



v. 19

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1 Preface

1.1 About This Manual

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

1.2 Safety and Special Notices

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

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

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

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



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

1.3 Contacting Us

Support: support: support@evosep.com

Sales: sales@evosep.com

1.4 Declaration of Conformity

We:

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



Apparatus model (P/N)	Evosep One (EV1000)	
Туре	General Laboratory equipment	
Manufacture site	Made in Denmark	
Manufacture year	From 2017	
Serial number	S000001 and later	NE DE

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

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

Applicable directives	•	EMC Directive 2014/30/EU
	٠	Low Voltage Directive (LVD) (2014/35/EU)
	•	RoHS 3 Directive 2015/863/EU
	•	WEEE Directive 2012/19/EU
	•	Regulation 2023/1230/EU – Machinery
The following harmonized standards and technical		EN61010-1: Safety requirements for electrical equipment for 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).

Joanna Freeke, PhD Product Manager

Freeke

July 11th, 2024



2 Introduction

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

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

- 1. More uptime with improved reliability and robustness owing to:
 - Partial elution that leaves impurities from each sample on the disposable tips that act as precolumns
 - Low pressure elution and gradient formation that cause less wear and tear
- 2. Increased productivity with higher duty cycle utilization owing to:
 - Fewer LC household steps
 - Minimized dwell time as gradient formation occurs at a high flow rate and close to the column
- 3. Increased performance with better data quality owing to:
 - Reduced cross-contamination using disposable tips
 - High flow rates during autosampler washing steps

The Evosep One technology is centered around the Evotip and integrates sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with a small plug of C18 stationary phase at the bottom of the tip. The Evotips are used to desalt and clean samples prior to LC-MS analysis; however, the traditional subsequent steps of eluting, drying down, re-suspending the samples from tips are completely omitted. Rather, the tips are loaded directly into Evosep One for analysis. This new, unique process leads to significantly less sample loss and variation, as well as much simpler and faster work flows. The Evosep One sample tray accommodates up to 6 racks of 96 tips, i.e. 576 rinsed samples may be lined up for fast, uninterrupted analysis. Upon sequence acquisition, the autosampler places one, pre-loaded sample tip at the time in-line with the solvent system, see Figure 1.



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

3 Installing the Evosep One Hardware

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

Important notes:

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

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

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

3.1 Lifting Instructions

Important notes:

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

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

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

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

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



8. The instrument can now be lifted by two persons. Please lift from underneath the instrument from each side.

3.2 Table and Trolley Requirements

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

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

Evosep One footprint for installing on trolley:

Depth	440 mm	17.3 in
Width	420 mm	16.5 in

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

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

3.3 Power Requirements

Line Voltage:

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

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

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

3.4 Temperature and Humidity Requirements

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



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

Important notes:

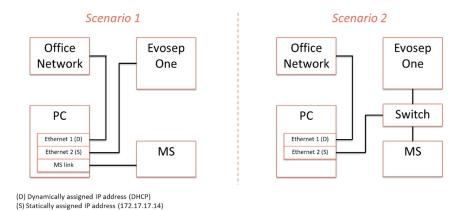
Analytical specifications require a temperature range of 22 ± 3 °C (72 ± 6 °F) with an operating humidity range of 20 - 80%, non-condensing.

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

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

3.4.1 Connecting the Ethernet Communication Cable and Checking Network Adapter Settings

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



Important notes:

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

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

🖳 🕨 Cont	crol Panel 🕨 Netwo	ork and Internet 🕨 Network Connections 🕨
Dis Surveyor N Unidentifie Broadcom	Surveyor MS Sta General Connection IPv4 Connec IPv6 Connec Media State: Duration:	Surveyor MS Properties Surveyor MS Properties Retworking Cc Internet Protocol Version 4 (TCP/IPv4) Properties General You can get IP settings assigned automatically if your network supports
	Details	The this capability. Otherwise, you need to ask your network administrator for the appropriate IP settings.

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

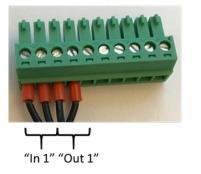
3.4.2 Connecting the Contact Closure Cable

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

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



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



Wire	Signal
Pin1	ln1 (-)
Pin2	In1 (+)
Pin3	Out1
Pin4	Out1

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

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

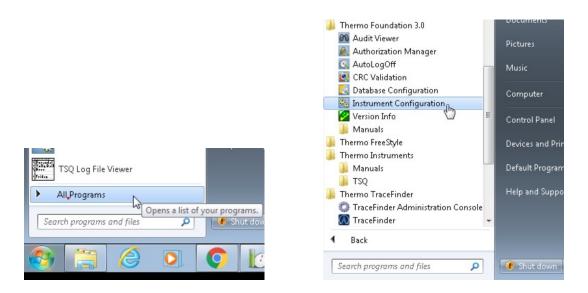
3.4.3 How to Remove Other LC Devices from MS System Configuration

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



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

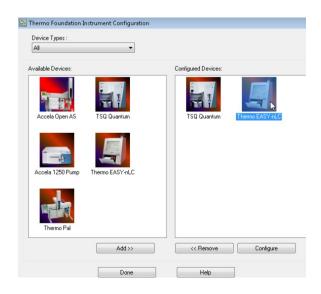
- 1. Close Xcalibur.
- 2. From Windows Start button, click "All Programs" and open the "Instrument Configuration" program. This can typically be found in one of the Thermo specific folders.



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

🖏 Т	Thermo Foundation	Instrument Configurati
	Device Types :	
	All	•
A	Available Devices:	
	100	

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



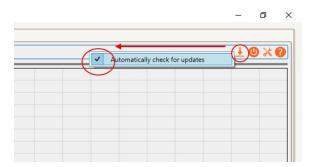
4 Installing Control Software

4.1 Automated Software Plugin Update

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

•ו
Software update check or download failed

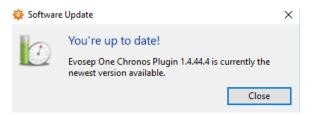
- 1. The software update process can be triggered in two ways:
 - Automatically during restart of Chronos/HyStar. The automated feature can be turned on/off by right clicking "Check for software update" button and checking /unchecking the box.



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

oftware update	@ X	0
ftware update		

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



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

🔅 Softwa	date	×
	new version of Evosep One Chronos Plugin is available!	
	sep One Chronos Plugin 1.4.44.4 is now available (you have 1.4.43.4). Would you to download it now?	
	ease 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	E
	- [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-225] Service-purge roop opdatering, brug kun solvenck til at purge roopet med (bone) [EVONE-222] Allow HD6750xConfigurer to update target without knowing, or specifying, ip information of the service servi	
	 [EVONE-208] Retention time reproducibility optimization #2, Gradient timetable precision (Decomposition of the second seco	
	>	
	kip this version Remind me later Install upda	te

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

🍄 Softwar	e Update	×
	Ready to install.	
		Install update

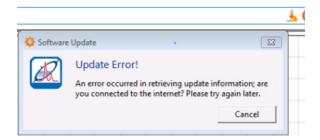
5. Press "Next" to proceed with the installation procedure.



🔀 Evosep One Chronos Plug	jin Setup	_		Х
	Welcome to the Evosep Plugin Setup Wizard	One Ch	ronos	
EVÖSEP	The Setup Wizard will install Evosep your computer. Click Next to contin Setup Wizard.			
	Back	ext	Canc	el

Important note:

The automatic software update will only work if the PC is connected to the internet. If not, the newest SW plugin can be downloaded manually from the Evosep webpage at <u>Evosep Support</u>.



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

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

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

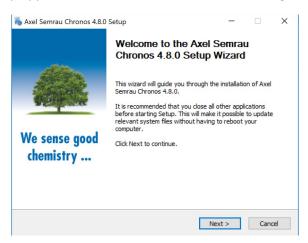
Chronos comes as a dongle-dependent version with limitations outlined below.

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



4.2.1 Installing Chronos

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



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

		continue set	tup, you need	a license key.	
Please en	ter your license	key			
8q6-9x8	-7vg-93				
Axel Semrau GmbH	_Co. KG				

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

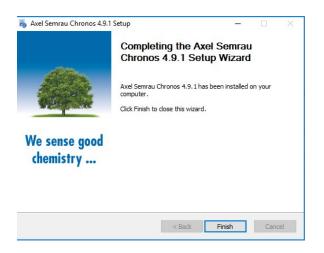
Axel Semrau Chronos 4.8.) Setup	-		×
	Choose Components Choose which features of Axel Sem to install.	rau Chronos 4	.8.0 you v	vant
Check the components you v install. Click Next to continue	vant to install and uncheck the compo	onents you dor	n't want to)
Select components to install: Space required: 142.0MB	Chronos Program files Example Methods Analyst Service Resta ChemStation interface CLF 2.5.0 export QLF 2.5.0 export QLF 3.1 export C	Description This section shortcut for Control plug	the Odor	a
Axel Semrau GmbH & Co. KG —	< Back	Next >	Can	- al
	< DACK	NEXU >	Can	Lei



6. Click "Install" to start the installation.

Axel Semrau Chronos	4.9.1 Setup	-	X
	Choose Start Menu Fold	er	
(Fight	Choose a Start Menu folder shortcuts.	for the Axel Semrau	Chronos 4.9.1
Select the Start Menu fo can also enter a name to Chronos	der in which you would like to cro create a new folder.	eate the program's sh	ortcuts. You
Accessibility			^
Accessories ADEweb.com			
Administrative Tools			
Chronos CutePDF			
Dell			
Dell Audio Dropbox			
HP			
Intel			*
Do not create shortcu			
xel Semrau GmbH & Co. K(

7. Click "Finish".



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



🕼 Evosep One Chronos Plu	gin Setup	-		Х
EVঊSEP	Welcome to the Evosep Plugin Setup Wizard	One Ch	ironos	
EVUSEP	The Setup Wizard will install Evosep your computer. Click Next to contin Setup Wizard.	o One Chron ue or Cance	os Plugin (I to exit th	on e
	Back	lext	Cano	el

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

Please read the follow	ing license agreement carefully		_	
	r - Software Lie	ense		
Agreemen	T			
-				
" <i>the software</i> " di and conditions of t terms and conditio	ep One Instrument Com istributed under this licens this license statement. If yo ons of this statement, you r	e is subject to ou do not agr	ee to a	rms
" <i>the software</i> " di and conditions of t	istributed under this license this license statement. If yo	e is subject to ou do not agr	ee to a	rms

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

Select which <u>Evosep</u> + 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
Whisper (BETA) Extended method	Whisper (BETA) methods are designed for highly sensitive, yet robust and reproducible analysis of low sample amounts.
Reset	Badk Next Can

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



🕼 Evosep One Chronos Plugin Setup	– 🗆 X
Destination Folder	EVŮSEP
Click Next to install to the default folder or click Change to choose	se another.
Install Evosep One Chronos Plugin to:	
C:\Program Files (x86)\Chronos\Plugins\	
Change	
Back	Next Cancel

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

艨 Evosep One Chronos Plugin Setup			_	Х
Options			EV 🛡	SEP
Select options below				
Overwrite Chronos configuration files				
General settings (General.cgs)				
Instrument settings (Instruments.ci	5)			
Tray settings (Trays.cts)				
	Back	Next	C	ancel

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

			_	~
🖶 Evosep One Chronos Plugin Setup		_		×
Ready to install Evosep One Ch	nronos Plugin	E	VŮSE	P
Click Install to begin the installation. installation settings. Click Cancel to e		change any of yo	bur	
	Deals	Trackell	Const	
	Back		Cancel	

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



		bnets	
User Network	IP: 192.168.167.206	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	

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

Ver. 1.603

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

륡 Evosep One Chronos Plugir	n Setup			_		\times
EVUSEP	Plugin S	ted the E Setup Wiz	zard			
		Back	1	inish	Cano	el

