set valve 6 pos 2-3	Syringe pumps	Drain pumps	Autosampler torque test	Loop flush*	Contact closure test*	Flow to column - 20% sol B^{\ast}
1 C:\Program Files (\Service.cam		none					

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

5.1.4 Creating a schedule

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

Schedule
Overlapped
Priority
🗌 Infinite loop
Create

5.1.5 Running a schedule

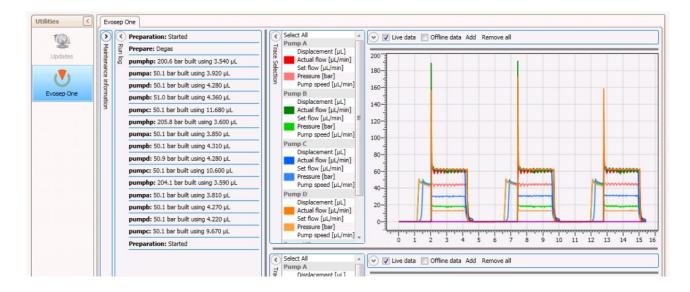
The schedule is now transferred to the schedule queue in the "Schedules and run control" section of the main menu.

Main menu <	Schedules	Run log Diagram Schedule timetable Sample status
Sample list	Execution Start queue	Demonstration Version
a	Stop	Schedule 1
Schedules and run control	Queue	
1	Schedule 1 queued	
Method editor		
1		
Settings		
<u></u>		
Activation		
		e N
		99 2011 19
		S S S S S S S S S S S S S S S S S S S
	Selected schedule	
	Start: 2017-11-02.14:11:07	
	End: 2017-11-02 14:11:08 Remaining: 00:00:01	
		0:00
	© ③ Al schedules Start: 2017-11-02 14:11:07	Runtime [min]
	End: 2017-11-02 14:11:08	Sample list line
	Remaining: 00:00:01	Analysis Method. Set valve 6. Pumps 1. Service True none
	Move up	
	Move down	Run status
	Remove	Autosampler status: Next action in:
Main menu	Clean up	Runtime: 00:00:00 Currently running:
Utilities		
My menu	Evosep One: not connected	100 %

The sample queue is started by pressing the "Start queue" button.

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

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





5.1.6 Aborting samples

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

Sample list Image: Constraint of the schedule of	Main menu 🔇	Schedules	Run log Diagram
Schedules and run control Stop 2017-11-06 103:143 Wethod edicor Schedule 1 done 2017-11-06 103:143 Schedule 2 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 3 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 4 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 5 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 6 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 7 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 9 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 5 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 6 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 6 done 2017-11-06 103:143 2017-11-06 103:143 Schedule 6 done 30en_100_new* done 30en_100_new* 2017-11-06 10:10-10 Schedule 6 done 33en_100_new* done 33en_100_new* done 30en_100_new* 30en_100_new* 30en_100_new* 30en_100_new* 30en_100_new* 30en_1	5 Sample list	Execution	2017-11-03 21:30:14 2017-11-03 21:33:52
Cueue 2017-11-06 10.31:40 Wethod odior Schedule 1 done Schedule 3 done Schedule 4 done Schedule 5 done Schedule 6 done Schedule 9 done Schedule 6 done Schedule 7 Schedule 6 Schedule 7 Schedule 7 Schedule 8 done Schedule 9 done Schedule 11 running Op new* done Schedule again for the remaining samples of this schedule Schedule 11 running On tot start any remaining samples at all Schedule 11 Schedule 11 running	ď		Stop 2017-11-06 10:31:26 2017-11-06 10:31:48 2017-11-06 10:31:48
Method editor Schedule 2 done 2017-11-06 104:00 Schedule 3 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 4 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 5 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 5 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 5 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 6 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 6 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 7 done 2017-11-06 104:00 2017-11-06 104:00 Schedule 9 done 30eq_100_new* done Schedule 9 done 30eq_100_new* done 3seq_100_new* done Schedule celecution control Image: Celecution control Schedule 11 running Celecution control Image: Celecution control Image: Celecution control Schedule 11 running Celecution control Image: Celecution control Image: Celecution control Schedule 11 running Celecution control Image: Celecution control Image: Celecution control Concel Schedule 11 running Celecution control Image:	Schedules and run control		2017-11-06 10:31:48 2017-11-06 10:31:49
Settings Schedule 5 done Schedule 5 done 2017-11-06 11:00:10 Schedule 6 done 2017-11-06 11:00:10 Schedule 9 done 3seq_100_new* done 3seq_100_new* done 3seq_100_new* done 3seq_100_new* done © Abort complete run immediately 3seq_100_new* done © On to start remaining samples of this schedule Schedule 11 running Cancel Schedule 11 running Cancel	Method editor	Schedule 2 done	2017-11-06 10:31:50 2017-11-06 10:46:03 2017-11-06 10:46:04
Activision Activisio Activision Activision Activision Activision Activision A		Schedule 5 done	2017-11-06 10:46:04 2017-11-06 10:46:04 2017-11-06 10:46:04 2017-11-06 11:00:09
Activation 3seq_100_new* done 3seq_100_new* done	9	Schedule 9 done	2017-11-06 11:00:14
3seq_100_new* done 3seq_100_new* done Schedule 11 running Ob not start any remaining samples at all Ok Cancel 2017-11-06 11:05:11 2017-11-06 11:05:11	Activation	3seq_100_new* done 3seq_100_new* done	22 © Abort complete run immediately 22 23
Schedule 11 running O Do not start any remaining samples at all OK Cancel 2017-11-06 11:05:11 2017-11-06 11:05:11		3seq_100_new* done	
			.6
2017-11-06 11:05:11			Cancel 6 21
			2017-11-06 11:05:11 2017-11-06 11:05:11 2017-11-06 11:05:12 2017-11-06 11:05:12

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

Main menu <	Schedules	
E	Execution	
Sample list	\triangleright	Start queue
		Stop
Schedules and run control	Queue	
	Schedule 1 done	
Method editor	Schedule 2 done	
Settings	Schedule 3 done Schedule 4 done Schedule 5 done Schedule 6 done	
A	Schedule 9 done 3seq_100_new* done	
Activation	3seq_100_new* done	
	Schedule 11 failed	

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

NOTE: Please be aware of the following:

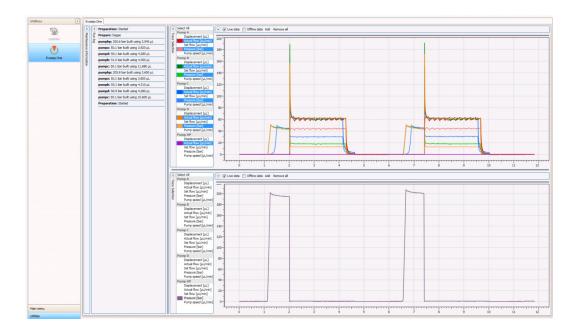
• If a failed schedule is not removed from the queue and new ones are entered below, Chronos will start by running the failed schedule before proceeding with the newly entered schedules.



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

5.1.7 Looking at graphs

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



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

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

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

Tips and tricks

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

Select Folder Crganize → New folder BusinessLogic ^ Diagnostics,201	PC > System (C:) > ProgramData > Evo	isep > EvosepOne > log:	\$ >	v ♂ Search	× م
← → ← ↑ → This Organize ← New folder One BusinessLogic ^		isep > EvosepOne > logi	\$ >	✓ Ŏ Search	logs ,O
Organize New folder					
One BusinessLogic ^	N				
BusinessLogic	A4				80 - 00
Diagnostics_201	Name	Date modified	Туре	Size	
	Diagnostics_2017-11-27_14-37-12	11/27/2017 2:37 PM	File folder		
Service_2017-11-	Diagnostics_2017-11-27_14-27-10	11/27/2017 2:27 PM	File folder		
Templates	Diagnostics_2017-11-27_14-14-26	11/27/2017 2:14 PM	File folder		
-	Diagnostics_2017-11-27_14-00-23	11/27/2017 2:00 PM	File folder		
🐉 Dropbox	Diagnostics_2017-11-27_13-45-59	11/27/2017 1:45 PM	File folder		
Evosep	Diagnostics_2017-11-27_13-35-46	11/27/2017 1:35 PM	File folder		
🚒 Evosep_Marketir	Diagnostics_2017-11-27_13-22-11	11/27/2017 1:22 PM			
	Diagnostics_2017-11-27_13-10-05	11/27/2017 1:10 PM			
ConeDrive	Diagnostics_2017-11-27_12-51-58	11/27/2017 12:51	File folder		
This PC	Diagnostics_2017-11-27_11-19-03	11/27/2017 11:19	File folder		
Desktop	Diagnostics_2017-11-27_11-04-22	11/27/2017 11:04	File folder		
Documents	Diagnostics_2017-11-27_10-54-55	11/27/2017 10:54	File folder		
	Diagnostics_2017-11-27_10-46-09	11/27/2017 10:46	File folder		
Downloads	Diagnostics_2017-11-27_10-38-08	11/27/2017 10:38	File folder		
) Music	Diagnostics_2017-11-27_10-21-48	11/27/2017 10:21	File folder		
Pictures	Diagnostics_2017-11-27_10-09-00	11/27/2017 10:09	File folder		
Videos	Diagnostics_2017-11-27_09-57-49	11/27/2017 9:57 AM			
System (C:)	Diagnostics_2017-11-27_09-47-59	11/27/2017 9:47 AM			
- Data (Da)	Diagnostics_2017-11-27_09-33-39	11/27/2017 9:33 AM	File folder		
Folder:	"Diagnostics_2017-11-27_13-22-11" "Diagno	ostics_2017-11-27_14-14-26	"Diagnostics_201	7-11-27_13-35-46*	
				61.11	
				Select F	Folder Cancel
	4				

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

Utilities	Evosep One	
Y		
Xcalibur reset	✓ Diagnostics_2017-11-27_14-14-26	х
Acdibur reset	✓ Diagnostics_2017-11-27_13-35-46	х
Updates	✓ Diagnostics_2017-11-27_13-22-11	x
Evosep One	Select All 64	

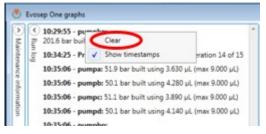
It is also possible to use drag-n-drop from Windows File Explorer to the Offline data section, as an alternative way to load the data.



5.1.8 Run log

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

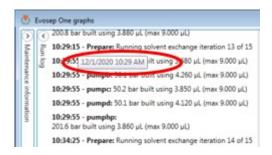
The run log can be flushed, by right clicking the log and pressing "clear".



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

۲	۲	10:29:55 - pumohan	
Ma	P	201.6 bar buil Clear	
inter	n log intenance inf	10:34:2 Pr 🗸 Show timestamps	station 14 of 19
anc		10:35:06 - pumpa: 51.9 car cont using 3.630) µL (max 9.000 µL)
e int		10:35:06 - pumpb: 50.1 bar built using 4.28	0 μL (max 9.000 μL)
0mm		10:35:06 - pumpe: 51.1 bar built using 3.890	μL (max 9.000 μL)
stio		10:35:06 - pumpd: 50.1 bar built using 4.14	0 µL (max 9.000 µL)

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



5.1.9 Maintenance information

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

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



- o Firmware version
- Displacement (total): Total pumped volume
- o Displacement (seal). Pumped volume since reset (if a pump seal was replaced)
- Instrument
 - $\circ \quad \text{Serial number} \quad$
 - $\circ \quad \text{Firmware version} \quad$
 - Analysis completed (by type)

ivosep One				
Software One: Evosep One Chror Robotic San Driver: 2.4.3 Firmware: 2.4.1				
One:	1.0.0.0			
Evosep One Chror	nos Plugin: 1.0.0.0			
🔿 Robotic San	ple Injection - RSI			
Driver: 2.4.3	(2.4.17311.1645)			
Firmware: 2.4.1	(2.4.17024.851)			
👻 Pump A				
✓ Pump B				
Pump D				
Pump HP				
Bus location:	3@172.17.17.1			
Product number:	1082			
Serial number:	7			
Firmware version:	44			
Type identifier:	0x0202			
Diagnostic codes:				
Displacement (tot	al): 43 mL			
Displacement (sea	al): 19 mL Reset			
Flow transform:	0, 1, 0, 0			
 instrument 				
Bus location:	2@172.17.17.1			
Product number:	1000			
Serial number:	19			
Firmware version:	16			
Type identifier:	0x0300			
Diagnostic codes:				
300 samples/day:				
200 samples/day:				
100 samples/day:				
60 samples/day:	0			
30 samples/day:				
Total analyses:	151			
Loop volume:	N/A µL			



5.1.10 How to import CSV files into Chronos

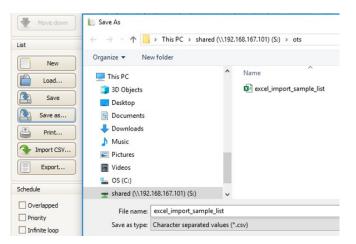
The general idea here is to use a mapping template to define the column matching between the CSV file you want to import, and the Chronos sample list columns. This example uses a CSV file saved from Chronos, but you can use any CSV file as source, as long as it contains the values to fill-in the columns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

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

n menu	Sample	Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur :
		1 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlat 1	6	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_6	
	💠 Add	2 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	7	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_7	
Sample list		3 C:\Program Files (x86)\Chronos\PI\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	8	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_8	
	Remove	4 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	9	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_9	
-		5 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	10	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_10	
and a second	Duplicate	6 C:\Program Files (x86)\Chronos\PI\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	11	C:\Thermo\Instruments\TSQ\Methods\11.SminLCM5_190219.meth	blank01_grad_11	
nedules and run control		7 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam		12	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	blank02_grad_12	
_	(Car none op	8 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam		13	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_13	
1	Move down	9 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	14	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_14	
		10 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	15	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_15	
Method editor	List	11 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	16	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_16	
	LIST	12 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	17	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_17	
¥=	New	13 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	18	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_18	
Settings	- Men	14 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvaSlot 1	19	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_19	
setungs	Load	15 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	20	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_20	
<u></u>	Save						
Activation	Save as	· · · · ·					
	Save as						
	Print						
	Timport CSV						

2. Then Click "Save as..." and choose "Character separated values" as type.

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



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



• O#	6	G .		2 2				
Home	Insert Draw	Page L	ayout	Formulas	Data	Review	w View	Help
	n Text/CSV	Recent	Sources		B	🔲 Que	ries & Connecti	ons
Fror	💶 Import Data							
Fror	← → ~ ↑	📜 = shar	ed (\\192.	168.167.101.	. ≥ ots	ڻ v	Search ots	
	Organize *	New folder						
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	3D Object	ts	a exc	el_import_sa	mple_list		6/28/20	19 10:0
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						Tools 🔹	Import	

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

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2 11	C:\Progra	m Files (x86	\Chronos\P	lugins\Evosep	One\Temp	lates\Xca	libur\Xcal	ibur 1 Evo	lot 1		16 C:\	Thermo	Instrumen	ts\TSQ\	Aethods\1	1.5minLCM	S_190219.meth	200fm	BSA 100	grad_16			C:\Xcal	ibur\meth	ods\6BSA	134 11 3G	.pmd
3 12	C:\Progra	m Files (x86	\Chronos\P	lugins\Evosep	One\Temp	lates\Xca	libur\Xcal	ibur 1 Evo	lot 1		17 C:\	Thermo	\Instrumer	ts\TSQ\/	Aethods\1	1.5minLCM	5_190219.meth	200fm	BSA_100	grad_17			C:\Xcal	ibur\meth	ods\6BSA	134_11_36	.pmd
				lugins\Evosep							18 C:\	Thermo	Instrumer	ts\TSQ\	Aethods\1	1.5minLCM	5_190219.meth	200fm	BSA_100	grad_18			C:\Xcal	ibur\meth	ods\6BSA	134_11_3G	.pmd
				lugins\Evosep													5_190219.meth		BSA 100							134 11 36	
				lugins\Evosep							20 C:\	Thermo	Instrumer	ts\TSQ\M	Aethods\1	1.5minLCM	5 190219.meth	200fm	BSA 100	grad 20			C:\Xcal	ibur\meth	ods\6BSA	134 11 36	.pmd

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

Save As					
← → • ↑ 🖡	> This PC > shared (\\1	92.1	68.167.101) (S:) > ots		
Organize • New	/ folder				
la OneDrive - Pers	onal	^	Name	Date modified	Туре
S This PC			excel_import_sample_list	6/28/2019 10:05	Microsof
3D Objects					
E Desktop					
Documents					
🔈 Downloads					
Music					
Nictures		ii.			
Videos					
👟 OS (C:)					
🐦 shared (\\192.	168.167.101) (S:)				
🔿 Network		~			
File name:	Modified_excel_import_s	samp	ble_list		
Save as type:	CSV (Comma delimited)				

6. To import sample list into Chronos, click "Import CSV..."

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



	Move down	9	C:\Program	Files	(x86)
€ ₽		10	C:\Program	Files	(x86)
Method editor		11	C:\Program	Files	(x86)
List		12	C:\Program	Files	(x86)
	New	13	C:\Program	Files	(x86)
Settings	, new	14	C:\Program	Files	(x86)
	Load	15	C:\Program	Files	(×86)
<u></u>	Save				
Activation	Save as				
	Print				
	F Import CSV				

- 7. In the CSV import window do following
 - a) Choose "Browse..." to select the desired "Chonos Method", this will ensure correct format of sample list columns, (E.g. for Xcalibur, use one of the Evosep Xcalibur methods)

Chronos Method: C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur	CSV Import				- 🗆 X
Chronos Method: C:\Program Files (x66)\Chronos\Plugins\EvosepOne\Templates\Xcalbur\Xca	CSV Mapping Template:				
C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur\Unitsmin, 8cm).cam CSV File to Import: S:\ots\Modified_excel_import_sample_list.csv D: Preview CSV File Use All Samples Number of Samples to Import: Save to Sample List	C:\Program Files (x86)\Chrono	s\Plugins\EvosepOne\Templates\import tem	plate.ccm		Browse
CSV File to Import: S^tots/Modified_excel_import_sample_list.csv Use All Samples Number of Samples to Import: Save to Sample List	Chronos Method:				
S-\sts \Modified_excel_import_sample_list.csv S-\sts \Modified_excel_import_sample_list.csv Use AI Samples Number of Samples to Import: Code Sample List	C:\Program Files (x86)\Chrono	s\Plugins\EvosepOne\Templates\Xcalibur\X	calibur 100 SPD (11.5min, 8cm).cam	Browse
S-\sts \Modified_excel_import_sample_list.csv S-\sts \Modified_excel_import_sample_list.csv Use AI Samples Number of Samples to Import: Code Sample List	CSV File to Import				
Use All Samples Number of Samples to Import: 1 🗘 Edit Template		_sample_list.csv			Browse
Use All Samples Number of Samples to Import: 1 🗘 Edit Template	Proview CSV Ele				
Save to Sample List			1		
· · · · · · · · · · · · · · · · · · ·	Use All Samples	Number of Samples to Import:	1	¥	Edit Template
· · · · · · · · · · · · · · · · · · ·					
E Sele	Save to Sample List				
					Select
See Import Import then Run Cancel	Namport 💦		Import then Run		Cancel

- b) Choose "Browse..." to select the "CSV File to Import".
- c) Click "Preview CSV File..." to see the correct column numbers in the sample list.

1	2	3	4	5
Column1	Analysis Method	Source Tray	Source Vial	Xcalibur Method
1	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 2	95	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.t
2	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 2	96	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
3	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	8	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.
4	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	9	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
5	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	10	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
6	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	11	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
7	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	12	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
8	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	13	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
9	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	14	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
10	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	15	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
11	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	16	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
12	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	17	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
13	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	18	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190215
14	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	19	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219
15	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	20	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS 190215

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

CS	V Prev	view	_			×
Г		1				^
	•	Analysis Method:Source Tray:Source Vial:Sample Name:Xcalibur Method:Xcalibur Filename:Xcalibur Processing:Xcalibur Output Dir,Comment				~
				_	_	Ť

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

Edit CS	SV Templa	te	-		\times
	s Header F Load now Mapping:	Now CSV Separator (use \t for tab):			
Column	Use	Sample List Column	CSV C	olumn No	
•	\checkmark	Source Tray	3		~
	\checkmark	Source Vial	4		~
	\checkmark	Sample Name	5		~
	\checkmark	Xcalibur Method	6		~
	\checkmark	Xcalibur Filename	7		~
	\checkmark	Xcalibur Processing	8		~
	\checkmark	Xcalibur Output Dir	9		~
	\checkmark	Comment	10		~
		2	Save	Car	icel

- e) Match sample list columns to headers. Make sure to check the "Use" column, or that data will not be included!
- f) Click "Save" to save the template, which can then be re-used for other imports.
- g) Click "Import" to add sample list to Chronos.

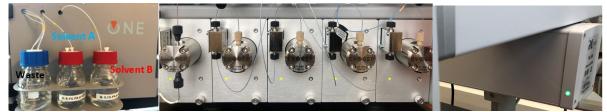
Preview CSV File	
Use All Samples	Number of Sample
Save to Sample List	

5.2 Evosep driver for control of Bruker MS

5.2.1 Instrument Preparation with Compass HyStar

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

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



2. Open Compass HyStar, click "Connect all instruments"

	View Options Compass			on View Options Comp lethod Set	
Compass Met	hod Set 🛄 Sample Tabl	 Maccuration 	Instruments	errod set 📲 sample ta	sole Ell-sochaola
HyStar	disconnected	Bruker OTOF MS	HyDiar	Ide	Bulker OTOF MS
deconnected	Evosep One	dicorrected	Fit ide	Evosep One	not ready shutdown
Connect al instruments	4	mio/010F-Q III		4	NICKOTOF-Q II
	HE REALING BAT	1		NE Pressure bar NO Films pLink	1
	Sample 0.00 / 0.00	~		Sample 0.00 / 0.00	-

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



5.2.2 Sample Acquisition with Compass HyStar

1. Make sure that Instrument Preparation has been performed.



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



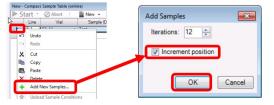
3. In HyStar, click the "Acquisition" Icon to open the HyStar Sample Table, then click "New" and click the small arrow in the first line under "Method Set" and choose "Use Individual Methods"

			mple Table	M, A	quisitior	🗋 👁 Quick Dat	a Vie	
			Instrumen	its				
			U.,	Char (0)		not condu		
New - Compass	Sample Table (online)						
Start -	Ø Abort	📄 New 👻	🗶 Delete 📔 Save 📗	Save As	Report	t - Import/Export -	🔅 Options 👻	
Line	Vial	Status	Sample ID	Inj.	Volume [µl]	Data Pa	h	Method Set
* 1		•		1	0	D:\Data\		
		•			0			Open Use Individual Methods

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

Wew - Compass	s Sample Table (or	nline)							
Start *	Ø Abort	New 🗸 🕽	🕻 Delete 🛛	💾 Save 🔛 Sa	ve As	📓 Report 👻 📔 Import	/Export 👻 🏟 Optio	ns 🔻	
Line	Vial	Sample ID	Inj.	Volume [µl]	Data Path	Method Set	Separation Method	Injection Method	MS Method
★ ✓ 1	S1-A1 -	test	1	1 0	D:\Data\ 🔹	-	100 samples per 🔻	Standard	• RC100.m •
	-			0	-	-	-		

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



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

New -	Compas	s Sample Table	(onlu	ne)				Save Sav	nple Table As	2			
► S	tart •	Ø Abort		📗 New 👻 💢 🛙	Delete	Save Save	Save As	lame:	Sample table quick start				
	Line	Vial		Sample ID	Inj.	Volume [µl]	Data Pa		Sample table quick start		Separation Method	Injection Met	MS Method
*	√ 1	S1-A1	*	Test	1	C	D:\Data\	Tag:	•	•	smp pr day	Standard	RC100.m
*	2	S1-A2	*	Test	1	C	D:\Data\	Description			smp pr day	Standard	RC100.m
*	3	S1-A3	-	Test	1	C	D:\Data\	Description	^ ^		smp pr day	Standard	RC100.m
*	V 4	S1-A4	*	Test	1	C	D:\Data\				smp pr day	Standard	RC100.m
*	₹ 5	S1-A5	*	Test	1	C	D:\Data\				smp pr day	Standard	RC100.m
*	6	S1-A6	*	Test	1	C	D:\Data\		-		smp pr day	Standard	RC100.m
*	7	S1-A7	-	Test	1	C	D:\Data\				smp pr day	Standard	RC100.m
*	8	S1-A8	*	Test	1	C	D:\Data\		OK Cancel		smp pr day	Standard	RC100.m
	0	C1.40	-	Test	1	0	D(Data)			- 1	eme er deu	Chandrad	BC100 m

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

Southie came douce score	compass sample rable (simile)			
🕨 Start 🝷 🖉 Abo	rt 📄 New 👻 Delete	Save Save	💾 Save As 🛛 📓 Report 👻	Import/Export -
		Volume [µl]	Data Path	Method Se
Start Sequence	Shutdown conditions: activated	0	D:\Data\	Evosep 100 smp pr day.m
Start Sequence	Duration: 2:55:30	0	D:\Data\	Evosep 100 smp pr day.m
	Expected end: 2018-06-19 19:05	0	D:\Data\	Evosep 100 smp pr day.m
Start Single		0	D:\Data\	Evosep 100 smp pr day.m
Acquisition		0	D:\Data\	Evosep 100 smp pr day.m
		0	D-(Data)	Everen 100 emp or day m

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



HyStar (1 / 12) injecting	Injecting Evosep One Injecting	Bruker OTOF MS ready operate	HyStar (1 / 12) injected	Run Evosep One	Bruker OTOF MS run operate
Time (min) 11.5 O.O Remaining Time 2 hours 42 min	HP Pressure 0.0 bar HP Plow 0.00 µL/min Method 100 samples per Sample 51-A1 0.00 / 0.00	micrOTOF-Q III	Time (min) 11.5 0.7 Remaining Time 2 hours 42 min	HP Pressure 398.9 bar HP Pressure 398.9 bar HP Pros 2.00 µL/min Method 100 samples per. Sample 51-A1 0.00 / 0.00	micrOTOF-Q III
Maint 100 samples	per day: Started per day: Sample position Slot per day: Started	1:2	Pump A Pump A Consplacement [µ1] Pump Set flow [µ1/min] Pump Seed [µ1/min] Pump Seed [µ1/min] Pump B Displacement [µ1] Actual flow µ1/min] Set flow [µ1/min] Pump Seed [µ1/min]		

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

Stop • O Abort	New 👻 💥	Delete
Stop Sequence	nple ID	Inj
Stop Sequence and Acquisition		
Stop Acquisition		
Stop All		-