When updating Chronos, please note the following:

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

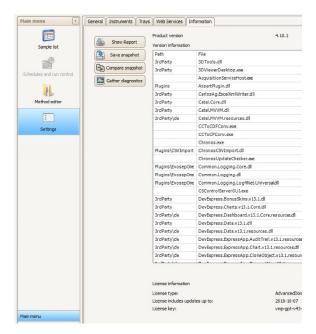


Utilities	Chronos Update	Checker	
X	Version	Release Date	About this version
Xcalbur reset	4.9.1	2017-10-23	Fixed Bugs: - The ReadBarcode task (- The ReadBarcode task (- In some cases Execute/ values: This happend if but actually a different v was not actively set. - The Heatex stirrer's ten - No understandable error barcode reader was not 1 - Some PAJ: arelated task task (no automatic link be - There was an incompet Failed tool change. - Parts of the user interf Workstation or Cairty ao Shet 4.7 - Very long sample lists u deloos, trav/val columns

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

Please er	iter your license key		
vmp-gpt	👗 Axel Semrau Chronos 5.0.0 Setup	×	
	This licence key is invalid or expired.		
	ОК		

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



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



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

Important note:

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

4.2.3 IP Configuration

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

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

4.3 Evosep Drivers for Control of Bruker MS

4.3.1 Installing ICF for Bruker Compass HyStar

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

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

Install
 Release Notes for ICF 5.6
 Release Notes for ICF 4.6
 Contact

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

😸 Bruker Plug-In for ICF 4.6 - InstallShield Wizard	×
License Agreement Please read the following license agreement carefully.	BRUKER
License Agreement	^
Bruker Daltonics Inc. and Affiliates ("BDAL")	
Product License Agreement and Limited Warranty	
Important: Please carefully read the License Agreement below before in the software product. The right to use the software product which acco- this Agreement(the "Software") is provided only on the condition that to this Agreement [Fuundo not arres to the tarms of this Agreement. It	mpanies you agree
I accept the terms in the license agreement	Print
\bigcirc I do not accept the terms in the license agreement	
InstallShield	
< Back Next >	Cancel

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

🗒 Bruker Plug-In for ICF 4.6 - InstallSh	eld Wizard
Network Services	BRUKER
Install Network Services	\sim
Install BootP service	
InstallShield	
	< Back Next > Cancel

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 wizard 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.	Please wait while the InstallShield Wizard installs Bruker Plug-In for ICF 4.6. This may take several minutes. Status:
	-
InstallShield < Back Cancel	InstallShield < Back Next > Cancel

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

📸 Bruker Plug-In for ICF 4.6 ·	InstallShield Wizard				
\sim	InstallShield Wizard Completed	# 0 Staylor Statution Spranet Statution Type of Statution Spranet Statution Spranet Statution (a) (3) (2) (3) (3) (3) (3) (3) (3) (3) (3) (3) (3		k 🗷 Dag	هم ها می • م
BRUKER		BRUKER INSTALLATION QUALIFICA		- <u>0</u> - 3 0 - 6	ago + Safaty + Tools + ⊕+
	The InstallShield Wizard has successfully installed Bruker Plug-In for ICF 4.6. Click Finish to exit the wizard.	Produce: Dinker Playsh for UCF 46 Date: 6262019 59:958 AM Worksteine: MCRE0_TOPQBI, Windows 7 Professional Service Pack 1 (NT Build 6.1.7601, IE.)			
	-	8) File(s) checked 0 File(s) compt Jaubennis Check Swcreeded			
		IQ passed	IQ failed		
		(circle appropriate) Remarks:			
		(ano, and)	(signature)		
	< Back Finish Cancel		EXPECTED CRC32:	FOUND CRC32:	RESULT
		C:/BDalSystemData/HyStar/LePlugin/CfgFiles/AgilentICFSystem.cfg	816EA610 Size: 267	CRC32: 816EA610 Size: 267	Checked O.K.

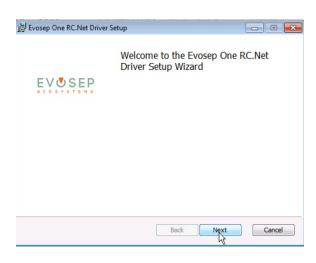
9. Click "Exit" to close the CD start menu.

	BRUKER
Plug-in for ICF	5.6 & ICF 4.6 • Install • Release Notes for ICF 5.6 • Release Notes for ICF 4.6 • Contact

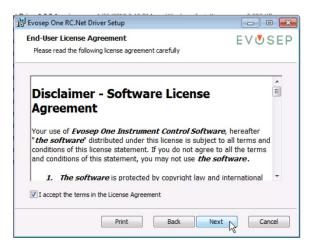
4.3.2 Installing the Evosep One RC.Net Driver 2.x.x.msi

Make sure the ICF plugin for HyStar is already installed.

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



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



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

	C 000 1/0
😸 Evosep One RC.Net Driver Setup	- • •
	EVOSEP
Click Install to begin the installation. Click Back to review or change any installation settings. Click Cancel to exit the wizard.	y of your
Back 😗 Install	Cancel

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

岁 Evosep One RC.Net Driver Setup			
Installing Evosep One RC.Net Driver	EVঊSEP	V Evosep One RC.Net Driver Setup	
🛞 User Account Control 🛛 🕰		Installing Evosep One RC.Net Driver	EVÖSEP
Please wait while the Se Do you want to allow for software on this some		Ve 😵 User Account Control	
software on this comp Status:		Please wait while the Se Do you want to a software on this	allow the following program to install computer?
Program name: Verified publisher:			me: Evosep One lisher: Evosep ApS zitor: Install 1.0.204 Evosep Biosystems English (United States)
		Show infor	S\Šoftware\HyStar\Evosep One RC.Net Driver 1.0.20.4.msi nation about this publisher's certificate
Back Next	Cancel	Hide details	Change when these notifications appear

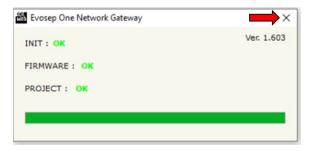
9. Choose which Evosep+ application you wish to install in addition to the standard methods.

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.	Whisper (BETA) methods are
Extended method	designed for highly sensitive, yet robust and reproducible analysis of low sample amounts.

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

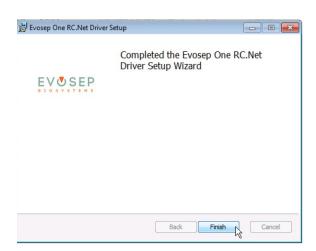
Adapter	S	ubnets	
User Network	IP: 192.168.167.200	5 Mask: 255.255.255.0	
Surveyor MS	IP: 172.17.17.14 Mask: 255.255.255.240 IP: 172.16.0.101 Mask: 255.255.0.0		

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



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





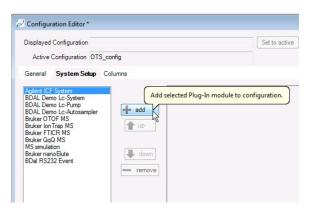
- 4.3.3 Create HyStar IFC Configuration for Evosep One
 - 1. Open Compass HyStar and click on the gearwheel icon to open the Configuration Editor.



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

Configuration Editor *				×
Displayed Configuration OTS_config Active Configuration OTS_config		Set to active	1 Export	Import
General System Setup Columns				
Aglent ICF System BDAL Demo Lc-System BDAL Demo Lc-Pump	Bruker OTOF MS			Settings
BDAL Drem L2 Advancer Buder Do Trag MS Buder TO Trag MS Buder TCR MS Buder TCR MS MS smulden Buder RS22 Event	micOTOFQ III SN 82288	88.203559 time of	fiset (sec.) 0	
Create a new confi	guration	Report	(Close

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





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

Displayed Configuration	Set to active	Export	
Active Configuration Evosep One micrOT)F		
General System Setup Columns		1	
SDAL Demo Lc-System SDAL Demo Lc-System SDAL Demo Lc-Autosampler	Agilent ICF System	🕖 Settings	
Hardware Configuration Dialog			0
 Agiere R.LSD. Agiere R.Net SDK Sample Drivers CTC Analytics LC Brosep Drivers Agieret 1100/1200/1260/1250 LC CTC Analytics RA CTC Analytics RA Agieret 1120/1220 LC Systems Agieret 1120/1200 LC Agieret 1120/1200 LC 			Evosep One (EVOSEP_ONE N/A)
		3 < Auto Configure	

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

Configuration Editor * Displayed Configuration Active Configuration OTS_c	onfig		Set to active	🟦 Export) 🛃 Imp	ort.
General System Setup C	olumns				
Agent IC System BRA Upon Lo System BRA Upon Lo System BRA Upon Lo Areno BRA Upon Lo Arconsomer Barker OTOP MS Barker ToTCR MS Barker ToTCR MS Barker ToTCR MS Barker TotCR MS Barker nanoEke BDal RS232 Event	up up down remove	Aglert ICF System SAMPLER EVOSEP_0 Buker OTOF MS	NE-	Settings	
New	Dpen	Save Save	As	Close	

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

Configuration Editor * Displayed Configuration Active Configuration OTS_config	Set to active	Export)	Connected MS Ins	strument
Aufer Comparison Oragona General System School Colum BOAL Demo Lo System BOAL DEMO System BOAL	(Settings	MS Instrument Ini Name Ser. No. Type Station ID Server Host	msControl detected Auto detect / connect to MS s'Auto detect / button to update the requested instrument information) formation micr0T0F-Q III 8228888.20359 otof-series 1 localhost
New 1	Cpen Save Save As	Close		OK Cancel

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



Displayed Configuration		Set to active	1 Export	
Active Configuration OTS_c General System Setup C				
	_	Save Configuration		
Aglent ICF System BDAL Demo Lc-System		File name	Date modified	
BDAL Demo Lc-Pump BDAL Demo Lc-Autosampler	- add	270318TestConf	3/27/2018 3:01 PM	
Bruker OTOF MS	-	290318_Conf_with_Evosep_One	3/29/2018 2:11 PM	
Bruker IonTrap MS	👚 up	ARGON_MU1	9/18/2017 5:10 PM	
Bruker FTICR MS Bruker QaQ MS		CaptiveSpray_TOF_nanoEute	9/25/2015 9:20 AM	
MS simulation		CaptiveSpray_TOF_nanoEute_160307	7/21/2016 9:10 AM	
uker nanoEute	🖊 down	CaptiveSpray_TOF_nanoEute150928	9/28/2015 9:50 AM	
BDal RS232 Event		CaptiveSpray_TOF_nanoEute150928_HT	10/5/2015 10:36 AM	
	- remove	CaptiveSpray_TOF_nanoEute150928_LT	10/5/2015 11:59 AM	
		CaptiveSpray_TOF_nanoEute150928_Toff	10/5/2015 11:47 AM	
		CaptiveSpray_TOF_nanoEute151217	12/17/2015 11:54 A	
		CaptiveSpray_TOF_nanoEute160120	1/20/2016 10:36 AM	
		configuration	3/19/2018 2:18 PM	
		CTC_ICF	11/21/2017 9:14 AM	
		* [•	
		Selected Name		
		Evosep One micrOTOF		
		-	OK Cancel	

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

Configuration Editor Displayed Configuration Evosep Active Configuration OTS_co		Make the displ	ayed configuration active.	
General System Setup Co Rota 255 States BDAL Cens Lo-Fum BDAL Cens Lo-Fum BDAL Cens Lo-Fum Bulker Ion Time MS Bulker Ion Time MS Bulker Ion Time MS Bulker Ion Time MS Bulker Ion States Bulker	Aglent ICF Sys add SAMPLER E D Buker OTOF N	/OSEP_ONE-	Settings Settings et (sec.) 0	Compass HyStar The configuration has been changed. In order to update the 'Active Configuration' HyStar will restart now.
New	Save	Save As Report	Close	ок

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

4.3.4 Create Evosep Separation Methods

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

🔟 Compass HyStar 4.1	- [Compass]				
Navigator Acquisition	n View Options Compa	ass H			
🕆 Compass 📄 Me	thod Set 📰 Sample Ta	ble	Method Set Editor. Method Set:	New	-
			Separation Method		
Instruments	Open the Method Set	Editor	Injection Method	Standard 🔹	Edit
HyStar	disconnected	Bru	MS Method		
disconnected	Evosep One	d	Scheduled Precursor List (SPL)		
	Offline		Processing Method		
Connect all instruments					

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	I	CF System Me	thod Dialog	
Separation Method Name: Total Runtime 500 min Edit sequisition time Available Modules Acquiation Time Missing method data		ICF Method Name Description Runtime	Auxiliary Traces 100 samples per day 11.5 min acquisition time 11.5 min	•

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

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

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

eparation Method	Name: 100 samples per da	м	
		Hardware Modules	
Total Runtin	me 11.50 min	Available Modules	
		Agilent ICF System	Edit Method
Edit acquisit	ion time		
Acquisition Time	e		
Start time	0.00 min		
Acquisition time range	11.50 min		
Stop time	11.50 min		

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

Separation Method	100 samples per day		= 🖌
Injection Method	Standard		• /
MS Method			
Scheduled Precursor List (SPL)			
Processing Method			
met:			
mert		The Days Dec Sector Store	

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.