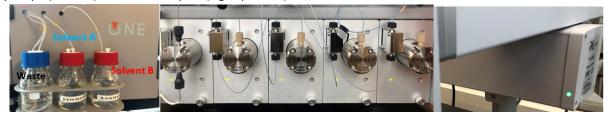
10. If something unforeseen happens during a maintenance script or a method any error messages can be cleared by right clicking the HyStar status view and choose "Clear Error"

Compass	Method Set 🛛 🔛 Sample Ta	able 🙀 Acquisiti
struments		
initialized	disconnected	Bruker OTOF MS
	Display Runtime	shutdown
	Stop Shutdown Counter Stop 'Prerun' Time	micrOTOF-Q III
	Reset System	
	Reset System Clear Error	

5.3 Evosep driver for control of SCIEX OS

5.3.1 Instrument Preparation with SCIEX OS

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



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



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



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

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



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



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

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

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

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

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

0 -	Batch	습 💠 🗒 🕑 🗄	A 🛄						
		Decision Rules 🗸 🗛	uto-Calibrate New	Open	✓ Save	♥ Print		View	Submit
Unt	itled 🧾 🙋						1-22		
					Save As Batch	X			
					Type a file name The batch is saved in the Batch folde Current Project:Evosep_project	r of the current project.	-	late Layout	Manage Samples
	Sample Name	MS Method	LC Method	Rack Type	Batches		ition	Data File	â
1	test_1	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	Name	Date Modified *		test_100SPD_	SWATH_1
2	test_2	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	evolutich2 evolutich2	2/15/2022 4:37:14 PM 2/24/2022 2:52:56 PM		test_100SPD_	SWATH_2
3	test_3	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	evolution evolution Training_150322	3/9/2022 9:23:56 AM 3/31/2022 9:14 18 AM		test_100SPD_	SWATH_3
4	test_4	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray				test_100SPD_	SWATH_4
5	test_5	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	File Name Type a file name		1	test_100SPD_	SWATH_5
		100500 CUNTURE IN 100 TEA	100 1 1			Save Cancel	í	1 100000	T

7. Press Submit to submit samples to sample queue.

O - Batch	습 🖗 🕮 🖄 🛆 🧮						Submit Samples
	Decision Rules V Auto-Calibrate New	Open	✓ Save	♥ Print	View	Submit	Confirm the selection of samples Total number of samples to be submitted: 6 of 6
							The oxiloanti X500 FSI Positive Calibration Solution, will be applied every 5 samples.

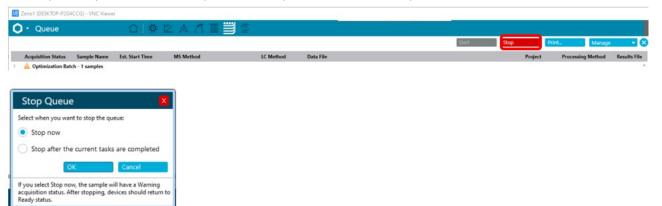
8. Go to the "Queue" and click "Start"

🗘 • Queue		合体	🗒 🖻 🖻 🔺						
						Start	Stop Print	Man	age 🔹 🗙
Acquisition Status	Sample Name	Est. Start Time	MS Method	LC Method	Data File	Project	Processing Method	Results File	Auto Processing S
🕔 Untitled - 8 sampl	es								A
		3/31/2022 10:28:48	100SPD_SWATH56vW_4			Evosep_p	roject		
0	test_1	3/31/2022 10:30:49	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_1	Evosep_p	roject		
0	test_2	3/31/2022 10:49:59	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_2	Evosep_p	roject		
0	test_3	3/31/2022 11:09:09	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_3	Evosep_p	roject		
0	test_4	3/31/2022 11:28:19	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_4	Evosep_p	roject		
0	test_5	3/31/2022 11:47:29	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_5	Evosep_p	roject		

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

	Device Details	
Queue	Device	
Acquisition sample	Device Name: Evisep One Agilent Integrated System Mandaturer: Agilent Firmware Version: Evisep One EVISEP, ONE - NUA	Evosep One graphs
	Serial Number: Evisep One EVOSEP_ONE - N/A Serial Number: Evisep One EVOSEP_ONE - n/a	100 samples per day: Started
Acquisition sample	Detailed Status	100 samples per day: Sample position Slot1:2
Acquisition queue		100 samples per day: Started
	Evosep One ?	
	®on ⊜off	non
Devices	EVÖSEP	utio
Devices	HP Pressure 0.7 bar HP Flow Show Graphs	2
	Method	

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





5.4 Evosep driver and sample Acquisition with MassHunter Software

- 5.4.1 Create MS method with MassHunter
 - 1. To create a method, open **"Offline Method Editor"**, and on the Evosep One tab select the desired chromatography method.

//	
line	Properties DA Evosep One QQQ
hod	Name 100 samples per day V
or	Description 30 samples per day 1064, EV1109
	IO0 samples per day 200 samples per day 300 samples per day Extended method 15 SPD High organic method 100 SPD (BETA) System and column wash Whisper100 20 SPD (BETA) Whisper100 40 SPD (BETA)
	Agilent MassHunter Acquisition Method Editor
	File Tools Method Help

Note the LC method run time for MS method timing.

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

_									-	
I	A 🔚	gilent MassHu	Inter Acquisition	Method Edi	tor					
	File	Tools M	ethod Help							
		💕 🛄 🚺	100SPD_S	ched 4minR	Twin_MR	M_BSA.n	n			
1	Prope	rties DA	Evosep One	QQQ	•				<u> </u>	1
5	Tur	0.110	0.TUNE.TUNE.X			Dumo		A	4	
5	litun	_	Browse	1	No limit/A: 11.5	s Pump m	in			2
	lon	source		Lime	filtering					
4→	HP	LC-Chip 💌			Peak widt	h 0.25	min			3
	Time	e segments		,						•
	#	Start / Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored			
	•	1 0	Dynamic MRM	To MS	200	0	7			



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

File Tools Method Help		
🗋 💕 🚰 💹 📄 100SPD_Sched_4	minRTwin_MRM_BSA.m V	â 😏

5.4.2 Sample Acquisition with MassHunter

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





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

	Agilent MassHunter Acquisition Worklist E
	File Tools Worklist Help
	- 🗋 💕 🖬 💹 🕞 -
1. Right click here	🗕 🗾 🛛 Sample Name 🛛 Sam
and and the second s	Add Multiple Samples
	Add Sample

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

Add Multiple Samples	X Add Multiple Samples	>
Sample Information Sample Position	Sample Information Sample Position	
Sample Name: BSA Append Counter Suffix Counter Number of digts: 2 Start Value: 1 Step: 1	P1 85 Exctp box Selection Origin Block Increment P2 96 Exctp box • Top left • Top left • Top right • Bock Increment P2 96 Exctp box • Top left • Top right • Bock Increment P3 96 Exctp box • Bottom left • Bottom right • Colum might • Colum might • Seperative • Number of samples Number of replicates	
Method Name: 800SPD_BSAm V Path: D:\MassHunter\Methods\Evosep\Standardised	12 1 Wetplate/Tray 1 1 2 3 4 5 6 7 9 10 11 4 1 2 3 4 5 6 7 8 9 10 11 4 1 2 3 4 5 6 7 8 9 10 11 4 1 3 3 • 3 • 7 8 9 10 11 4	

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

2 Mark column and Right click



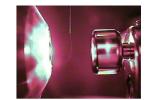


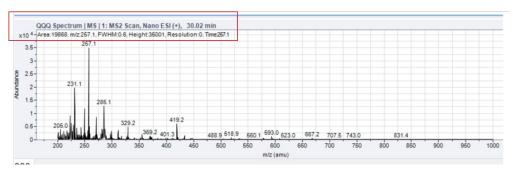
5. To run LC checks/preparations prior to MS run. Right click for drop down method. E.g. use" flow-tocolumn" at 1000nl/min to check spray for 100 SPD LC method.

		Diagnostics	×	Preparation	×
Right Click		Evosep One self-dia	gnosis procedures	Procedure for prepa samples	ring the LC system for running
Agilent MassHunter Workstation File View Sample Worklist Context: Acquisition	Method Tools Help	Pump A-D: Restriction: Tip seal:	-	Pump preparat Align solve	none Degas ow: Solvent exchange
Evosep One ?	_ = QQQ	HP system:		System and column w	Run Cancel
10 m Coff EVV SE HP Pressure 258.6 HP Flow 0.41 Method Sample 0.007000		HPLC Chip		Procedure 1 samples Pum Flow to colu	or preparing the LC system for running preparition none Utigs solvering: mn / die Bow none 4000 «Imim (10 mm) 1000 «Imim (10
Chromatogram Plot	Park Autosampler			1.0	Idle flow (250 nl/min)
		TIC			

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

	Agilent MassHunter Workstation Data Acquisition File View Sample Worklist Method Tools Hel	
	Context: Acquisition V Layout: Agilent_Service.	
	Instrument Status	
5. Save the method for future use		 4. If editing method on the fly click apply 3. Set Scan
		Range
	0.00/0.00 Instrument Inject	
	Method Editor	
	V I I I I I I I I I I I I I I I I I	V V Apply 😒
	Properties DA Evosep One Q.Q.Q. Tune file	Acquisition Source Chromotogram Instrument Diagnostics Scan segments End Mass Scan Time Fragment/or Cell Accelerator Voltage Polarity > 200 1000 500 166 5 /Positive
2. Select	- Ion source - Time filtering	
MS2 Scan	HPLC-Chip Peak width 0.07 min	
	Time segments Deta Deta Stored # Start Scan Type Div Valve Deta Stored > 1 0 MS2 Scan To MS 0 Immediate	
1. Open		
Method	•	
Editor	Method Editor Worklist Sample Run	





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

ETTE ·	A. 21. 21.2 21.3 21.4 21.8	31.6
	Metod Edu	🗸 Apply 😒
Data	Sample Name Sample Position	Method
Acquisition	1 P1-A1 D1MassHunter(Methods)Evosep(De	velopment/Hela()
	2) V P1-A2 D.\MassHunter(Methods\Evosep\De	velopment/Hele/

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

	? _ =	QQQ	900.4	5	900.3	1	\$
Right Click	Standby					dist	Worl
	•	() On Off	18052022_0	- II 🖻		1	
		HPLC Ch	Sample F	Name	Sample	Г	
1 ()	StandBy		P1-A1				1
	✓ On	HPLC Ch	Sample F	Name		Г 	1

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



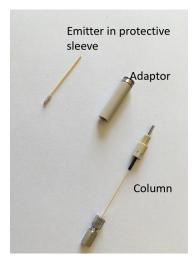


6 Configuration, source, column, and emitter

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

6.1 Thermo Scientific EASY-Spray source

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

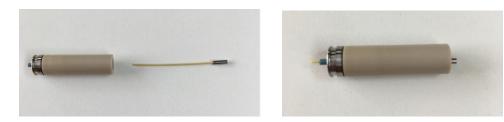


How to set up

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



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



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



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



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



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





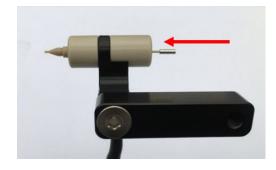
6.2 Thermo Scientific Flex source

How to set up

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



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



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



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

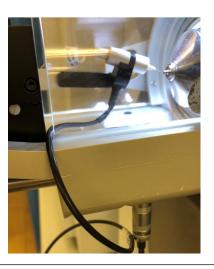




Note:

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

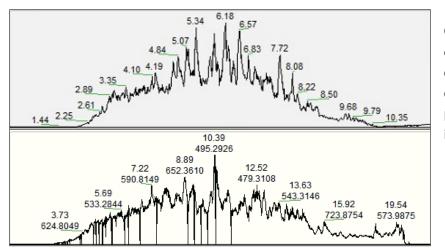






6.3 Thermo Scientific FAIMS Pro interface

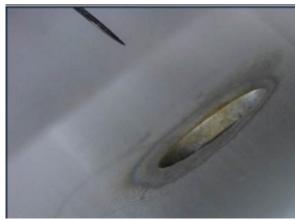
Optimal spray stability is a critical parameter in LC/MS and becomes even more crucial in combination with the FAIMS Pro Interface. This is challenged after continuous analysis of hundreds of samples and the electrospray becomes unstable leading to loss of signal and lower identifications. The spray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



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

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

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





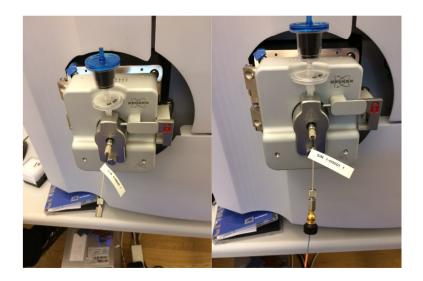
Before cleaning

After cleaning



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

6.4 Bruker Daltonics CaptiveSpray source.



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

6.5 Agilent Nanospray source.

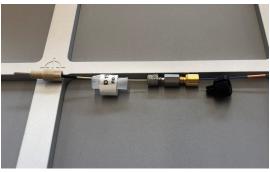
Evosep Column, Agilent Needle emitter EV1117, Agilent sleeve adapter EV1116 and Agilent Needle sleeve assembly





How to set up

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



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





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



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

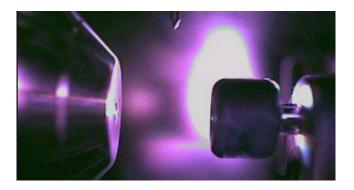




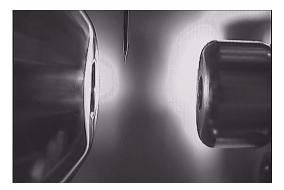
5. Now assemble the needle sleeve assembly and remove the protective sleeve from the emitter.



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



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



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

Recommended start source conditions:

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

• Capillary voltage 1700V

Method Editor	
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Properties DA QQQ	
Tune file C Stop time _20KV_2021_07_02.TUNE.TUNE XML C No limit/As Pump	Acquisition Source Chromatogram Instrument Diagnostics Source parameters Gas Temp: 200 *C 200 *C High Pressure RF 200 V 150 V
Image: HPLC-Chip Image: Peak width Image: Output of the segments Time segments Image: Head of the segments Image: Div Valve Delta Delta Delta Delta EMV (+) EM	Gas Flow: 11 //min 11.0 //min Low Pressure RF 110 V 60 V Copy Paste Paste Paste Paste
	Capillary: 1700 V 1428 nA
	Chamber Current 0.18 µA

6.6 Sciex Optiflow ion source in microflow regime.

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

How to set up

1) Carefully insert a "1-10 μ L/min" electrode into the Micro probe.



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



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



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



6.7



7 Preparing the Evosep One for use

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

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

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

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

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

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



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

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

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

in menu <	Sample	Analysis Method	
	4 Add		
Sample list	Remove	Add Sample(s)	×
F	Duplicate	Method Prepare	
Schedules and run control	Move up	Number of samples	
A	Move down		
Method editor	List	Add C	lose
1	New		
Settings			
	Save		
	Saye as		

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

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

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

7.1 Degas pumps

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



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

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

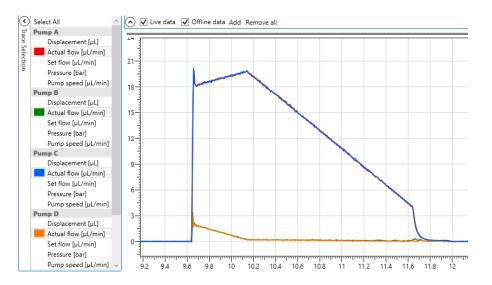
7.2 Solvent exchange of pumps

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

7.3 Align solvents

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

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





7.4 Flow to column

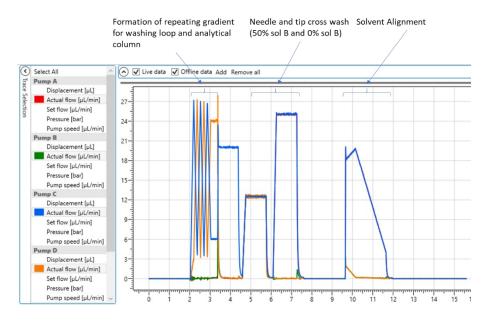
Before running the first sample, it is recommended to tune the MS ion source conditions, according to MS manufacturer guidelines to secure a stable electrospray. For this purpose, use the "Flow to column" program, which encodes an automated procedure for delivering solvent-A to the analytical column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

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

7.5 System and column wash

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

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





ielect All	🔿 🗌 Live data 🗹 Offline	data Add Remove al	1		×		
ump A							
Displacement [µL]	420				1		
Actual flow [µL/min]	400-			 		 	1
Set flow [µL/min]							
Pressure [bar]	380-						
Pump speed [µL/min]	360-						
ump B	-						1
Displacement [µL]	340-						
Actual flow [uL/min]	320						
Set flow (µL/min)	-						
Pressure [bar]	300-						
Pump speed [µL/min]	280-						
Pump C	-						
Displacement [µL]	260-						
Actual flow [µL/min]	240-						
Set flow [µL/min]							
Pressure [bar]	220-						
Pump speed [µL/min]	200-						
Pump D	-						
Displacement (µL)	180-						
Actual flow (µL/min)	160-						
Set flow [µL/min]	-						
Pressure [bar]	140-						
Pump speed [µL/min]	120-						
Pump HP	4						
Displacement [µL]	100-						
Actual flow [uL/min]	80-						
Set flow [µL/min]	-						
Pressure [bar]	60-						
Pump speed [µL/min]	40						
e amp appea (hr) minj	4						
	20						
	0						

Pump HP delivering the repeating gradient to the analytical column with a constant pressure of 400 bar

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

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

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

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

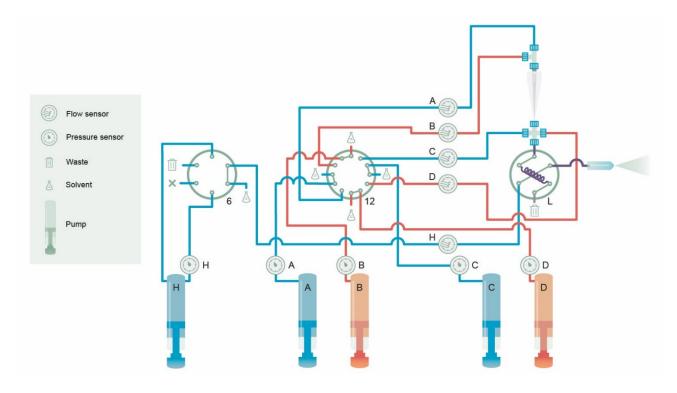
🔊 Options 🛛 🗙
Procedure for preparing the LC system for running samples
Degas:
Purge:
Align solvents:
Flow to column: none v nl
System and column wash: 🗹
Run Cancel

8 Running Samples using Evosep One

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

8.1 Separation principle

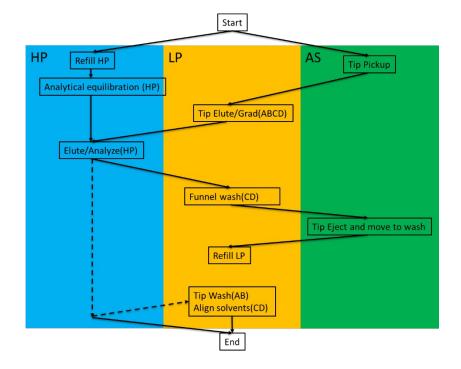
The Evosep One technology is centered around the Evotip and integrates sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with a small plug of C18 stationary phase at the bottom of the tip. The Evotips are used to de-salt and clean up the samples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



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



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



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

Once the Evotip is sealed in-line with the solvent system, a gradient from pumps A and B runs through the Evotip and sequentially elutes the adsorbed analytes (Tip Elute/Grad(ABCD)). While the gradient, with the embedded and pre-separated analytes, elutes from the Evotip, a secondary gradient from pumps C and D continuously modify the composition of the initial A/B gradient to generate an offset gradient that ensures optimal chromatographic performance at the analytical column, see figure 3. Pumps A+B deliver a partial gradient which is sufficient to sequentially elute the analytes of interest but still leave all the high-molecular contaminants behind which are then discarded with the Evotip after the analysis. A high organic wash (80% ACN) volume is introduced just after the gradient using Pump D, bypassing the Evotip, to efficiently wash the analytical column. It takes approximately one minute at 20-40 μ l/min (<20 bar) to create the preformed and offset gradient with the embedded analytes and position it precisely in the storage loop (ID100 μ m, 30 μ l), see figure 3.

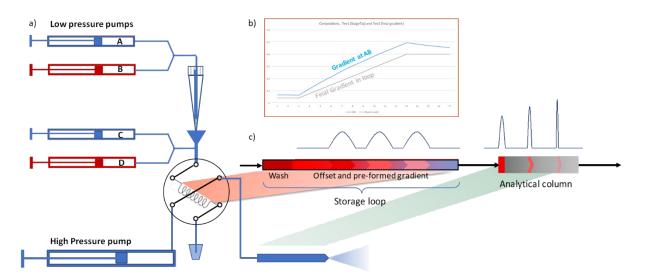


Figure 3: Evosep One a) Simplified plumbing diagram of Evosep One. b) Illustration of the A/B gradient running through the Evotip and the following C/D modified gradient resulting in an offset gradient for optimal focusing at the analytical column. c) Illustration of the preformed and offset gradient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

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



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

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

Table 1: Evosep One Methods.

8.2 Sample acquisition

Chromatographic Data system (CDS) and generic methods

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

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

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

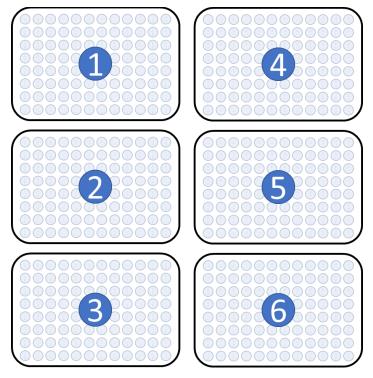


Add Remove Dupkcate Move up Move down	Add Sample(s) Method C:Program Fil Number of samples	es (x86)/Chronos/Plugins/EvosepOne/Te rei 1 =	a				
t	🔯 Open Method						
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Save	Evosep_MPI	Generic	2017-10-30 14:44	File folder	0.00		
Save as	Frølundfoto	Xcalibur	2017-10-30 14:44	File folder			
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Print	Outlook	Diagnose.cam	2017-10-09 11:54	Chronos Analysis	2 8		
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						Open 😽	Cancel
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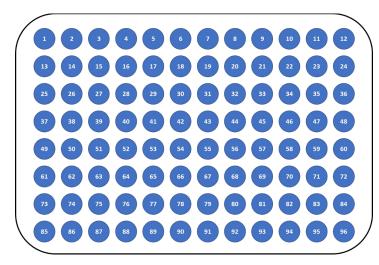
- Integrated LC-MS mode
 - a. The appropriate sample method is chosen firstly based upon MS CDS vendor and secondly required sample/day throughput (Table 1)

1	Samp	le	Analysis Method		Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	xcalibur Processing	Xcalibur Output Dir
Ш			1 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 samples per day (11	.5 min).cam	EvoSlot 1	1				
	- - +	<u>A</u> dd								

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

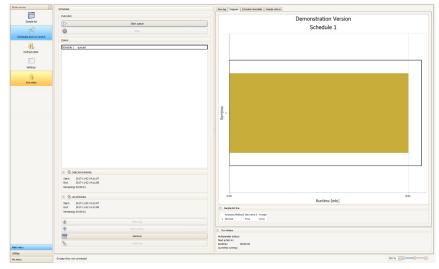


c. Source vial position (1-96) must be specified using the dropdown menu,



- d. For Thermo MS; "Xcalibur Method" the MS acquisition method must be specified. MS acquisition time must be correlate with the individual LC-MS methods, as specified in table
 1.
- e. For Thermo MS; "Xcalibur Filename" the MS data filename(s) must be specified.
- For Thermo MS; "Xcalibur Processing" the MS data post processing method *can* be specified.
- g. For Thermo MS; "Xcalibur Output Dir" the MS data directory must be specified.
- h. When the sample list is completed, create a schedule and start the analysis. Chronos will send the sample information to Xcalibur and once the MS is in "waiting for contact closure status", the Evosep One sample separation will start.





4

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

Analysis Method

- 1 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur MS standby.cam
- Standalone LC mode
 - a. The appropriate sample method is chosen based on the required sample/day throughput (Table 1)

Sample	Analysis Method 🔺	Source Tray	Source Vial
	1 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Generic\100 samples per day (11.5 min).cam	EvoSlot 1	1
<u> 슈</u> dd			

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

Schedule
Overlapped
Priority
Infinite loop
Create

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

in menu 🤾	Schedules	Duning Dagram Schedule snetable Sample status
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	These down	E la seña Nararen telas Natarios
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f. The Evosep One starts the sample separation produce and sends a contact closure signal to the MS CDS, when the sample elution begins.

Tips and tricks:

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

Nalbe Flasses M/2.1% Tot 2 C	nun control	Load Eana Sana Sana	Methodinane: Xualit Description:	tur 60 samples pr	r day (21 mir)												Show invisible task p
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- Save the method in the same name.
- When submitting new samples with the updated method, the user can now browse from the specified default directory.

Main menu <	Sample	Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	xcalibur Processing	Xcalibur Output Dir
Ē	<u>ер A</u> dd	1 C:\Pron).cam	EvoSlot1	1	C:\Thermo\Instruments\TSQ\Methods\		C:\Thermo\Instruments\TSQ\Methods\	D:\
Sample list	Remove							

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

Sample		Analysis Method	Tool	Source Tray	Source Vi	al Sample Name	Xcalibur Method	Xcalibur Filename	xcalibur Processing	Xcalibur Output Dir	Volume
다.	1	C:\Pron).cam		EvoSlot1	1	Copy meth	od with increme	nt of vial no			0
Remove									_		

Copy row	\times
How many times should this row be copied?	OK
	Cancel
2	



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

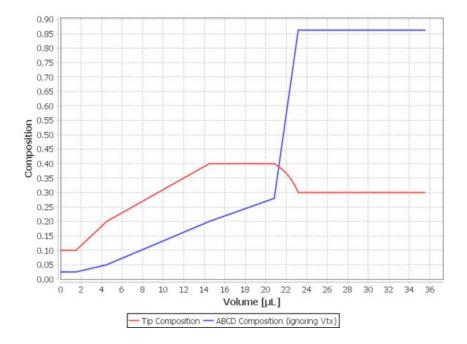
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• If you often run the same analysis using the same methods, the sample list can be saved and reloaded instead of created from scratch.