	Evosep_100_JCF.m II JP.m	4/6/2018 3:11 PM	File folder	
	100 Samples Evosep One.m	3/29/2018 2:15 PM	File folder	
	Bruker-100_270318.m	3/27/2018 3:14 PM	File folder	
	😹 GeometryFiles	3/27/2018 2:34 PM	File folder	
Method name:	Evosep 100 smp per day			
Save as type:				
Comm	ent			

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



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

4.3.5 Create Evosep One Tray Type and Sample Table

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

🕷 Compass HyStar 4.1 -	[Compass]			
Navigator Acquisition	View Options Compa	ss Help		Sample Table Editor. Sample Table: New*
😭 Compass 📘 Meth	od Set 🛛 🔛 Sample Tak	le 🛃 Acquisition	👁 Quick D	[]] 🖉 + × 🏨 Agilent 100 Vial Holder (Vials_100) 😅 🗸
Instruments	Ope	n the Sample Table E	ditor (Offline)	년 Configure
HyStar	disconnected	Bruker OTOF MS		
disconnected		disconnected		
	Evosep One			
	Offline			
Connect all instruments	HP Pressure bar	micrOTOF-Q III		

2. Choose "Evosep" as the "Tray type". Select "96Evotip" for "Slot 1" - "Slot 6". Click "OK".

Tray Configur	ration		🔲 Tray	Configuration	
Tray type: Eve Agi Agi Agi Agi CTC CTC CTC CTC CTC CTC CTC CTC	sep crown lerr 100 Val Holder err Matsampler Holder err Val Holder CPAL3 Tay Holder CPAL3 Tay Holder CPAL3 Tay Holder CPALXT MT Holder CPALXT MT Holder CPALXT MT Holder		Tray typ Slot 1: Slot 2:	96Evotp	•
	C-PALXT Pelier Stack C-PALXT TrayX		Slot 3: Slot 4:	96Evotp	
			Slot 5: Slot 6:	96Evotp	
		OK Cancel			OK Cancel

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

⊙ S1				O 52				O 53						O 54		
Ne	w •	늘 Open	ж	Delete	Save	Save /	As	Report -	Import/Export -	Op	tion	is •		-		
	Line	Vial		Status	Sample ID	Inj.	Volume [µl]		Data Path			Method Set	Separation Metho	bd	Injection Met.	MS Method
•	✓ 1	S1-A1	*		test	1		0 D:\Data\		*			100 samples per day	*	-	
			*					0		-		Open		-	-	
											~	Use Individua	al Methods			
												Evosep 60 sm	np per day.m			
												Evosep 100 s	mp per day.m			

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



- 5. Click "Close" to close the Sample Table Editor window.
- 4.3.6 Flow to Column 20% Solvent B Enables MS calibration
 - The service program, Flow to column 20% sol B, is intended to aid MS calibration. It will run 20% solvent B to the transfer line at 250 nl/min for 10 min. The program requires a Evotip in position S1-A1.

4.4 Evosep One Driver for SCIEX OS

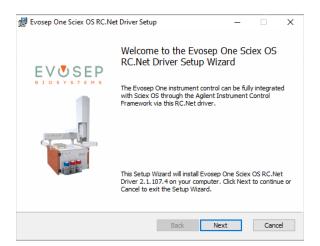
4.4.1 Installation Prerequisites

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

Services (Local)				
Clearcore2 Service	Name	Status	Startup Type	Description
Stop the service Restart the service	🎑 Clearcore2 Service	Running	Manual	Required for Mass Spectrometer acquisition.
	🖏 Client License Servic		Manual (Trigger Start)	Provides infrastructure support for the Microso
	CNC K	D	M	The CNC (contraction of the board to also)

4.4.2 Installation Procedure

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



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

End-User License A	Agreement	EVO	SE
Please read the follow	wing license agreement carefully		
Disclaime Agreemen	r - Software Lico t	ense	^
" <i>the software</i> " of conditions of this I and conditions of t	ep One Instrument Contra distributed under this license icense statement. If you do n this statement, you may not	is subject to all terr not agree to all the i use <i>the software</i> .	ns and terms
	ware is protected by copyric	tht law and internati	onal 🗡
_	in the License Agreement		

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

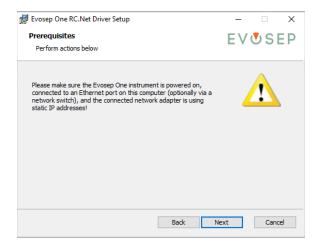
🕼 Evosep One Sciex OS RC.Net Driver Setup	– 🗆 X
Evosep+ applications Select which Evosep+ applications you need according their install state in the list below.	EV USEP*
Evosep + application selection is also available fro OS RC.Net Driver [®] folder in Windows Start.	om a shortcut in the "Evosep One Sciex
Whisper (BETA) Extended method 15 SPD X V High organic method 100 SPD	Whisper (BETA) methods are designed for highly sensitive, yet robust and reproducible analysis of low sample amounts.
Reset	Back Next Cancel

Important notes:

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

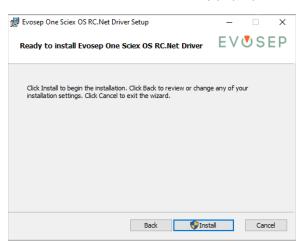
During the user training, the +applications, and how to select them from the Evosep folder from the Windows Start menu, should be discussed.

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





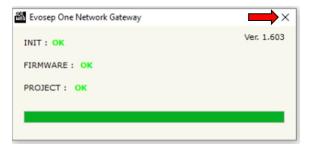
7. Click "Install" to begin the installation. Click "Yes" to any pop-up windows during the install.



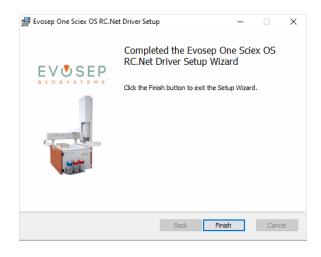
8. Click show details (if window not fully visible) then click "Yes" to allow the program to install the software.

Evosep One RC.Net Driver Setup			
Installing Evosep One RC.Net Driver	EVঊSEP	Evosep One RC.Net Driver Setup	EVÖSEP
West Account Control Image: Control Please wait while the St Image: Control Image: Contro Image: Control Image			
Status: Program name: Verified publisher: Show det/3s		Verifie Verifie Verifie Progra	Im name: Evosep One d publisher Ivosep ApS min locatin: Install 1.0.204 Evosep Biosystems English (United States) S.Software Hybrah (Socep One RC.Net information about this publisher's certificate
Back	Cancel	Hide details	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". Close the dialog by clicking the "X".



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



- 4.4.3 Create SCIEX OS Hardware Configuration for Evosep One
 - 1. Start SCIEX OS this will automatically start the Clearcore2 Service.
 - 2. Select "Configuration".



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



4. Click the "Add" button on the toolbar.

Add

5. On the "Device" dialog, select "Integrated System" and "Agilent Integrated System". Click "Settings...".

Device	×
Select the device and then adjust the com	munication settings to test the device.
Type Integrated System	~
Model Agilent Integrated System	✓ Settings
Test Device	
	Save <u>C</u> ancel

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

Version: 1.0.0 Manufacturer: Agilent Simulate Device Agilent Settings Communication IP Address 172 • 17 • 1 Full View Configure Configuration Available Devices Iso Pump Qual Pump Qual Pump Qual Pump Qual Pump Qual Pump Column Comp. Sampler Low Flow Fung Pump Pres Pump Oluster Column Comp. Sampler Low Flow Fung Pump Pres Pump Oluster Column Comp. Sampler Low Flow Fung Pump Pres Pump Oluster Column Comp. Sampler Low Flow Fung Pump Pres Pump Cluster Column Comp. Sampler Low Flow Fung Pump Pres Pump Cluster Column Comp. Sampler Low Flow Fung Pump Pres Pump Cluster Column Comp. Sampler I Exosep One[EVDSEP_ONE nig] Remove Fund Remove Fund					
Version: 10.0.0 Manufacturer: Agilent Simulate Device Agilent Settings Communication IP Address 172 17 17 1 IP Address 172 17 17 1 IP Address Setected Devices ISo Pump Dual Pump Qual Pump Qual Pump Qual Pump Qual Pump Qual Pump Configuration Add Simpler 1: Evosep One[EVOSEP_ONE_nig] Market Pump Column Comp. Sampler 1: Evosep One[EVOSEP_ONE_nig] I Add Simpler 1: Evosep One[EVOSEP_ONE_nig] I Add Simpler 1: Evosep One[EVOSEP_ONE_nig] Add Simpler 1: Evosep One[EVOSEP_ONE_nig] I Add Simpler 1: Evosep One[EVOSEP_ONE_nig] I Configuration I Add Simpler 1: Evosep One[EVOSEP_ONE_nig] I Configuration 1: Evosep One	Device D	iver			
Analacturer: Agilent Simulate Device Agilent Settings Communication IP Address 172 17 17 1 Auto Configuration Available Devices Iso. Pump Bm. Pump Bm. Pump Bm. Pump College Pump College Pump Low Flow Sampler HP Sampler Low Flow Sampler Low Flow HiP Sampler Low Flow HiP Sampler Continues	Name:	Agilent Integrated System			
Simulate Device Agilent Settings Communication IP Address 172 17 1 Paddress 172 17 1 Paddress 172 17 1 Pathology 172 17 1 Pathology 172 17 1 Pathology 172 17 1 Pathology 172 1 Pathology	Version:	1.0.0.0			
Aglient Settings Communication IP Address 172 17 17 1 Futo Configuration Available Devices Iso Pump Oust Pump Oust Pump Prep. Pump Cluster Column Comp. Sampler Prep. Sampler Low Flow HIP Sampler Prep. Sampler Continues	Manufacturer:	Agilent			
Iso. Pump Bin. Pump Dout Pump Doug Pump Perse, Pump Cluster Column Comp. Sampler Low Flow Sampler Low Flow HilP Sampler Low Flow HilP Sampler Low Flow HilP Sampler Column Comp. Sampler Low Flow HilP Sampler Comfigure		ngs			
DAD		172 • 17 •	17 . 1	Auto Configu	ure

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

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	Cano	cel	

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

Test Device

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

Device X
Adjust the communication parameters and then test the device.
Type Integrated System
Model Agilent Integrated System
Test Device The test was successful.
Device Display Names
Integrated System Evosep One
: Autosampler - Evosep One
Save

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



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

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	ft 🕐
Evosep One	٧
ZenoTOF™ 7600 System (simulation)	<u> </u>
Calibrant Delivery System	٣
MS Check	+-
Direct Control	
Standby	Gquilibrate

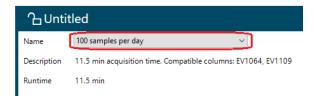
- 4.4.4 Create SCIEX OS LC Methods for Evosep One
 - 1. In the top-left drop-down menu, click "LC Method".