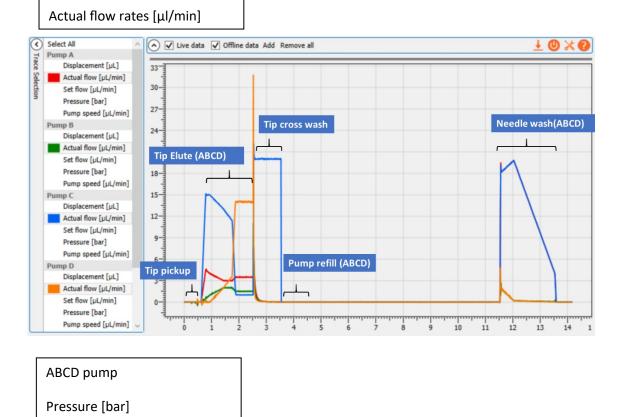


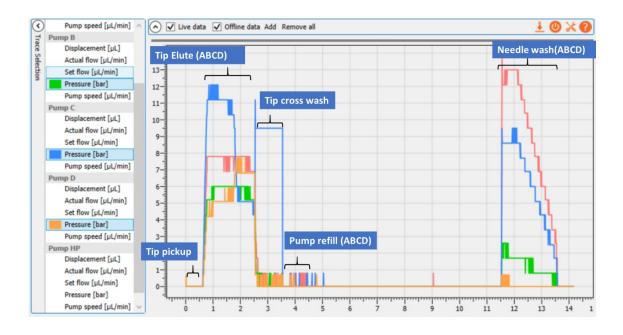
8.3 Example pump data

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

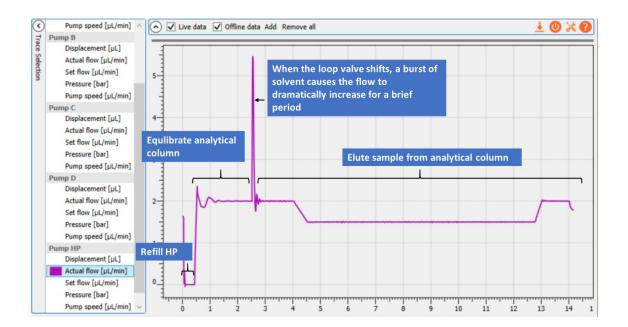


ABCD pump





HP pump Actual flow rate [µl/min]



HP pump

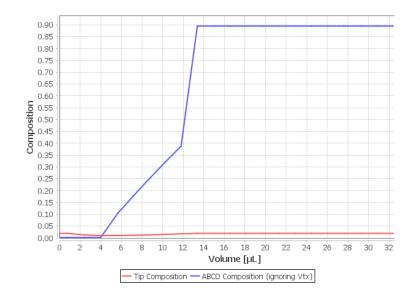
Pressure [bar]

		Equibrate analytical
	Pump speed [µL/min] ^	column 🛛 🔹 🗚 Add Remove all 👘 Elute sample from analytical column 🔜 🚣 🙂 💥 😮
Trace	Pump B	
Ce	Displacement [µL]	
ele	Actual flow [µL/min]	400-
Selection	Set flow [µL/min]	
-	Pressure [bar]	360-
	Pump speed [µL/min]	
	Pump C	320-
	Displacement [µL]	
	Actual flow [µL/min]	280- When the loop valve shifts, the un-
	Set flow [µL/min]	pressurized loop volume (30 μl)
	Pressure [bar]	240 causes the pump HP pressure to drop
	Pump speed [µL/min]	a while
	Pump D	200-
	Displacement [µL]	
	Actual flow [µL/min]	160
	Set flow [µL/min]	
	Pressure [bar]	120-
	Pump speed [µL/min]	
	Pump HP	80-
	Displacement [µL]	Refill HP
	Actual flow [µL/min]	
	Set flow [µL/min]	
П	Pressure [bar]	
-	Pump speed [µL/min] ~	
	Pump speed [pL/min]	0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

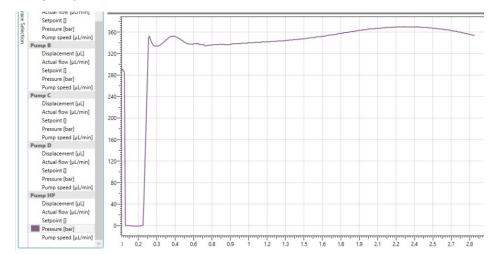
Below is a collection of the gradients and sample HP pump pressure and flow profiles for the different Evosep One methods

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

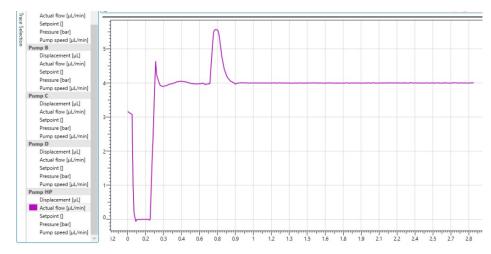
o Gradient



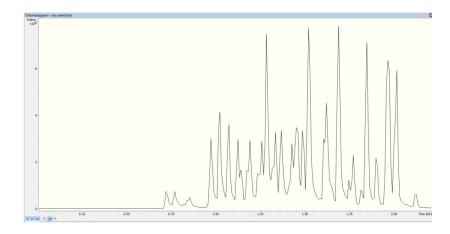
o Pump HP pressure



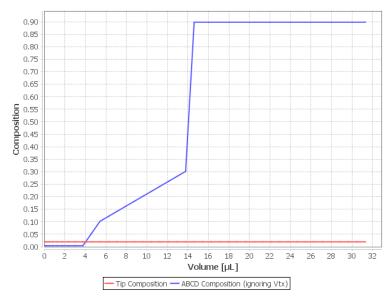
o Pump HP flow



o Example base peak chromatogram, tryptic BSA digest

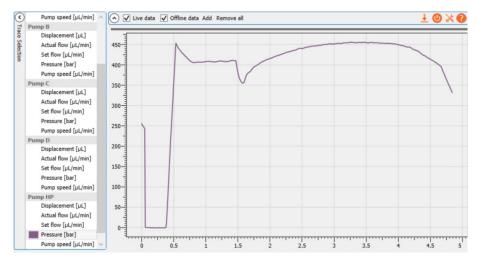


• 300 SPD method

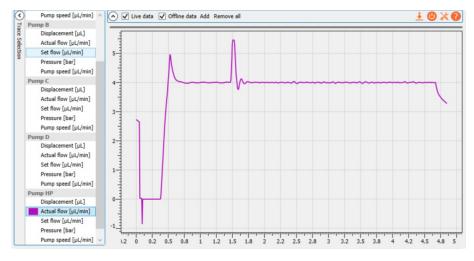


o Gradient:

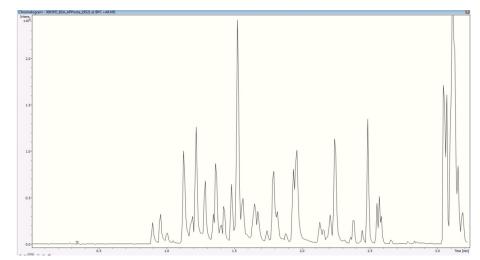
o Pump HP pressure



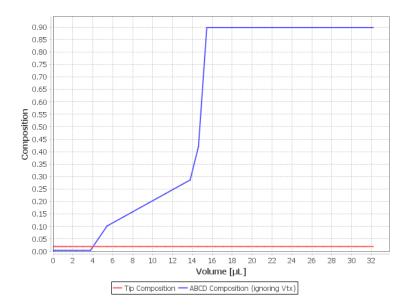
o Pump HP flow



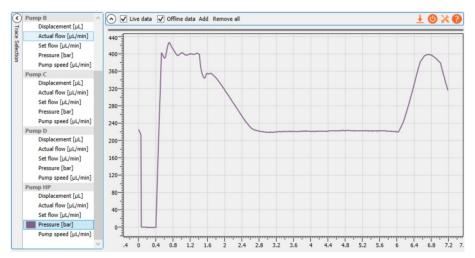
o Example base peak chromatogram, tryptic BSA digest



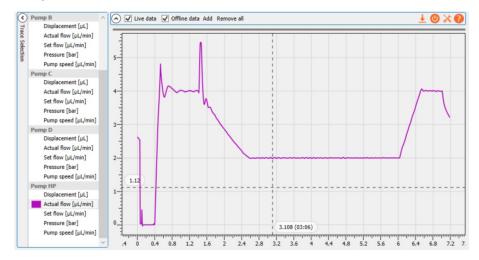
- 200 SPD
 - o Gradient:



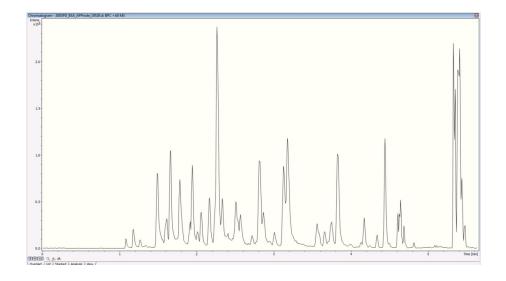
o Pump HP pressure:



o Pump HP flow:

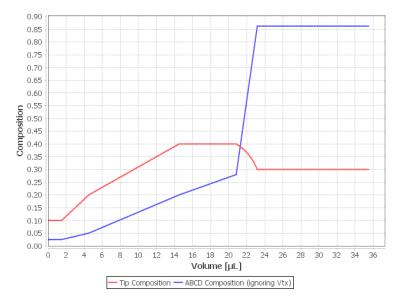


• Example base peak chromatogram, tryptic BSA digest

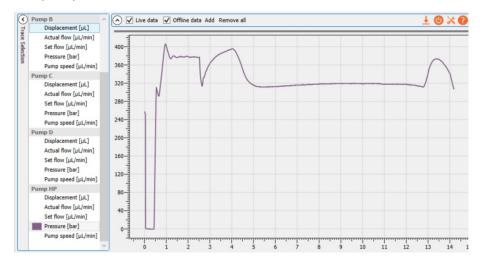


• 100 SPD method

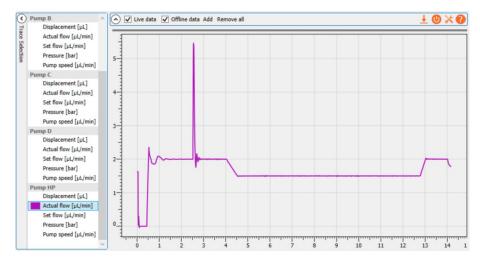
o Gradient:



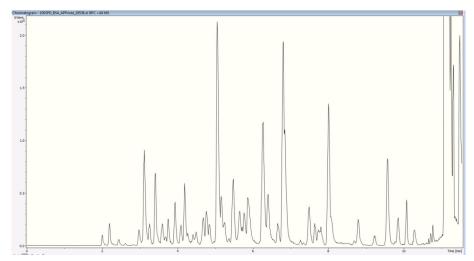
• Pump HP pressure:



o Pump HP flow:



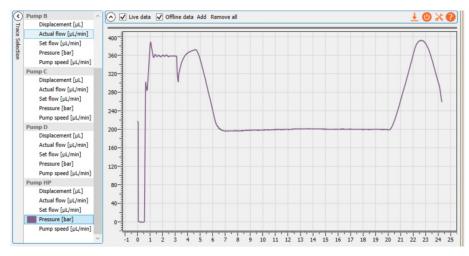
• Example base peak chromatogram, tryptic BSA digest



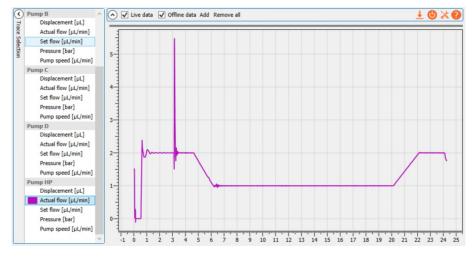
- 60 SPD method
 - o Gradient:



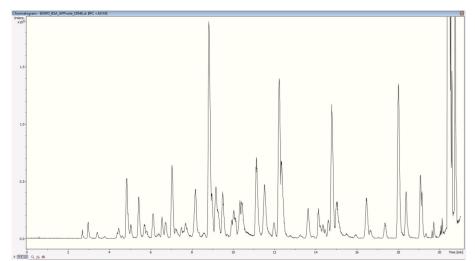
o Pump HP pressure:



o Pump HP flow:

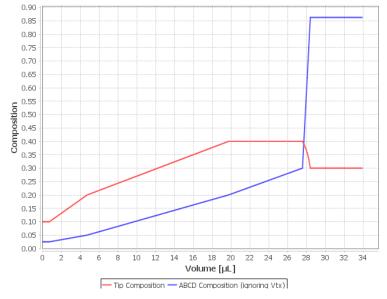


o Example base peak chromatogram, tryptic BSA digest

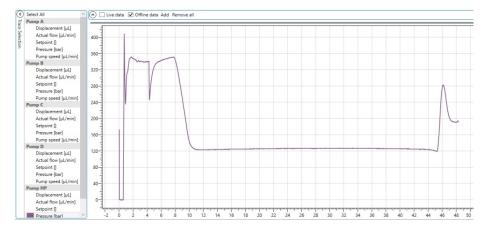


• 30 SPD method

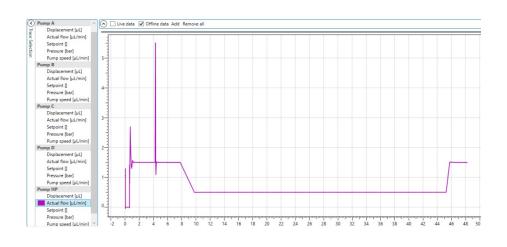
o Gradient:



• Pump HP pressure:

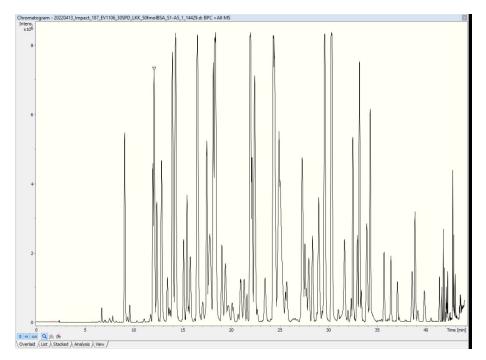


• Pump HP flow:

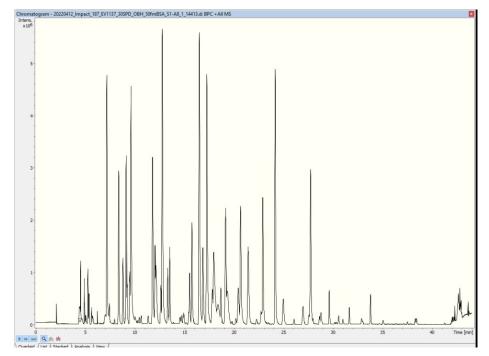




• Example base peak chromatogram, EV1106, tryptic BSA digest



o Example base peak chromatogram, EV1137, tryptic BSA digest



9 Troubleshooting

9.1 Evotip troubleshooting.

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

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

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

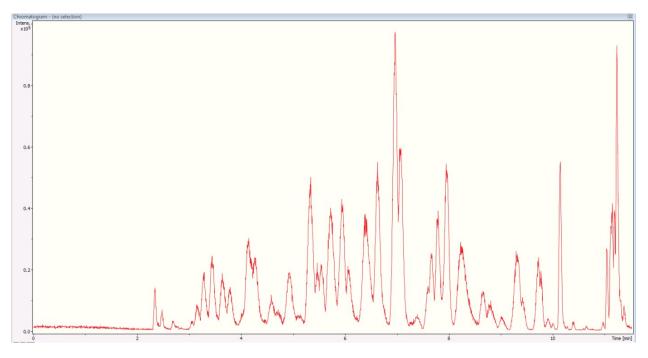


Figure 2 200 fmol BSA correctly loaded with the SOP

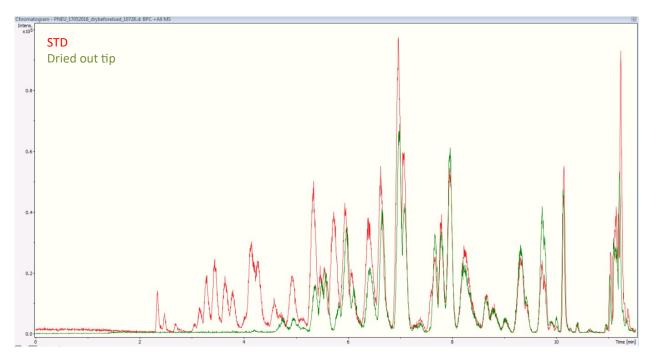


Figure 3 Tip dried out before load

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

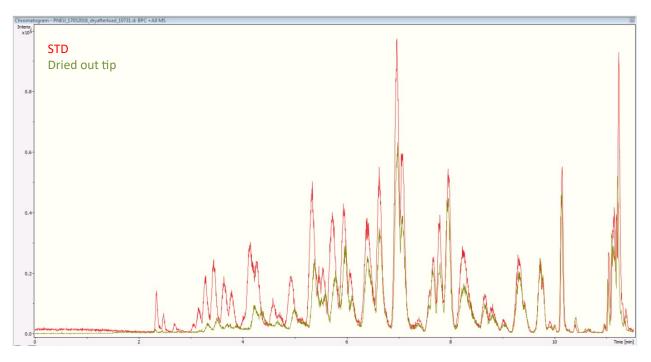


Figure 4 Tip dried out after load

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



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

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

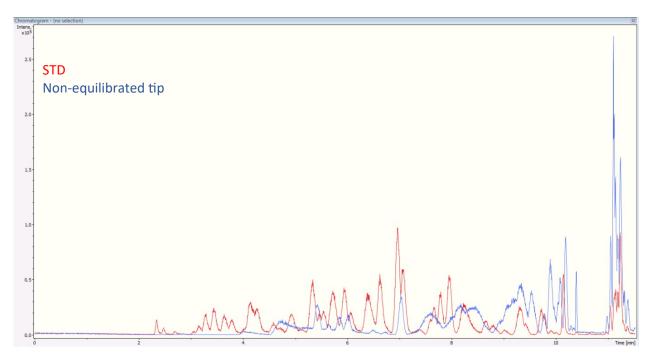


Figure 5 Tip which has not been equilibrated correctly before load of sample

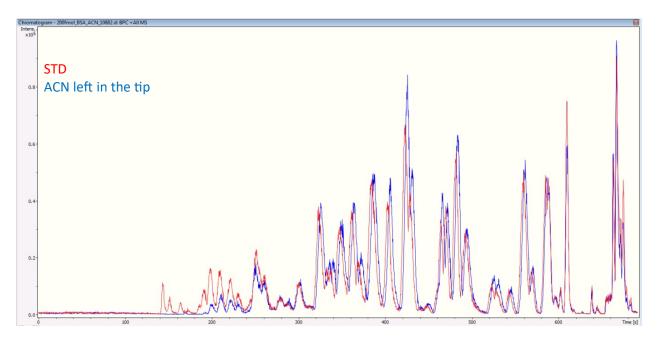
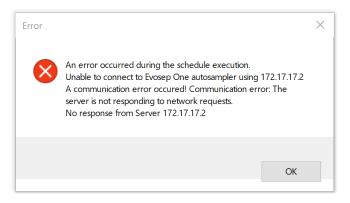


Figure 6 ACN left in the tip after the wash step

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

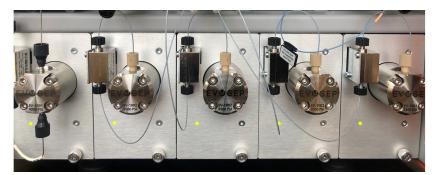
9.2 How to troubleshoot connection problems between PC and Evosep One

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





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



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

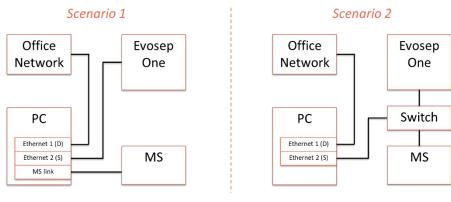


If LEDs are off, please check that power cords are correctly inserted from the power outlet and up to the Evosep One, and that the power switch on the autosampler power supply is switched on.

9.2.2 Check Lan connection

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





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

Please verify that.

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

9.2.3 Check network adapter set up.

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

- a. The ethernet card is set up to use a fixed IP address.
- b. A subnet has been generated with IP address 172.17.17.14 and subnet mask 255.255.255.240 for the "Internet Protocol version 4(TCP/IPv4)"
- 1. If the ethernet adapter is not set up to use a fixed IP address, the plugin installer will not be able to add the subnet. In that case change the ethernet card to use a fixed IP address, and manually configure its settings as per below description:

IP address 172.17.17.14 and subnet mask 255.255.255.240



Retwork Connections	v		_
← → → ↑ 😰 > Control Panel > Network and Internet	> Network Connections	~ Ō	Search Network Conne
Organize - Disable this network device Diagnose this	connection Rename this connection View status of this connection Change settings of this conne	ction	1
Network 4	ments mtified network R) Ethernet card connected to Evosep C		
Instruments Status	Instruments Properties Internet Protocol Version 4 (TCP/IPv4) Networking Sharing	Properties	×
General Connection	Connect using: Vou can get IP settings assigned autom this capability. Otherwise, you need to for the appropriate IP settings.		
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Activity	Constant Protocol Version 6 (TCP/IPv6) Constant Properties Install Uninstall Properties	esses:	· ·
Bytes: 2,491,205,536 4,259,217,533 Properties Disable Diagnose	Description Transmission Control Protocol/Internet Protocol. The default wide area network protocol that provides communication across diverse interconnected networks. Use of the provides communication		Advanced
Close	OK Cancel		OK Cancel

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

Sent Sent Received Bytes: 1,605,671,773 2,743,207,334 Operation Image: Sent Sent Sent Sent Sent Sent Sent Sent	Network Connections			×
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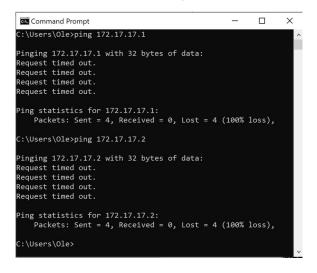
9.2.4 Ping hardware units

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

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

C:\Windows\system32\cmd.exe	
C:\>ping 172.17.17.1	
Pinging 172.17.17.1 with 32 bytes of data: Reply from 172.17.17.1: bytes=32 time<1ms TTL=64 Reply from 172.17.17.1: bytes=32 time<1ms TTL=64 Reply from 172.17.17.1: bytes=32 time<1ms TTL=64 Reply from 172.17.17.1: bytes=32 time<1ms TTL=64	
Ping statistics for 172.17.17.1: Packets: Sent = 4, Received = 4, Lost = 0 (0% Approximate round trip times in milli-seconds: Minimum = Oms, Maximum = Oms, Average = Oms	loss),
C:\>ping 172.17.17.2	
Pinging 172.17.17.2 with 32 bytes of data: Reply from 172.17.17.2: bytes=32 time<1ms TTL=128 Reply from 172.17.17.2: bytes=32 time<1ms TTL=128 Reply from 172.17.17.2: bytes=32 time<1ms TTL=128 Reply from 172.17.17.2: bytes=32 time<1ms TTL=128	
Ping statistics for 172.17.17.2: Packets: Sent = 4, Received = 4, Lost = 0 (0% Approximate round trip times in milli-seconds: Minimum = 0ms, Maximum = 0ms, Average = 0ms C:\>	loss),

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





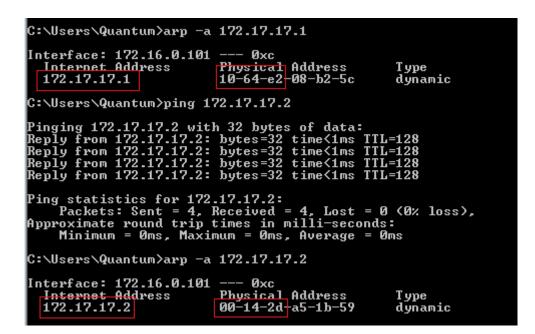
4. To verify that the Ping reply is coming from the Evosep One hardware and not another piece of hardware with the same IP address, run following commands

arp -a 172.17.17.1

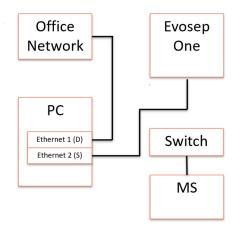
And check that the physical address has prefix 10-64-e2

arp -a 172.17.17.2

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



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





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

9.3 Error messages

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

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

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

	Error messages
1)	Instrument at 172.17.17.1:2 gateway target device failed to respond
2)	Pumpa at 172.17.17.1:7 gateway target device failed to respond, or
	Pumpb at 172.17.17.1:6 gateway target device failed to respond, or
	Pumpc at 172.17.17.1:5 gateway target device failed to respond, or
	Pumpd at 172.17.17.1:4 gateway target device failed to respond, or
	Pumphp at 172.17.17.1:3 gateway target device failed to respond, or
	all of the above
3)	An error occurred the schedule execution A communication error occurred! Communication error: The
	server is not responding to network requests.
	No response from Server 172.17.17.2 or 172.17.17.1
4)	[pumpa 172.17.17.1:7 (6/5/4/3)] An existing connection was forcibly closed by the remote host
5)	Pump(hp,a,b,c,d) A connection attempt failed because the connected party did not properly respond a
	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/in 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 suppor or This software does not support the device firmware - please upgrade the Evosep One instrument s 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 127.0.0.1:64001. This could happen if there is another application already listening on this endpoint 127.0.0.1:64001. This could happen if there is another application already listening on the ethernet cab connected and that it's not locked by the handheld terminal 12) Pump(h,b,c,d) - Pressure overload 13) Pump(a,b,c,d) - Pressure overload 14) MoveTorQbject(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 (xx min) - Pump HP does not contain sufficient solvent to perform the analys 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 samp	
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21) IPump(hp.a.b.c.d)) tan detection tault	
22) [Pump(hp,a,b,c,d)] drive not responding	



23) Couldn't verify method script authencity

24) Flow sensor offset

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

9.4 Error messages regarding communication issues with Chronos and Evosep plugin

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

Main menu		Main menu	
Utilities		Utilities	
My menu	Evosep One: not connected	My menu	Evosep One: connected

Error message:

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

Possible cause:

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

Action:



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

Error message:

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

Pumpb at 172.17.17.1:6 gateway target device failed to respond



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

Possible cause:

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

Action:

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

Error message:

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

Possible cause:

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

Action:

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

Error message:



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

Possible cause:

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

Action:

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

Error message:

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

Possible cause:

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

Action:

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

Error message:

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

Possible cause:

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



Action:

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

Please note:

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

Error message:

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

Possible cause:

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

Action:

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

Error Message:

8) The software does not support the device hardware/firmware - please contact Evosep support! or

The device firmware must be updated to be used with this software - if this does not happen automatically next time the software is connected to the hardware, please contact Evosep support!

or

This software does not support the device firmware - please upgrade the Evosep One instrument software package to obtain compatibility with the connected hardware!



Possible Cause

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

Action

- Update the Evosep One software on the PC to the latest version and connect to the Evosep One from that PC.
 - Before updating the SW please read the release note for more information on new features, changes etc. in the software

Error Message:

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

Possible cause:

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

Action:

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

Error Message:

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

Possible cause:

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

Action

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

9.5 Error messages regarding hardware

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

Error Message:

11) Tip expected but not present

Possible cause:

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

Action:

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

Error Message:

12) Pumphp – Pressure overload

Possible cause:

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

Action:

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

Error Message:

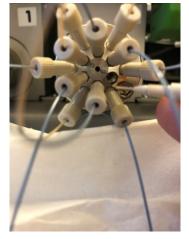
13) Pump(a,b,c,d) – Pressure overload

Possible cause:

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

Action:

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



Error Message:

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

Possible cause:

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



Action:

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

Error Message:

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

Possible cause:

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

Action:

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

Error Message:

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

Possible cause:

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

Action:

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



Error Message:

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

Possible cause:

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

Action:

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

Error Message:

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

Possible cause:

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

Action:

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

Error Message:

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

Possible cause:

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

Action



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

Error Message:

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

Possible cause

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

Action

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

Error Message:

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

Possible cause

• Pump fan faulty or not connected

Action

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

Error Message:

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

Possible cause

• Faulty pump PCB (Printed Circuit Board)

Action

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



Error Message:

23) Couldn't verify method script authencity

Possible cause

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

Action

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

Error message:

24) Flow sensor offset

Possible cause:

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

Action:

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

Error message:

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

Possible cause:

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

Action:



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

9.6 Schedule / Sample not starting / Contact closure problems

9.6.1 Troubleshooting tips for Xcalibur set-up.

How it works:

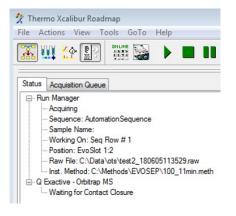
The Evosep One is set up to wait for the MS to be ready before starting a sample run, this feature ensures that the Evosep One does not run any samples if the MS is not ready to acquire data.

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

2018-06-05 11:19:30	Logging to file C:\ProgramData\Evosep\EvosepOne\logs\Runlog Schedule 5
2018-06-05 11:19:49	Communication to LC"Evosep One" established
2018-06-05 11:19:49	Starting "Schedule 5"
2018-06-05 11:19:49	Xcalibur: Ready to download method.
2018-06-05 11:19:49	Xcalibur: Acquisition request submitted
2018-06-05 11:22:21	[Info] 200 samples per day (5.6 min): Started
2018-06-05 11:22:23	[Info] 200 samples per day (5.6 min): Sample position EvoTray:EvoSlot 1:1
2018-06-05 11:29:43	[Info] 200 samples per day (5.6 min): Completed
2018-06-05 11:35:28	Xcalibur: Ready to download method.
2018-06-05 11:35:28	Xcalibur: Acquisition request submitted
2018-06-05 11:35:30	[Info] 200 samples per day (5.6 min): Started
2018-06-05 11:35:31	[Info] 200 samples per day (5.6 min): Sample position EvoTray:EvoSlot 1:2
<	

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

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



Typical issue 1:

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

Possible cause:

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

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

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

```
2018-06-06 13:26:18 Logging to file C:\ProgramData\Evosep\EvosepOne\logs\Runlog Schedule 7 20
2018-06-06 13:26:38 Communication to LC"Evosep One" established...
2018-06-06 13:26:38 Starting "Schedule 7"...
2018-06-06 13:26:38 Xcalibur: Ready to download method.
2018-06-06 13:26:38 Xcalibur: Acquisition request submitted
2018-06-06 13:26:40 [Info] 100 samples per day (11.5 min): Started
2018-06-06 13:26:41 [Info] 100 samples per day (11.5 min): Sample position EvoTray:EvoSlot 1:1
2018-06-06 13:26:44 [Info] Info: One or more pumps low on solvent, refilling now...
2018-06-06 13:26:45 [Info] Prepare: Degas
2018-06-06 13:28:14 [Info] pumpc: 50.2 bar built using 1.340 µL
2018-06-06 13:28:14 [Info] pumpa: 52.8 bar built using 1.520 µL
2018-06-06 13:28:16 [Info] pumpb: 50.2 bar built using 2.510 µL
2018-06-06 13:28:17 [Info] pumpd: 51.0 bar built using 2.650 µL
2018-06-06 13:28:21 [Info] pumphp: 202.5 bar built using 5.080 µL
2018-06-06 13:51:30 Xcalibur: Ready to download method.
2018-06-06 13:51:30 Xcalibur: Acquisition request submitted
2018-06-06 13:51:37 [Info] 100 samples per day (11.5 min): Completed
2018-06-06 13:51:37 [Info] 100 samples per day (11.5 min): Started
2018-06-06 13:51:38 [Info] 100 samples per day (11.5 min): Sample position EvoTray:EvoSlot 1:2
```

Action:

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

Typical issue 2:

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

Possible cause:

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

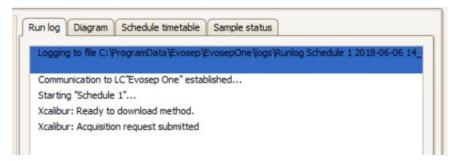
Action:

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



Typical issue 3:

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



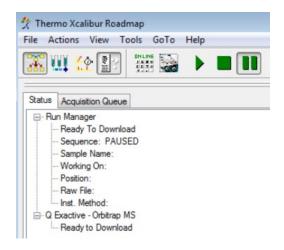
Possible cause:

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

	Analysis Method	Source Trav	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur Sample Na	Xcalibur
1	C:\Program Files (x86)\Chr\Xcalib (x 100 samples per day (11.5 min).cam	EvoSlot 1		C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_030717.meth			C:\Xcalib
2	C:\Program Files (x86)\Chr\Xcalibur 100 samples per day (11.5 min).cam	EvoSlot 1	2	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_030717.meth			C:\Xcalib
3	C:\Program Files (x86)\Chr\Xcalibur 100 samples per day (11.5 min).cam	EvoSlot 1	3	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_030717.meth	200fm_BSA_100_grad_3		C:\Xcalit

Action:

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





Typical issue 4:

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

Possible cause:

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

Action:

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

9.7 Hardware troubleshooting

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

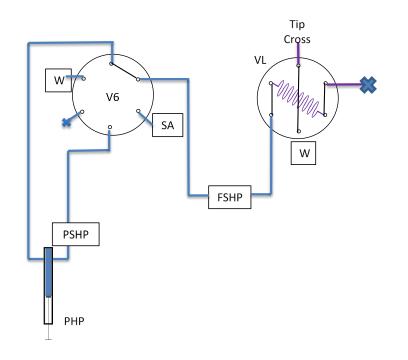
9.7.1 Leak in the HP system

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

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

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

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



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

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

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

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

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

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

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



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

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

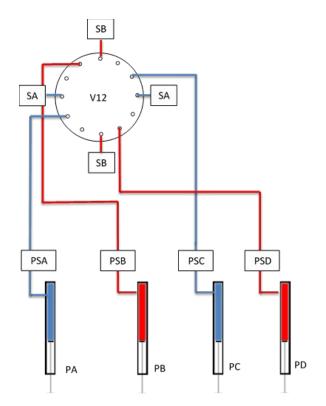
- If the script fails, exchange the rotor seal, and re-run the script.
- If the script fails, call for assistance.

9.7.2 Leak in the LP system pump to V12 area

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

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

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





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

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

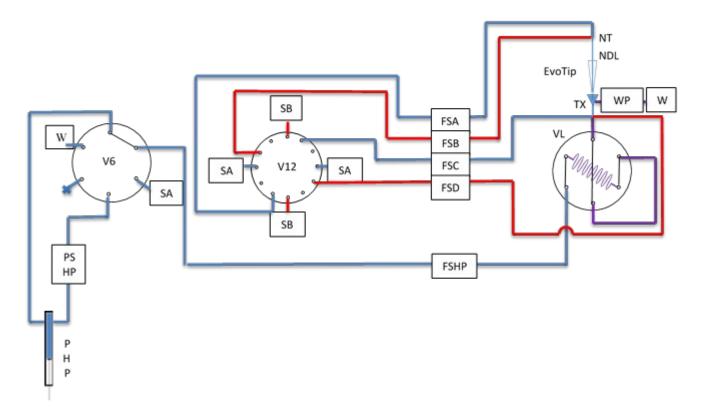


9.7.3 Leak in the LP system, Tip seal area

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

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





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

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

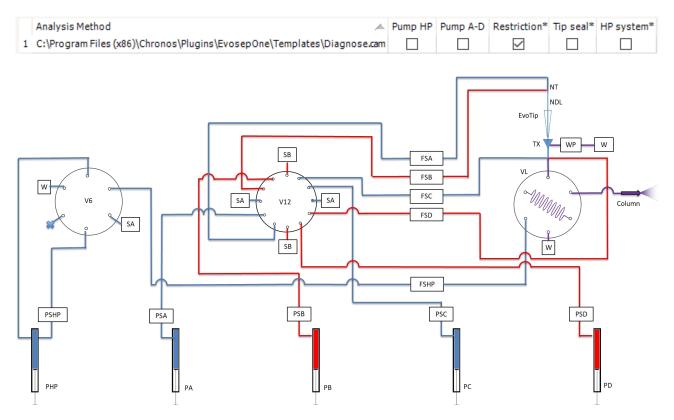


A leak is observed with a flow that is too high on FSHP, flow > 0.10 μ l/min, and the script for the HP system pass.

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

9.7.4 High restriction in the system

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



The restriction test is divided in 3 parts:

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



- 1. Disconnect the tubing on valve V12, flush the valve port with ethanol, to remove residual material and reconnect the tubing.
- 2. Exchange the tubing.
- **ii.** Re-run the script. If the restriction is still too high, call for assistance.
- 2. High pressure system
 - a. A pump HP pressure above 150 bar indicates a partially blocked high-pressure subsystem
 - i. Remove the transfer line and re-run the script.
 - ii. If the pressure drops to less than 70 bar, the transfer line is blocked or partly blocked. Install a new transfer line and re-run the script to confirm the blocked transfer line.
 - iii. If pressure is still >70 bar, remove the line FSHP to VL and re-run the script.
 - iv. If the pressure drops to less than 40 bar, the line FSHP to VL is most likely blocked or partly blocked. Install a new line and re-run the script to confirm the blocked line.
 - v. If the pressure is still >40 bar, replace the line going from the V6 to FSHP and re-run the script.
 - vi. If the pressure remains >40 bar call for assistance.
- 3. Tip interface and loop
 - a. A pressure above 50 bar for either pump A/B/C/D indicates a partial restriction of the tip interface or the loop
 - i. Disconnect the loop on the loop valve, flush the valve ports with ethanol to remove residual material and reconnect the loop.
 - ii. Exchange the loop. Re-run the script. If the restriction is still too high, call for assistance.



10 Routine Maintenance

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

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

10.1 Recommended maintenance schedule

10.1.1 Daily Maintenance

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

10.1.2 Weekly Maintenance

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

10.1.3 Prepare instrument for storage

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

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

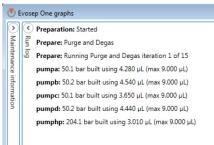


- Place both A and B line in solvent B bottle as illustrated above
- Run the Prepare Pump preparation -Solvent Exchange
- Park autosampler in lock position
- Switch off instrument

When starting up after storage

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

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



• Move the A tubing back into bottle A.



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

11 Replacing spare and wear parts

11.1 Recommended wear part list

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

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

Please find pictures of all parts in our web shop

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

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

11.2 Power off the instrument

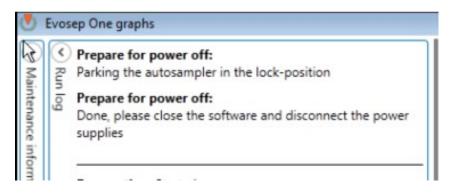
- 1. Stop any running procedures including idle flow.
- 2. Go to the Graph viewer window.