	Configuration
Acquis	ition
	Batch
	Queue
	MS Method
ð	LC Method
	MS Tune
Proces	sing
A	Explorer
°,	Analytics
Manag	ement
菜	Configuration
U	Library
1	Event Log

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".



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



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

Important note:

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

4.5 Evosep One Driver for Thermo Chromeleon / Xcalibur

4.5.1 Chromeleon vs SII for Xcalibur

SII for Xcalibur is basically a Chromeleon installation repackaged. You should choose to install either one or the other, but <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 the operating system and environment in general, follow the requirements for the version of Chromeleon or SII for Xcalibur, which the driver will be running under. Currently, Windows 10 64-bit US English is supported.

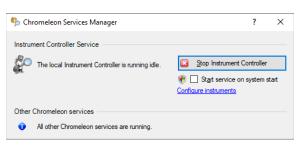
For Thermo SII for Xcalibur 1.7, Thermo Foundation 3.0 SP2 or later is required.

No specific Xcalibur version is mentioned by the SII installer, but Release Notes state that validation was done with Xcalibur 4.5 and 4.5 SP1.



4.5.3 Prerequisites

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



4.5.4 Chromeleon/SII for Xcalibur License

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

Chromeleon Administration Console (DESKTOP-OL77A2	:H)			
Corror Romoor Cortolor Locrose Cortolor Cortose Cort	Clief Licenses Assign client licenses to user accounts or ins <u>Instanent Controller Licenses</u> Assign licenses to instrument controllers. <u>Manage Licenses</u> Verw, add, activate and remove license cod		lers.	
Manage Licenses			?	×
License codes				_
Code	Status	Version	Add	
CMEI111002-10EA-P8CV-BD1F-TR02-G2TK-NHGR-XAPA	Time-limited (Time-limited license expires 13-D	7.3.2	Remove	
			Activate	
				_
			Details	
			Close	

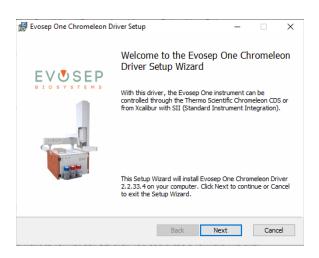
3. Under "Instrument Controller Licenses", make sure that at least one Class 3 instrument is allowed.

S Chromeleon Administration Console (DESKTOP-0L77A2H)							
License Manager Licenses Licenses Instrument Controller Licenses		Assign licenses to instrumer		ises			
		Drag a column header here to	group by that col	umn.			
		Instrument Controller	Status	Instrument Class 1	Instrument Class 2	Instrument Class 3	3 Ac
	▶	DESKTOP-OL77A2H	🗸 ок	0 🗸	0 🗸	1 -	
	*			•	*	•	ļ



4.5.5 Installation Procedure

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



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

d Evosep One Chromeleon Driver Setup	- 🗆 X
End-User License Agreement	EV U SEP
Please read the following license agreement carefully	LVUJLF
Disclaimer - Software Licens Agreement	e
Your use of <i>Evosep One Instrument Control So</i> " <i>the software</i> " distributed under this license is su conditions of this license statement. If you do not a and conditions of this statement, you may not use if 1. <i>The software</i> is protected by copyright la	bject to all terms and gree to all the terms the <i>software</i> .
$\ensuremath{\ensuremath{\square}}\xspace$ I accept the terms in the License Agreement	
Print Back	Next Cancel

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



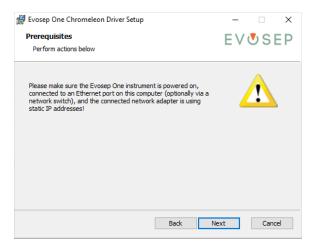
🛃 Evosep One Chromeleon Driver Setup	– 🗆 X
Evosep+ applications Select which Evosep+ applications you need acc their install state in the list below.	ess to by setting
Evosep + application selection is also available fr Chromeleon Driver* folder in Windows Start.	om a shortcut in the "Evosep One
Whisper (BETA) Extended method 15 SPD X • High organic method 100 SPD	Whisper (BETA) methods are designed for highly sensitive, yet robust and reproducible analysis of low sample amounts.
Reset	Back Next Cancel

Important notes:

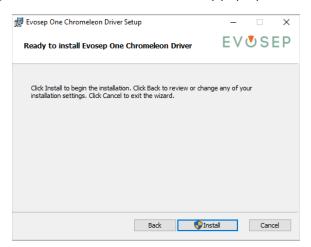
The +applications are not installed during a standard installation as Evosep prefers that the customer actively chooses the +applications they need.

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

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



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





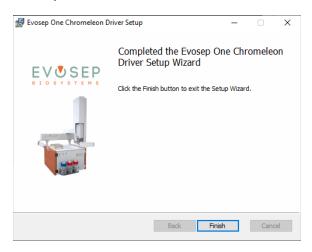
8. Click "Show details" (if window not fully visible) then click "Yes" to allow the program to install the software.

By Evosep One RC.Net Driver Setup			
Installing Evosep One RC.Net Driver	EV Ö SEP	Evosep One RC.Net Driver Setup	
😵 User Account Control 🛛 🕰		Installing Evosep One RC.Net Driver	EV®SEP
Please wait while the Se Do you want to allow Software on this comp Status:			to allow the following program to install this computer?
Program name: Verified publisher: Show det		Verifies Progra	m name: Evocep One I publisher: Evocep ApS m location: Install Evocep Biosystems English (United States) Si Software HyStar Roosep One RC.Net Information about this publisher's certificate
Back Next	Cancel	Hide details	Change when these notifications appear

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

osep One Chronos Plugin Setup	—	
		40S
SW67508 Ethernet Update	>	<
INIT : OK	Ver. 1.004	
FIRMWARE : OK		
PROJECT : OK		
		-
		1

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

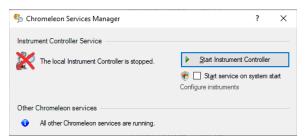


4.5.6 Checking Driver Installation



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

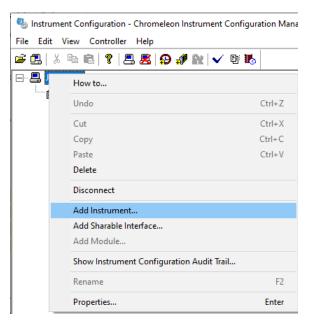
- 4.5.7 Create Chromeleon Hardware Configuration for Evosep One
 - 1. From the "Chromeleon Services Manager" window, click "Start Instrument Controller".



2. Click "Configure instruments".

🎭 Chromeleon Services Manager	? ×
Instrument Controller Service	Stop Instrument Controller Configure instruments
Other Chromeleon services All other Chromeleon services are running.	

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



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

0	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

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

🍰 Add module to instrument			×
Instrument JPLAPTOP_1 Manufacturers: Evocep Biosystems Fluke	^	Modules: Vosep One	_
Futuke Gilson HP Isco Kontron Markes Markes Perkin Elmer Polymer Laboratories Rarin Rheodyne Shordex S	*		
		OK Cancel	

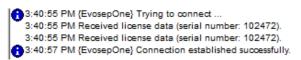
7. On the Evosep One Driver Configuration dialog, you can set the basic instrument settings:

💀 Evosep One Drive	er Configura –	-	×
Device Configuration			
Device Name:	EvosepOne		
Pumps Address	172.17.17.1		
Pal Address	172.17.17.2		
Auto Idle-flow			
Simulation mode			
	ОК	Cance	el

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



- Auto idle-flow: Start idle-flow after a few minutes of inactivity.
- Simulation mode: Use for testing without an Evosep One device present. This will offer some very basic methods for testing, including emitting generated pump trace data.
- 8. When satisfied with the configuration, click "OK" to close the dialog.
 - The message window should show something like this:



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

e g	Instrun	nent Co	nfiguration -	Chrom	eleon Instrun	
File	Edit	View	Controller	Help		
	Impor	t			Ctrl+I	
	Save I	nstallati	ion (JPLAPTC	OP)	Ctrl+S	
	Expor	t				
	Print Ctrl+P					
	Recen	t File				
	Lock (Client				
	Exit				Alt+X	

- 10. Upon successful configuration, you may close the window.
- 4.5.8 Configuring Thermo Scientific SII for Xcalibur

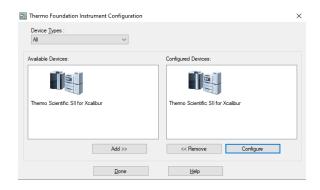
Important note:

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

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



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



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

SII for Xcalibur (Configuration	×
	<u>C</u> onfigure Device	
	Selected instrument system:	
	JPLAPTOP_1 v	
	Controlled by <u>e</u> xternal autosampler	
ОК	Cancel <u>H</u> elp	

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

4.5.9 Important – When Upgrading from an Earlier Driver Version

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

😘 Services						- 0	×
File Action View	Help						
(+ +) 📰 🖾 🖸	à 🗟 📲 📰 🕨 🗰 💷 🕪 👘						
🔍 Services (Local)	O Services (Local)	-					
	Thermo Foundation Acquisition	Name	Description	Status	Startup Type	Log On As	^
	Service	Chermo 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:	Control Con	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:



	Acquisition Server	×
Acquisition Server (Thermo Scientific SII for Xcalibur) × Information:Problem detected during Stop Analysis. Please delete the current acquisition queue.	Thermo Scientific SII for Xcalibur device reported a failure during Prepare For Run command The sequence has been paused. To resume the list, go to the "Actions" main menu	
	and uncheck the "Pause Analysis" menu item.	

4.5.10 Create LC Methods for Evosep One

This section describes how to create LC methods through Xcalibur.

1. In Xcalibur, click "Instrument Setup".



- 2. Click "Next".
 - The run time can be ignored here as it is set automatically when selecting the Evosep One method next.

	X 8					2010/2010/07		-
ĩei		ttings for System.					Ş	K
hermo científic SII for	20.000 Diagnost	specify the run tim	(0.100100)	00.000 min]				
	No	Channel			Select all channels			

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

ame	100 samples per day	•
escr	iption 11.5 min acquisition time. Compa	ble columns: EV1064 EV1109
untir		
No	Channel	
1	Pump_A_Displacement	
2	Pump_A_Actual_flow	
3	Pump_A_Setpoint	
4	Pump_A_Pressure	
5	Pump_A_Pump_speed	
6	Pump_B_Displacement	
7	Pump_B_Actual_flow	
8	Pump_B_Setpoint	
9	Pump_B_Pressure	
10	Pump_B_Pump_speed	
11	Pump_C_Displacement	
12	Pump_C_Actual_flow	
13	Pump_C_Setpoint	
14	Pump_C_Pressure	
15	Pump_C_Pump_speed	
16	Pump_D_Displacement	
17	Pump_D_Actual_flow	
18	Pump_D_Setpoint	
19	Pump_D_Pressure	
20	Pump_D_Pump_speed	
21	Pump_HP_Displacement	
22	Pump_HP_Actual_flow	
23	Pump_HP_Setpoint	
24	Pump_HP_Pressure	
25	Pump_HP_Pump_speed	

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

4.6 Adding Specialized Applications to the Evosep One

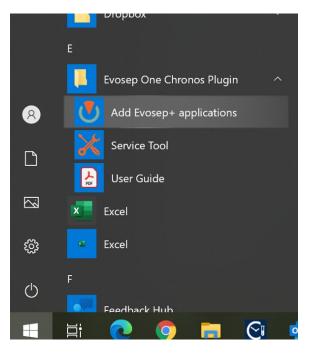
The Evosep One is preconfigured with 6 standard methods with throughput ranging from 500 to 30 samples per day. In addition, it is now possible to add several specialized methods.