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

🖲 E	Evosep One graphs			
) M	([Run log is empty]	V V Live data Offline data Add Remove all) ف	$\mathbb{Q} \times \mathbb{O}$
Maintena	are set	0.04	Park autosampler	Park autosampler in lock-position
ince inform	ection.		Do you want to park the autosampler in the lock-position?	
rmation			OK Cancel	

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



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

11.3 Replacing the HP Pump cassette

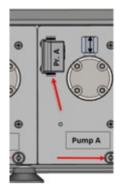
- 1. Remove the left side panel
- 2. Power off the instrument
- 3. Disconnect Viper tubing from top port on HP pressure sensor
- 4. Disconnect Viper tubing from top port on HP pump



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

11.4 Replacing the LP Pump cassette

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



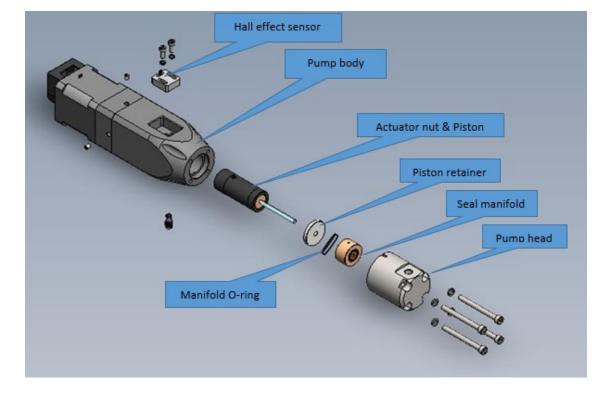
- 4. Gently pull out the pump cassette from the instrument by pulling the knurled nut, be careful not to kink or break the surrounding tubing
- 5. Insert pump cassette in opposite order



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

11.5 Replacing HP/LP pressure sensor

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



11.6 Replacing pump piston seals including seal manifold

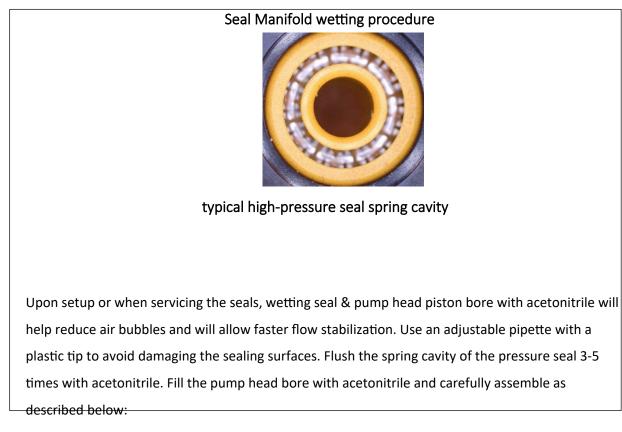
- 1. Run service script to fill pumps, which will fully retract piston within the pump house
- 2. Disconnect tubing from pump head



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

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

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



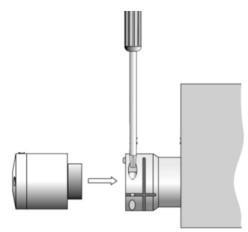
- 6. To install: First insert the seal manifold in the pump head with the O-ring pointing outwards and make sure to align the manifold drain slots vertically
- 7. Now take the pump head with the manifold and slide the manifold and pump head onto the piston
- 8. Tighten the 4 pump head screws finger-tight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

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

11.7 Replacing a valve stack (Field Service)



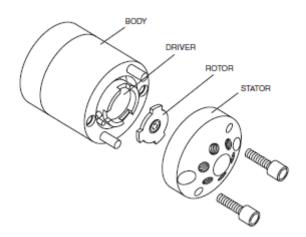
- 1. Disconnect all tubing from the stator port
- 2. For replacement of Valve 6, run the service script called "Set valve 6 pos 2-3"
- 3. Unscrew the Torx screw at the valve adapter, which holds the valve to the valve drive
- 4. Install the replacement valve and reassemble the above steps in the reverse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

11.8 Replacing valve drive actuator (Field Service)





11.9 Replacing a valve rotor and or valve stator



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

11.10 Tubing and fittings

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

Viper and nanoViper tubing



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

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

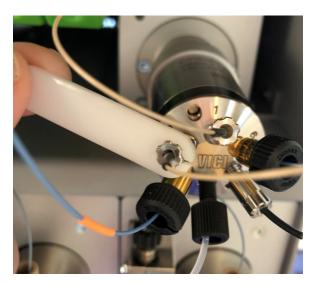
NanoConnect tubing





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

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



Peek

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

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

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



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



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



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

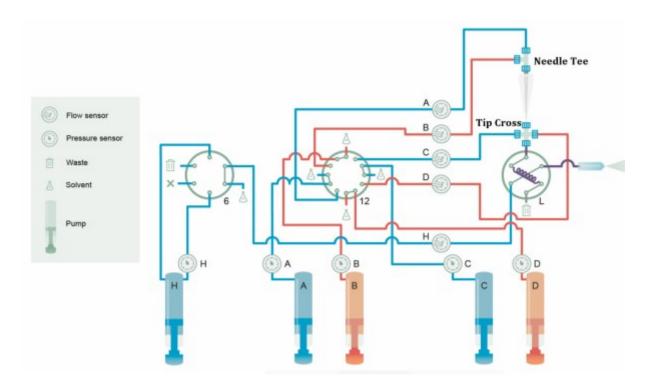
Tubing for Viper compatible ports on LP pressure sensors, comes with a 10-32 coned fitting for 1/32" OD tubing.



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



Tubing diagram and ports valve port positions



	Valve Drive 1		Valve Drive 2		Valve Drive 3
	Valve 6 tubing position	Val	ve 12 tubing position	Val	ve Loop tubing position
P		64		D 4	The
1	Pump HP	P1	Solvent B Tee	P1	TipCross



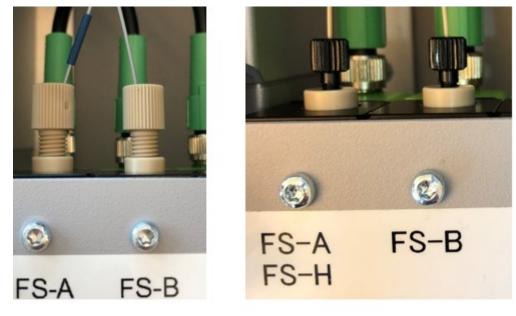
P 2	Waste	P2	Pressure sensor B	P2	Loop
Р 3	Blank	Р3	Flow sensor B	P3	Flow sensor HP
Р 4	Pressure sensor HP	P4	Solvent A Cross	P4	Waste
Р 5	Solvent A Cross	Р5	Pressure sensor A	Р5	Loop
Р 6	Flow sensor HP	P6	Flow sensor A	P6	Transferline
		P7	Solvent Tee		
		P8	Pressure sensor D		
		Р9	Flow sensor D		
		P1 0	Solvent C Cross		
		P1 1	Pressure sensor C		
		P1 2	Flow sensor C		

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

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



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



Flat bottom version

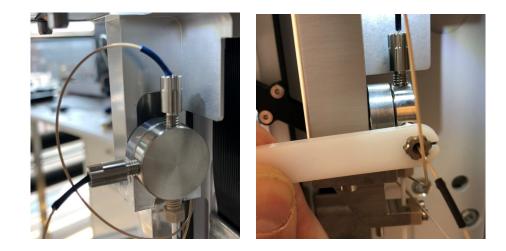
6-40 coned version

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





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



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



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





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

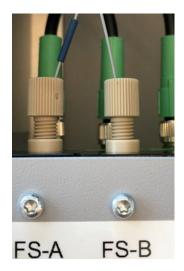


10. To connect new tubing,

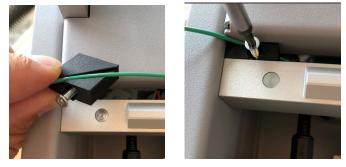
- 11. From the Pal Terminal select "RobotArmLeft"
- 12. Press "Options" and select "Change Syringe"
- 13. Press "Move" to move the needle to the exchange position
- 14. Push the tubing with the two metal fittings up through the small opening, do this from underneath and up.
- 15. Route the other end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







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

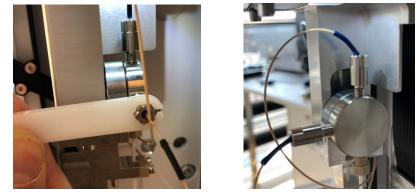


17. Put the black sleeve on the green tubing into the small groove on the black tubing holder and route the tubing on the backside of the Tee holder, put the black cover back on the tubing holder using a T6 screwdriver.





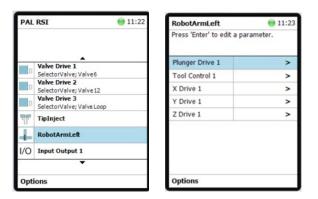
18. Now connect tubing A and B from the stainless steel Tee and tighten with the torque wrench please note that tube A has a blue label and sitting vertical and tubing B has a black label and is orientated horizontal



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

11.12 Replacing the needle

1. From the Pal Terminal select "RobotArmLeft"



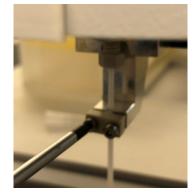
2. Press "Options" and select "Change Syringe"

RobotArmLeft	11:36	
Press 'Enter' to edit a p	arameter.	
Plunger Drive 1	>	
Tool Control 1	>	
X Drive 1	>	
Y Drive 1	>	
Z Drive 1	>	
Change Syringe		
Change Tool		
Home PALhead		
Move To Home		

3. Press "Move" to move the needle to the exchange position



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



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



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



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

11.13 Replacing the needle tee

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

11.14 Replacing the tool

1. From the Pal Terminal select "RobotArmLeft"

PAL	RSI	😝 11:22	RobotArmLeft	😑 11:23
			Press 'Enter' to edit a pa	arameter.
			Plunger Drive 1	>
0	Valve Drive 1 SelectorValve; Valve6		Tool Control 1	>
1	Valve Drive 2 SelectorValve; Valve 12		X Drive 1	>
10	Valve Drive 3 SelectorValve; Valve Loop		Y Drive 1	>
7	TipInject		Z Drive 1	>
L	RobotArmLeft	a and the		
/0	Input Output 1			
	•			
Opti	ons		Options	

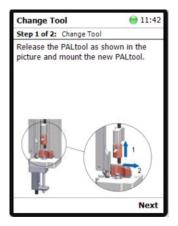
2. Press A to select "Options" and select "Change Tool"

RobotArmLeft	📵 11:40
Press 'Enter' to edit a p	arameter.
Plunger Drive 1	>
Tool Control 1	>
X Drive 1	>
Y Drive 1	>
Z Drive 1	>
Change Syringe	
Change Tool	
Home PALhead	
Move To Home	
Select	

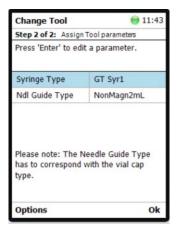
3. Press "Move" to move the tool to the exchange position



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



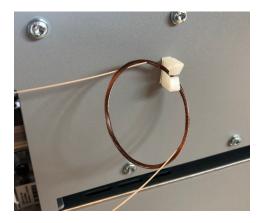
- 8. Install new tool and connect tubing and needle
- 9. Press next on terminal
- 10. Verify that syringe type = GT Syr1 and Ndl Guide =NonMagn2mL



11. Press ok to move robot arm to home position

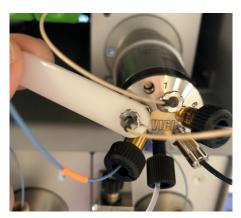
11.15 Replacing the loop

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





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



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

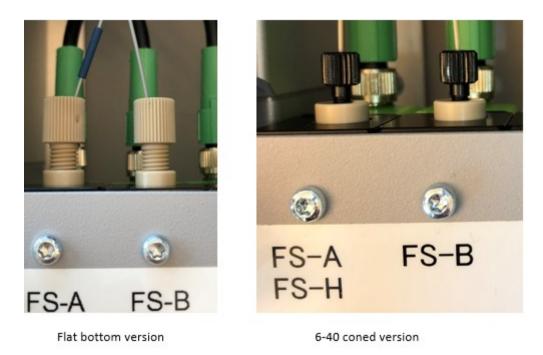
Note:

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

11.16 Replacing a flow sensor

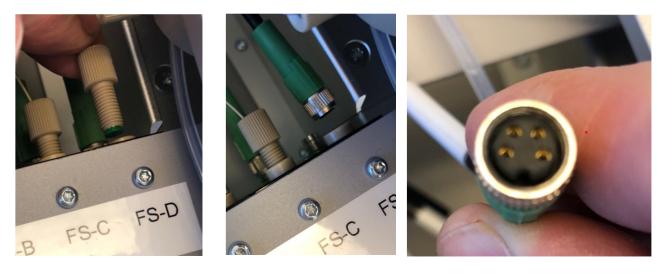
Please note that the low-pressure flow sensor (FS-A-D) comes in two versions using two different fittings

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



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

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





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



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

🙋 Add Sample(s)		
Method	Calibrate 😶	
Number of samples		
	Add Close)

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



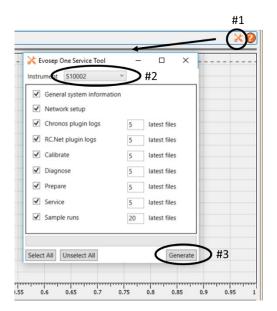
 If a low-pressure sensor (FS-A-D) has been replaced schedule and run the Tip seal test to verify that the tubing connections around the flow sensor is leak free. If the high-pressure sensor (FS-HP) has been replaced schedule and run the Diagnose HP system leak test.

12 Support, service and warranty

12.1 How to request technical support

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

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





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

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

12.2 Remote support via TeamViewer

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

😔 Evosep Support			×	
EVU	S	Е	Ρ	
Allow Remote Con	trol		\$	
Please tell your Evosep specialist the following ID and Password to connect to your desktop:				
	80 6	60 0	62	
PASSWORD		984	48	
www.teamviewer.com	[Cano	el	
Ready to connect (section)	ure con	nection)		

12.3 How to arrange for a service visit

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

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

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

12.4 Product warranty

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



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

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

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