Important note:

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

Methods must be manually enabled by the following procedure:

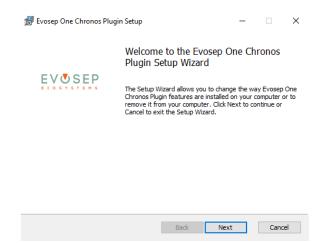
- 1. Close the Evosep One control software (Chronos/HyStar).
- 2. Depending on what software is being used on the Evosep One, choose the appropriate folder from the Windows Start menu. I will be one of:
 - Evosep One Chronos Plugin
 - Evosep One HyStar Driver
 - Evosep One SCIEX OS Driver
 - Evosep One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



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

Important note:

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



5. Click the "Extended method" icon.

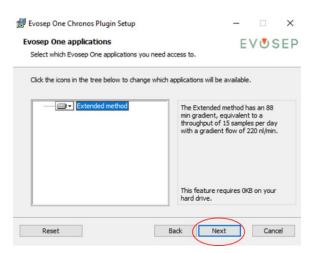


vosep One Chronos Plugin Setup		-		
osep One applications		E	V 🛡 S	5 6
Select which Evosep One applications you r	eed access to.	_		
Click the icons in the tree below to change	which applications will be av	ailable		
x • Extended method	The Extended me min gradient, equ throughput of 15 with a gradient fo	ivalent sample	t to a es per day	
	This feature frees hard drive.	up 32	KB on you	ır
		_		

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

🖟 Evosep	One Chronos Plugin Setup	-		×
	One applications hich Evosep One applications you need access to.	E	VŪS	EP
Click the	icons in the tree below to change which applications will be	available	2.	
	X - Extended method The Extended	method h	as an 88	
	Will be installed on local hard drive		to a per day	
	B Entire feature will be installed on local hard dri	ive	20 nl/min.	
	× Entire feature will be unavailable			
	This feature fr hard drive.	ees up 32	2KB on you	ır
Res	et Back Nex	ĸt	Cano	:el

7. Click "Next".



8. Click "Change".





- 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.

• → • ↑ 📘	< Evo	sepOne > Templates > Generic	~	õ	Search Gene	eric	P
Organize 🔻 New	v folde	ŕ				BE • 🔲	
🐉 Dropbox	^	Name	D	ate m	odified	Туре	
		30 SPD (44min, 15cm)	20	20-03	-26 08:43	Chronos A	nalysis
 OneDrivé 		60 SPD (21min, 8cm)	20	20-03	-26 08:43	Chronos A	nalysis
This PC		100 SPD (11.5min, 8cm)	20	20-03	-26 08:43	Chronos A	nalysis
3D Objects		200 SPD (5.6min, 4cm)	20	20-03	-26 08:43	Chronos A	nalysis
Desktop		300 SPD (3.2min, 4cm)			3-26 08:43	Chronos A	
Documents		Extended method (88min, 15cm, 15SPD	$\sum 20$	20-04	-30 05:57	Chronos A	nalysis
Downloads							
Music							
E Pictures							
Videos							
1 OS (C:)							
	~	<					

13. For Compass HyStar, go to the Section 4.3.4 "Create Evosep Separation Methods" and follow the procedure for creating a separation method for the 88 minute Extended method.

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

5 Instrument Software Control

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

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

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

> * 🕇 📙 « P	lugins > EvosepOne > Templates	✓ [™] Search Temp	olates 🔎
Organize 🔻 New fol			lii • 🔟 🤇
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 I
Desktop	Diagnose	2020-03-26 08:43	Chronos Analysis M
Documents	Prepare	2020-03-26 08:43	Chronos Analysis N
Downloads	Service	2020-03-26 08:43	Chronos Analysis I
J Music	System and column wash	2020-03-26 08:43	Chronos Analysis I
E Pictures			
Videos			
🛀 OS (C:) 🗸	<		
File	name: Prepare	V Analysis me	ethods (*.cam)

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

5.1.1 Running Samples

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

- 1. A method is selected
- 2. A sample list is composed (method(s) and sample position(s))
- 3. The sample list is scheduled for run
- 4. The schedule is executed

5.1.2 Methods

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



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

5.1.3 Sample Lists

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

1. Click the "Add" button.



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

	Files (x86)/iChronos/Plugins/EvosepOne/J'e 😔						
uniber of samples	10 Open Method						
	$e \rightarrow - \uparrow \blacksquare u$	OS (C:) > Program Files (x86) > Chro	nos > Plugins > EvosepOne >	Templates	νð	Search Templates	, p
	Organize - New fo	older				(iii • [. 6
	😹 Evosep_LF_test	^ Name	Date modified	Type ~	Size		
	🎘 Evosep_Merketir	Generic	2017-10-30 14:44	File folder			
	at Evosep_MPI	Xcalibur	2017-10-30 14:44	File folder			
	A Faslundfoto	Calibrate.com	2017-10-03 07:46	Chronos Analysis	1	KB	
	G OBH	Ciagnose.cam	2017-10-09 11:54	Chronos Analysis	2	KB	
	Outlook	Prepare.cam	2017-10-03 07:48	Chronos Analysis		t KB	
	a. Screenshats	Service.cam	2017-10-24 13:30	Chronos Analysis	2	108	
	🐴 OneDrive						
	This PC						
	Desktop						
	Documents						
	Downloads						
	EVOSEP_NAS						
	h Music						
	Pictures						
	Videos						
		*					
	Fie	e neme: Prepare.cam				Analysis methods (".cem)	~

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

🔯 Add Sample(s)		×
Method	Prepare	•••
Number of samples		
	Add g	<u>C</u> lose

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

 Analysis Method
 Source Tray
 Source Vial
 Sample Name
 Xcalibur Method
 Xcalibur Filename
 Xcalibur Post Acquisition Program
 Xcalibur Output Dir
 Comment

 1
 C:\Pro...9).cam
 EvoSlot 1
 1
 C:\X...\Methods
 C:X...
 C:X...

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



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

• System and column wash method:

• Source Tray (1-6) and Source Vial (1-96) must be specified.

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

• Diagnose methods:

• Select subsystem test, e.g. "Pump HP" and/or "HP system" method, by using the checkbox(es).

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

• Calibrate methods:

- Select method(s) by using the checkbox(es).
- The "Flow sensor ABCD" calibration script performs a multipoint flow sensor calibration of the low pressure pumps A, B, C and D.
- The "Flow sensor HP" calibration script performs a multipoint flow sensor calibration of the high pressure pump HP.
- The "Loop volume" calibration script measures the exact volume of the sample loop. The calculated volume is used in the sample runs to ensure higher analyte retention time accuracy. If the loop is replaced, the calibrate/loop volume script must be re-run.

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

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

 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*

 1
 C:\Program Files (...\Service.cam
 none
 Image: Contact closure test*
 Flow to column - 20% sol B*

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

5.1.4 Creating a Schedule

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

Schedule					
Overlapped					
Priority					
Infinite loop					
S Create					

5.1.5 Running a Schedule

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

Hain menu 🔇	Schedules	Run log Dagram Schedule tmetable Sample status
Internet int	Unidat	Term myor textua enade sequence.
	© Steends around See 201-062-061267 See 201-062-061268 Konneyer (SE002) © Or Annexer See 201-062-061268 See 201-062-061268 See 201-062-061268 See 201-062-061268 See 201-062-061268 See 201-062-061268 See 201-062-062-06268 See 201-062-06268 See 201-06268 See 201-06268	See Rundtme (min) See Rundtme Rund
Main menu Utilites	Citanup -	Ruthrei: 00.00.00 Gunetty running:
	Eveno One: not connected	

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



Q	\mathbf{O}	Preparation: Started	Select All
	Mai	Prepare: Degas	9 Displacement [u]
Updates	Maintenance	g pumphp: 200.6 bar built using 3.540 µL	
(1)		pumpa: 50.1 bar built using 3.920 µL	Set flow (pu/min) Pressure (bar) 180-
0	info	pumpd: 50.1 bar built using 4.280 µL	5 Pump speed [µL/min] 160-
vosep One	information	pumpb: 51.0 bar built using 4.360 µL	Pump B Disolacement [uL]
	9	pumpc: 50.1 bar built using 11.680 µL	Actual flow [µL/min]
		pumphp: 205.8 bar built using 3.600 µL	Set flow (µL/min) = 120-
		pumpa: 50.1 bar built using 3.850 µL	Pump speed (µL/min) 100-
		pumpb: 50.1 bar built using 4.310 µL	Pump C
		pumpd: 50.9 bar built using 4.280 µL	Displacement [µL] 80-
		pumpc: 50.1 bar built using 10.600 µL	Set flow (µL/min) 60-
		pumphp: 204.1 bar built using 3.590 µL	Pressure [bar] Pump speed [ul/min]
		pumpa: 50.1 bar built using 3.810 µL	Pump D
		pumpb: 50.1 bar built using 4.270 µL	Displacement [µL] 20-
		pumpd: 50.1 bar built using 4.220 µL	Set flow [µL/min]
		pumpc: 50.1 bar built using 9.670 µL	Pressure (bar)
		Preparation: Started	
		rreparation: Started	C Select All C Whee data C Offine data Add Remove all Define data Add Remove all Define data Add Remove all

5.1.6 Aborting Samples

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

Main menu 🤇	Schedules		Run log Diagram :
	Execution		2017-11-03 21:30:14
Sample list	00	Pause	2017-11-03 21:53:52 2017-11-03 21:53:57
<i>•</i>		Pause	2017-11-06 10:31:26
		Stop	2017-11-06 10:31:48
Schedules and run control			2017-11-06 10:31:48
Schedules and run condition	Queue		2017-11-06 10:31:48
			2017-11-06 10:31:49
	Schedule 1 done		2017-11-06 10:31:50
Method editor	Schedule 2 done		2017-11-06 10:46:03
	Schedule 3 done		2017-11-06 10:46:04
1/2	Schedule 4 done		2017-11-06 10:46:04
Settings	Schedule 5 done		2017-11-06 10:46:04
Decailgs	Schedule 6 done		2017-11-06 11:00:09
.0	Schedule 9 done		2017-11-06 11:00:14
	3seq_100_new* done	Schedule execution control	
Activation	3seq_100_new* done		52
	3seq_100_new* done	Abort complete run immediately	2
	3seq_100_new* done	O Do not start remaining samples of this sch	
	3seq_100_new* done	Queue this schedule again for the rema	4
	3seq_100_new* done		6
	Schedule 11 running	O Do not start any remaining samples at all	56
			.6
		OK Cancel	6
			11
		I	2017-11-06 11:05:11
			2017-11-06 11:05:11
			2017-11-06 11:05:11
	Selected schedule		2017-11-06 11:05:12

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

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

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

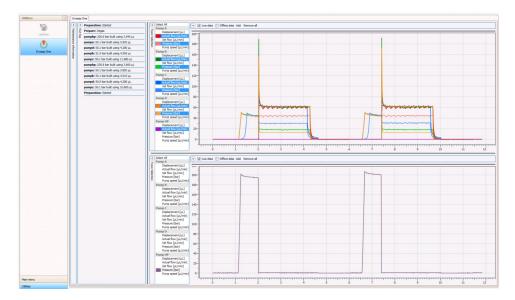
Important note:

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

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

5.1.7 Looking at Graphs

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

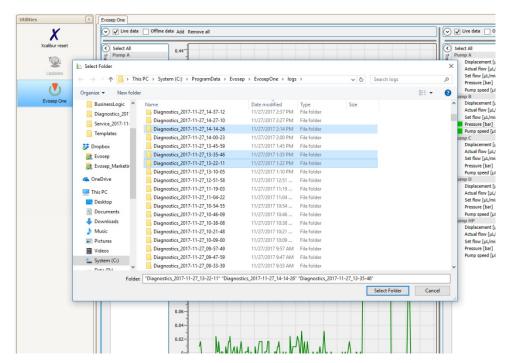


2. The following traces can be selected for each pump by a single left click on the side bar:

Name	Description
Displacement [µl]	Pump piston position. Pumps are full when Displacement is 0 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 for each pump.
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 an individual pressure sensor for each pump. The pressure limits are 500 bar and 100 bar for the Pump HP and Pump A/B/C/D, respectively.
Pump speed [µl/min]	The Pump speed graph shows the actual pump piston velocity. It may differ from the set point when the flow feedback control is utilized, e.g. during the gradient formation.

Tips and tricks

- 1. The graph viewing area is divided into two plots that can be resized by left mouse clicking the intersection and dragging. Double left clicking a graph type on the side bar will enable or disable the specific graph type for all pumps.
- 2. The graphs can be zoomed-in by "mousing over" the area of interest while holding down the "Shift" button + left mouse click.
- 3. It is possible to view previous pump traces by clicking the "Offline data" check box. Select one or more sample folders and click "Select Folder". Alternatively, you can drag-and-drop files from Windows File Explorer to the "Offline data" section.



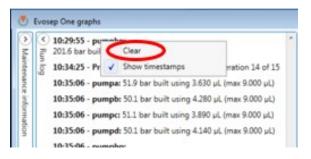


4. The opened sample folders are visible in the "Offline data" list. Individual data files can be selected/unselected using their respective checkboxes.

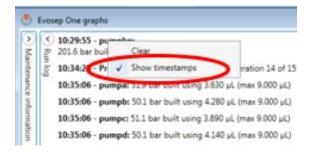
Utilities	Evosep One	
Y	✓ Live data ✓ Offline data Add Remove all	
Xcalibur reset	✓ Diagnostics_2017-11-27_14-14-26	x
Acaibur reset	✓ Diagnostics_2017-11-27_13-35-46	x
Updates	✓ Diagnostics_2017-11-27_13-22-11	x
Evosep One	Select All 64 Pump A Diplacement [µ] Diplacement [µ] 64	

5.1.8 Run Log

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

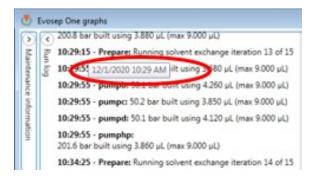


3. The User can choose to enable/disable timestamps in the log by right clicking the log and selecting/deselecting the "Show timestamps" option.



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





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

Copy message	>
Сору	Ctrl+C
Clear	
Show timestar	nps

5.1.9 Maintenance Information

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

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

vosep One							
Software							
One: 1.0.0.0							
Evosep One Chronos Plugin: 1.0.0.0							
Robotic Sample Injection - RSI							
\bigcirc	Driver: 2.4.3 (2.4.17311.1645)						
Firmware: 2.4.1	· ·						
	(
🕑 Pump A	✓ Pump A						
Pump B	✓ Pump B						
Pump C							
Pump D							
Pump HP							
Bus location:	3@172.17.17.1						
Product number:	1082						
Serial number:	7						
Firmware version	1: 44						
Type identifier:	0x0202						
Diagnostic codes	:						
Displacement (to							
Displacement (se	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:							
30 samples/day:	44						
Total analyses:	151						
Loop volume:	N/A µL						

5.1.10 How to Import CSV Files into Chronos

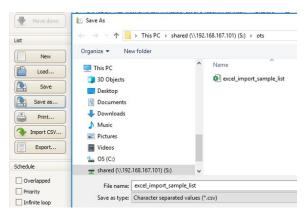
The general idea here is to use a mapping template to define the column matching between the CSV file you want to import and the Chronos sample list columns. This example uses a CSV file saved from Chronos, but you can use any CSV file as source if it contains the values to fill-in the columns described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

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

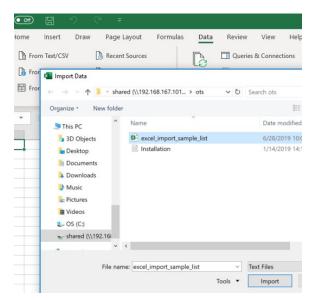


n menu 🤇	Sample		Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalbur S
			1 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	6	C:\Thermo\Instruments\TSQ\Methods\11.SminLCMS_190219.meth	200fm_BSA_100_grad_6	
	4	Add	2 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	7	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_7	
Sample list			3 Ci\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	8	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_8	
		Remove	4 Ci\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	9	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_9	
Ð			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	
			6 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	11	C:\Thermo\Instruments\TSQ\Methods\11.SminLCMS_190219.meth	blank01_grad_11	
thedules and run control			7 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	12	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	blank02_grad_12	
_	(m.		8 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	13	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_13	
1	- M		9 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	14	C:\Thermo\Instruments\TSQ\Methods\11.5minLCM5_190219.meth	200fm_BSA_100_grad_14	
d h			10 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 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	EvoSlot 1	16	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_16	
	LOI		12 C:\Program Files (x86)\Chronos\Pl\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	17	C:\Thermo\Instruments\TSQ\Methods\11.SminLCMS_190219.meth	200fm_BSA_100_grad_17	
\$ <u>-</u>		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		THEN	14 Ci\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	
securigs		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	
6	2	Save						
Activation	🔁 s	ave as						
	-	Print						
	Ime	ort CSV						

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



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



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

AutoSave 💽 🛱 🧐 🤆 🖛	Book1 - Excel		Ole Tang Sørensen 🔟 🔳 😑
File Home Insert Draw Page Layout Formulas Data Review View Help	₽ Search		영 Share 🖓 Co
$ \begin{array}{ c c c c } \hline & X \\ \hline P_{dete} & D \\ \hline & & \\ \hline \hline & & \\ \hline \\ \hline$	⊡ Merge & Center ~ \$ ~ % 9 % Conditional F Formatting ~	Jornat as Cell Table - Styles - yles Cells	$\begin{array}{c c} \Sigma & & & & & & \\ \hline \Sigma & & & & & & \\ \hline \blacksquare & & & & \\ \hline \blacksquare & & & \\ & & & & \\ & & & \\ & & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & &$
644 · IX · A			
A 8	C D E	FG	н
		Xcalibur Filename 🛛 💌 Xcalibur Sample Name 💌	Xcalibur Processing
1 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\6BSA_134_11_3G.pmd
2 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\68SA_134_11_3G.pmd
3 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\68SA_134_11_3G.pmd
4 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\68SA_134_11_3G.pmd
5 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo		200fm_BSA_100_grad_10	C:\Xcalibur\methods\6BSA_134_11_3G.pmd
6 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\68SA_134_11_3G.pmd
7 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo		blank02_grad_12	C:\Xcalibur\methods\68SA_134_11_3G.pmd
8 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\6BSA_134_11_3G.pmd
0 9 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo			C:\Xcalibur\methods\68SA_134_11_3G.pmd
 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur1 Evo 	ot 1 15 C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_15	C:\Xcalibur\methods\68SA_134_11_3G.pmd
2 11 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo	ot 1 16 C:\Thermo\instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_16	C:\Xcalibur\methods\68SA_134_11_3G.pmd
3 12 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo	ot 1 17 C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_17	C:\Xcalibur\methods\68SA_134_11_3G.pmd
4 13 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo	ot 1 18 C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_18	C:\Xcalibur\methods\68SA_134_11_3G.pmd
5 14 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo	ot 1 19 C:\Thermo\instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_19	C:\Xcalibur\methods\68SA_134_11_3G.pmd
6 15 C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 1 Evo	ot 1 20 C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.meth	200fm_BSA_100_grad_20	C:\Xcalibur\methods\68SA_134_11_3G.pmd

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

Save As				
← → × ↑ 🖡 > This PC > shared	(\\192.168.1	67.101) (S:) > ots		
Organize • New folder				
ConeDrive - Personal This PC S 3D Objects Desktop Desktop Documents Downloads Music Pictures Videos CS (C)		lame ₽ excel_import_sample_list	Date modified	Type Microsof
shared (\\192.168.167.101) (S:)				
Network File name: Modified_excel_imp	✓ ort_sample_li	ist		
Save as type: CSV (Comma delimi	ted)			

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

AL.	Move down	9 C:\Program Files (x86)
		10 C:\Program Files (x86)
Method editor	List	11 C:\Program Files (x86)
	List	12 C:\Program Files (x86)
¥=	New	13 C:\Program Files (x86)
Settings	INCIV	14 C:\Program Files (x86)
Securigs	Load	15 C:\Program Files (x86)
4	Save	
Activation	Save as	
	Print	
	Tmport CSV	

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

CSV Import				- 🗆 ×
CSV Mapping Template: C:\Program Files (x86)\Chrono	os\Plugins\EvosepOne\Templates\import tem	olate.ccm		 Browse
	os\Plugins\EvosepOne\Templates\Xcalibur\X	calibur 100 SPD (11.5min, &	cm).cam	Browse
CSV File to Import: S:\ots\Modified_excel_import.	_sample_list.csv			Browse
Use All Samples	Number of Samples to Import:	1	× V	Edit Template
Save to Sample List				Select
🔷 Import		Import then Run		Cancel

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

1	2	3	4	5
Column 1	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.n
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.r
3	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur\00 SPD (11.5min, 8cm).cam	EvoSlot 1	8	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.n
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.n
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.r
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.r
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.r
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.r
9	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	14	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_190219.r
10	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur\Xcalibur\U00 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 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\00 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_190219.
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.r
15	C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Xcalibur\Xcalibur 100 SPD (11.5min, 8cm).cam	EvoSlot 1	20	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS 190219.

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

CSV	Previ	ew	-	-		×
Г	_	1			-	^
	•	Analysis Method;Source Tray;Source Vial;Sample Name;Xcalibur Method;Xcalibur Filename;Xcalibur Processing;Xcalibur Output Dir;Comment				~

• Click "Edit Template..." to ensure the CSV Separator is set to the character used in your CSV file.

Edit C	SV Templa	te	-	- 🗆	×
_	is Header F Load now n Mapping:	kow CSV Separator (use \t for tab):			
	Use	Sample List Column	С	SV Column N	o.
•	\checkmark	Source Tray	3		\sim
	\checkmark	Source Vial	4		~
	\checkmark	Sample Name	5		\sim
	\checkmark	Xcalibur Method	6		\sim
	\checkmark	Xcalibur Filename	7		\sim
	\checkmark	Xcalibur Processing	8		~
	\checkmark	Xcalibur Output Dir	9		~
	\checkmark	Comment	10)	~
			Save	Ca	ncel

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



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

S:\ots\Modified_excel_import_s	sample_list.csv
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 the Evosep solvent levels (waste, solvent A, and solvent B; left picture) and LEDs displaying power for the pumps (middle picture) and autosampler (right picture).
 - The LEDs must be green.



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





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



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



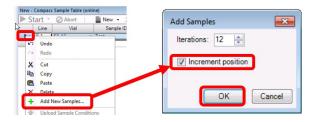
3. In HyStar, click the "Acquisition" Icon to open the HyStar Sample Table. Click "New" and click the small arrow in the first line under "Method Set". Choose "Use Individual Methods".

		Vie	Quick Data	n a	auisitia		ple Table	m				
					quistae	- [[[]]]						
						ents	Instrume					
			not condu									
					+				P	nple Table (on	C	
		🛱 Options 🔹	Import/Export -	oort -	Rer	Save As	Save	X Delete	New -	Abort		
Method Set			Data Path		Volume [µl]	Inj.	Sample ID		Status	Vial	Line	
	*			D:\Data\	(1				*	₹1	ł
Open	• Open)	(-		
	-									•		

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

Start v ⊘Abort New v X Delete Save B Save As Report v Import/Export v Options v Line Vial Sample ID Inj. Volume [µ] Data Path Method Set Separation Method Injection M	
	ethod MS Meth
★ ✔ 1 S1-A1 v test 1 0 D\/Data\ v v 100 samples per.v Standard	▼ RC100.m

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



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

St	art -	Abort		🖹 New 🔹 💥 D	elete	Save 🔛	Save As	ame:	mple Table As				
	Line	Vial		Sample ID	lnj.	Volume [µl]	Data Pa		Ageithe rene donce seart		Separation Method	Injection Met	MS Method
*	√1	S1-A1	- 1	Fest	1	C	D:\Data\	Tag:	•	5	smp pr day	Standard	RC100.m
*	12	S1-A2	- 1	Test	1	C	D:\Data\	Description		5	smp pr day	Standard	RC100.m
*	13	S1-A3	- 1	Fest	1	C	D:\Data\	Description	^ ·	s	smp pr day	Standard	RC100.m
*	14	S1-A4	- 1	Test	1	C	D:\Data\			5	smp pr day	Standard	RC100.m
*	15	S1-A5	- 1	Fest	1	C	D:\Data\			5	smp pr day	Standard	RC100.m
*	16	S1-A6	- 1	Fest	1	C	D:\Data\		-	5	smp pr day	Standard	RC100.m
*	17	S1-A7	- 1	Test	1	C	D:\Data\			8	smp pr day	Standard	RC100.m
*	18	S1-A8	- 1	Fest	1	C	D:\Data\		OK Cancel	5	smp pr day	Standard	RC100.m
	19	\$1.09	- 1	Feet.	1		D-(Data)				emo or day	Standard	PC100 m

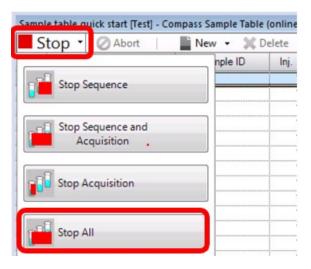
7. Mark the first line in the sample table by clicking the black triangle in Line 1. Click "Start" and select "Start Sequence".

🕨 Start 🔹 🖉 Abo	rt 📄 New 👻 🗶 Delete 🛛	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: 2018-06-1919:05	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
1		0		Euseen 100 emp pr day m

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

HyStar (1 / 12)	Injecting	Bruker OTOF MS	HyStar (17	/ 12) Run	Bruker OTOF MS
injecting	Evosep One	ready operate	injected		run operate
Time (min) 11.5 0.0 Remaining Time 2 hours 42 min	HP Pressure 0.0 bar HP Plow 0.0 00 µL/min Method 100 samples per Sample 51-A1 0.00 / 0.00	micrOTOF-Q III	Time (min) 0.7 Remaining T 2 hours 42 t	11.5 HP Pressure 398.9 bar HP Flow 2.00 µL/m Time Swhole 100 samples per Sample Sample	micrOTOF-Q III
	Evosep One graphs 100 samples per d 100 samples per d 100 samples per d 100 samples per d	ay: Sample position Slot1:2	Constraints of the second seco	A placement [µ1] placement [µ1] mol flow [µ1/min] source [µ1/min] splacement [µ1] µplacement [µ1] ual flow [µ1/min] source [µ2] mo speed [µ1/min]	

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



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

Compass	Method Set 🔡 Sample Ta	able 🛃 Acquisitio
nstruments		
HyStar	disconnected	Bruker OTOF MS
initialized		not ready
	Display Runtime	shutdown
	Stop Shutdown Counter	micrOTOF-Q III
	Stop 'Prerun' Time	micro ror q m
	Reset System	
	Clear Error	
	Error Logbook	-

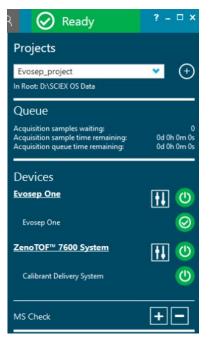
5.3 Evosep Driver for Control of SCIEX OS

5.3.1 Instrument Preparation with SCIEX OS

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



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



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





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



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



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

				-					
Sample Name	MS Method	LC Method	Rack Type	Rack Position	Plate Type	Plate Po	Vial Position	Data File	
 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 1 and drag down to add samples. Adjust Sample Names, Vial Positions, and Data File values.

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. Click "Save" and save the Batch Table with an appropriate name.

) -	Batch	습 💠 🗒 🕑 🕯	≤ <u>∧</u> <u>⊞</u>							
		Decision Rules 🗸	Auto-Calibrate New	Open	✓ Save	 Print 		View	Submit	
Unt	itled 🧰 🙋									
					Save As Batch					
					Type a file name The batch is saved in the Batch fold Current Project:Evosep_project	er of the current project.		ate Layout	Manage Samp	oles
	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_S	WATH_1	1
2	test_2	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	evobatch2 evobatch1	2/15/2022 4:37:14 PM 2/24/2022 2:52:56 PM		test_100SPD_S	WATH_2	
3	test_3	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	evolutchi Training_150322	3/9/2022 9/23:56 AM 3/31/2022 9 14:18 AM		test_100SPD_S	WATH_3	
4	test_4	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray		1/31/2002 11 10 10 MM U		test_100SPD_S	WATH_4	
5	test_5	100SPD_SWATH56vW_400_750	100 samples per day	Evosep One tray	File Name Type a file name			test_100SPD_S	WATH_5	1
			100 1 1	r 0 .		Save Cancel				Ŧ

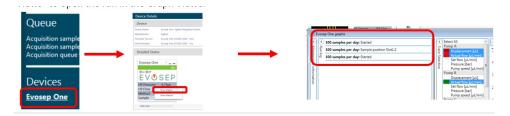
7. Press "Submit" to submit the Batch to sample queue.

🗘 - Batch	습 🖗 🕮 🖄 🚊 🚊						Submit Samples
	Decision Rules 🖌 Auto-Calibrate New	Open	♥ Save	• Print_	View	Submit	Total number of samples to be submitted 6 of 6
							The calibrant X500 R3 Positive Calibration Solution, will be applied every 5 samples.

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

🕽 - Queue		合一幕	🗒 🖻 🖻 🔺	<u></u>						
						Start	Stop	Print	Man	age 🔹 🗙
Acquisition Status	Sample Name	Est. Start Time	MS Method	LC Method	Data File	Pr	oject	Processing Method	Results File	Auto Processing
Untitled - 8 samp	es									A
	Cal	3/31/2022 10:28:48	100SPD_SWATH56vW_4		Cal	Evo	sep_project			
0	test_1	3/31/2022 10:30:49	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_1	Eva	sep_project			
0	test_2	3/31/2022 10:49:59	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_2	Eva	sep_project			
0	test_3	3/31/2022 11:09:09	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_3	Evo	sep_project			
0	test_4	3/31/2022 11:28:19	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_4	Eva	sep_project			
0	test_5	3/31/2022 11:47:29	100SPD_SWATH56vW_4	100 samples per	test_100SPD_SWATH_5	Evo	sep_project			

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



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





5.4 Evosep Driver and Sample Acquisition with MassHunter Software

5.4.1 Create MS Method with MassHunter

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

	Agilent MassHunter Acquisition Method Editor	
	File Tools Method Help	
		· 💼 😒
Offiline	Properties DA Evosep One QQQ	
Method	Name 100 samples per day	
Relitor	Description 30 samples per day 60 samples per day 1064, EV1109	
	100 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	
		v 🛍 匀
	Properties DA Evosep One QQQ	
	Name 100 samples per day 🗸	
	Description 11.5 min acquisition time. Compatible columns: EV1064, EV1109	
	Runtime 11.5 min	

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

	Agilent MassHunter Acquisition Method Editor	
	File Tools Method Help	
	🗋 🎷 🔛 🛃 🖻 100SPD <u>_Sched_4mi</u> nRTwin_MRM_BSA.m	
	Properties DA Evosep One QQQ	- 1
5	Tune file Stop time A ItunesHED_Minus20.TUNE.TUNE.XML C No limit/As Pump	
	Browse 68	_ 2
	Ion source	
4	HPLC-Chip Peak width 0.25 min	3
	Time segments	
	# Start / Scan Type Div Valve Delta Delta EMV (+) EMV (-) Stored	
	▶ 1 0 Dynamic MRM To MS 200 0 🔽	

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

		Help		
) 💕 🖬 🤅	A 👌 1	00SPD_Sched_4minRTwin_MRM_BSA.m	~	1

5.4.2 Sample Acquisition with MassHunter

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



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

	Agilent MassHunter Acquisition Worklist E
	File Tools Worklist Help
	i 🗋 i 💅 🖬 💹 i 🖻 i
1. Right click here 💻	Sample Name Sam
	Add Multiple Samples
	Add Sample

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

Sample Information Sample Por	sition	
	210011	
Current Configuration		
Select Well-plate or Vial Tray	Plate/Tray Type	
Select Tray	96 Evotip box	
	Selection Origin	Block Increment
P2: 96 Evotip box	Top left O Top right	Row major
	0.000	O Column major
P5: 96 Evotip box	O Bottom left O Bottom nght	O Serpentine
P6: 96 Evotip box	Number of samples Num	ber of replicates
	12 1	
Well-plate/Tray		
1 2 3 4	5 6 7 8 9	10 11
0000	00000	10 (11 (12
	Seted Tray Exceed Dire tray P1 56 Evolp box P2 56 Evolp box P3 56 Evolp box P5 56 Evolp box P5 56 Evolp box P5 56 Evolp box	Select Tray Selec

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

	1	2 Mark co	olumn and Right	t click				Agiler	nt Massi	Hunter Acquisition V
Data File	+	Fill			•	Down	4	File	Tools	Worklist Help
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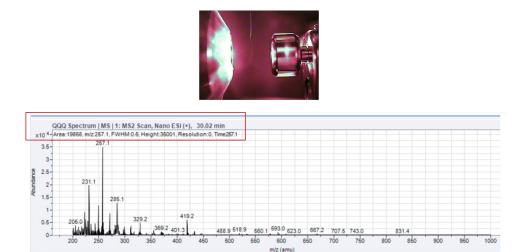
- 5. To run LC checks/preparations prior to the MS run, right click for drop down method.
 - For example, use "flow-to-column" at 1000 nl/min and check the spray for 100 SPD LC method.



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0.00 / 0.00 Show Manual Ins 250 n/min (10 min) Chromatogram Plot Park Autosampler 100 n/min (10 min) 100 n/min (10 min)	Not Ready On ©off EVUSSED HP Pressure 258.6 HP Flow 0.41 Method Sample 0.00/0.00 Not Ready Calibration Diagnostics Preparation Service Cancel Maintenance Pro Show Graphs Show Manual	Run Cancel Standby HPLC Chip	The second

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

	Agilent MassHunter Workstation Data Acquisition	
	File View Sample Worklist Method Tools Hel	
	Context: Acquisition V Layout: Agilent_Service.	
	Instrument Status	
	Evosep One QQQ	
	Injecting	
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	Browse 6d C 1 min	Segment Name Start Mass End Mass Scan Time Fragmentor Cell Accelerator Voltage Polarity 200 1000 500 166 5 Positive
2. Select	Ion source	
MS2 Scan	HPLC-Chip ▼ Peak width 0.07 min	
WISZ Scall	Time segments	
	# Start Scan Type Div Valve Delta Delta Stored	
	Time EMV (+) EMV (+) ▶ 1 0 MS2 Scan To MS 0 0 IV	
1. Open		
Method	•	
Editor	Method Editor Worklist Sample Run	



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

	Method Edit
Data	Sample Name Sample Position Method
Acquisition	1 P1-A1 D1MassHunter(Methods)Evosep\Development/Heli
	2 v P1-A2 D:\MassHunter\Methods\Evosep\Development\Hel

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

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	1		18052022_0	●On Off		
	Г	Sample Name	Sample F	- HPLC	On On	
1			P1-A1		StandBy	
					standay	

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





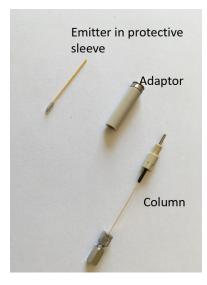
6 Configuration, Source, Column, and Emitter

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

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

6.1 Thermo Scientific EASY-Spray Source

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



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



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



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



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



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



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

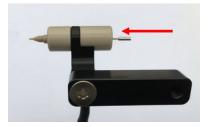


- 6.2 Thermo Scientific Flex Source
 - 1. Carefully remove emitter from the box by using your fingers or a pair of tweezers.



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





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



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



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



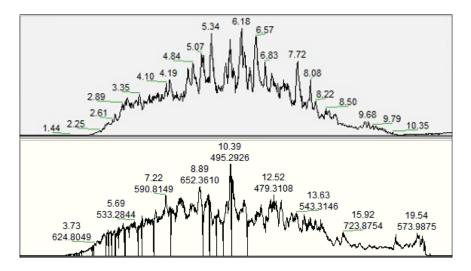


6.3 Thermo Scientific FAIMS Pro Interface

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



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



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

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



Before cleaning

After cleaning

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



6.4 Bruker Daltonics CaptiveSpray Source





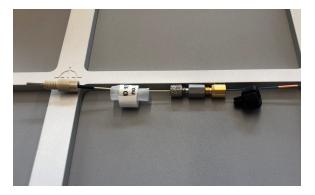
1. Run Connect the column to the CaptiveSpray source and connect the transfer line to the column.

6.5 Agilent Nanospray Source

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



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



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



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



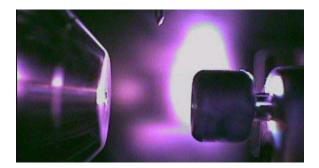
4. Insert the sleeve adapter with the column into the needle sleeve assembly. To ensure the correct position of the sleeve adapter and emitter, push the sleeve adapter towards the end of the needle sleeve assembly until the column peek nut is stopped by the small narrowing in the needle sleeve assembly,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



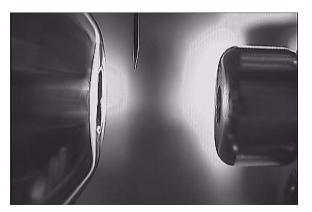
5. Now assemble the needle sleeve assembly and remove the protective sleeve from the emitter.



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



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



- 8. Run the flow to column from the Evosep One and adjust needle position, ionization voltage, etc. to get stable spray.
- 9. The recommended source conditions are shown below:
 - Gas Temp: 200 °C
 - Gas Flow: 11 L/min
 - Capillary: 1700 V

Method Editor		
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HFLC-Drip IF Peak width [0.03] min Time segments If Scan Type Div Valve Delta EMV (-) Delta EMV (-) Delta EMV (-) Stored 1 0 Dynamic MRM To Waste 400 0 IF	Positive Negalive Capilay: [700 V [720 V [428 nA	Low Pressure RF 110 V 60 V Copy Paste
	Chamber Current 0.18 µA	

6.6 Sciex Optiflow Ion Source in Microflow Regime

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

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



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



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





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



6.7

7 Preparing the Evosep One for Use

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

7.1 Hardware Configuration for Standard and Zoom Mode

Important notes:

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

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

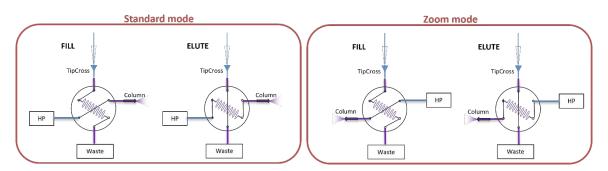
Whisper Zoom methods improve on the existing Whisper methods with significant chromatographic improvements in terms of peak shapes, intensity, reproducibility, and throughput. Running the system in Zoom mode requires a hardware change.

Standard mode:

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

Zoom mode:

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



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



		Add Sample(s)	×
		Method	Instrument configuration	
		Number of samples		
			de Add	Glose
hronos (Demo Ver in menu 🔇	rsion)	New Sample List*	de Add	Glose
n menu 🔇	Sample	Analysis Method	A	Loop mode
	-	Analysis Method		Loop mode

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

	Idle
HP Press HP Flow Method Sample 0.00 / 0.	Calibration Diagnostics Preparation Service Instrument configuration
	Cancel Maintenance Procedure
Table (onl	Show Graphs
ew • ×	Show User Guide
Sta	Park Autosampler
10 Instrumen	t configuration X
Evosep One	operating mode configuration
Loop mode:	Show current *
	Show current
	Set to standard Cancel

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

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	.5 bar 58 µL/min 1ode to Zoom	Covery Dire Graph Weret Reconcert 10143 Concrete to data services O Instrument configuration: Started Set a Zoom: Set a Zoom: Provide the log value is configured in the following way: Provide the set of the log value is configured in the following way: Provide the set of the log value is configured in the following way: Provide the set of the log value is configured in the following way: Provide the log value is configured in the log value in the log value is configured i
Cancel		

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



- Degas: prepares the pumps to run samples if the instrument has been idle for more than 6 hours
- Solvent exchange: purges the pumps
- Align solvents:
 - Prepares the system flow lines if the instrument has been idle for more than 6 hours
- Flow to column:
 - Sets a flow to the column. This is useful when setting up the MS spray conditions
- System and column wash:
 - Cleanses the system and analytical column of contaminants
- 8. The Evosep One automatically assesses the need for doing preparative actions to ensure optimal instrument performance without the need for User interaction. Under these circumstances, the system autonomously initiates the proper system preparation tasks immediately preceding sample analysis.
 - Degas is initiated if the system has been idle for more than 6 hours.
 - Align solvents is initiated if:
 - The system has been idle for more than 6 hours
 - The previous procedure was aborted
 - The previous procedure caused the solvents in the flow lines and the ceramic needle to be unaligned
- 9. Additionally, all the preparation programs can be manually executed by adding a sample using the "Prepare" method (C:\Program Files (x86)\Chronos\Plugins\EvosepOne\Templates\Prepare.cam) in the "Sample list" panel.

ain menu <	Sample	Analysis Method	
	- <u>₽</u>	1	
Sample list	Remove	Lo Add Sample(s)	×
F	Duplicate	Method Prepare	
Schedules and run control	Move up	Number of samples	
H	Move down		
Method editor	List	- Add	Close
3			
Settings	New New		
	Load		

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



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



7.2 Degas Pumps

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

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

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

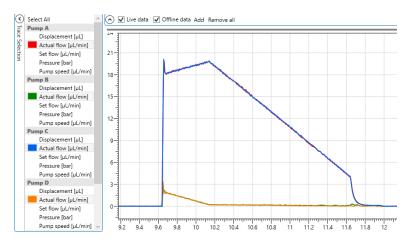
7.3 Solvent Exchange of Pumps

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

7.4 Align Solvents

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

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



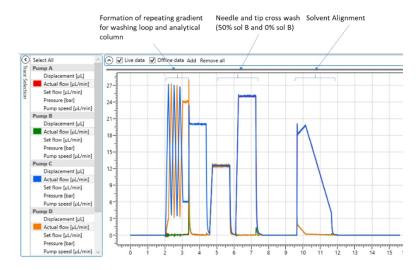
7.5 Flow to Column

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

7.6 System and Column Wash

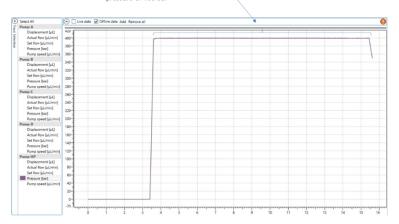
The "System and column wash" program can be used if the Evosep One autosampler, loop, or analytical column has been heavily contaminated from running a dirty sample. The program occurs in four steps and the duration varies according to the backpressure of the connected analytical column (typically 10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

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





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 ways:

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



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

🐼 Options 🛛 🗙
Procedure for preparing the LC system for running samples
Degas: 🗌
Purge:
Align solvents:
Flow to column: none 🔍 nl
System and column wash: ✔
Run Cancel

7.7 Idle Flow

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



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

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

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Sample list	Skin Caramel	Always save run logs								
-	Language English	Log folder: C:\ProgramData\Evosep\Evosep	Dne\Chronos run logs\							
edules and run control	Standard folders	Activate trace mode								
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8 Running Samples using Evosep One

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



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

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

8.1 Separation Principle

The Evosep One technology is centered around the Evotip and integrating sample preparation with LC-MS. The Evotip is essentially a disposable trap column in a pipette tip format with a small plug of C18 stationary phase at the bottom of the tip. The Evotips are used to de-salt and clean up the samples prior to LC-MS analysis; however, the traditional subsequent steps of eluting, drying down, and resuspending the samples from the tips are completely omitted. Instead, the tips are loaded directly into the Evosep One for analysis. This new process leads to significantly less sample loss and variation, as well as simpler and faster workflows. The Evosep One sample tray accommodates up to 6 racks of 96 tips, i.e. 576 rinsed samples may be lined up for fast analysis. See Figure 1 for the Evosep One plumbing diagram.

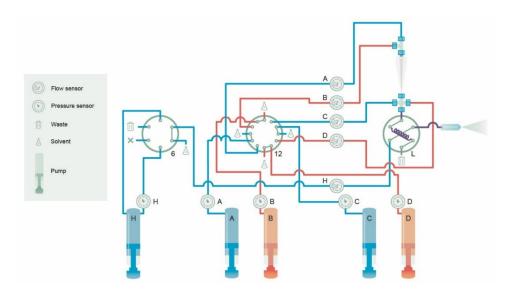


Figure 1. Evosep One plumbing diagram. H: High-pressure Pump, A/B/C/D: Low-Pressure pumps, 6: 6 port high-pressure solvent valve, 12: 12 port low-pressure solvent valve, L: Loop Valve.

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

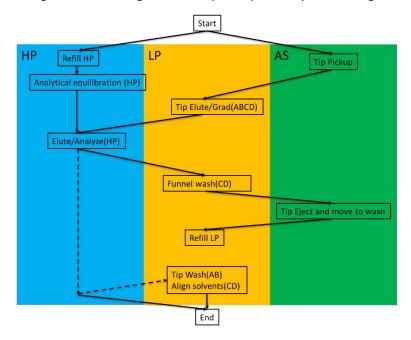


Figure 2. High level sample acquisition process diagram. The acquisition cycle can be divided into three sections: Blue – High pressure, pump HP; Yellow – low pressure, pump A,B,C,D; and, Green: autosampler actions.

Once the Evotip is sealed in line with the solvent system, a gradient from pumps A and B runs through the Evotip and sequentially elute the adsorbed analytes (Tip Elute/Grad(ABCD)). While the gradient, with the embedded and pre-separated analytes, elutes from the Evotip, a secondary gradient from pumps C and D continuously modify the composition of the initial A/B gradient to generate an offset gradient that ensures optimal chromatographic performance at the analytical column, see Figure 3a. Pumps A+B deliver a partial gradient sufficient to sequentially elute the analytes of interest, but filter out all the high-molecular contaminants to be discarded with the Evotip after the analysis. A high organic wash (80% ACN) volume is introduced just after the gradient using Pump D, bypassing the Evotip, to efficiently wash the analytical



column. It takes approximately one minute at 20-40 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 3b.

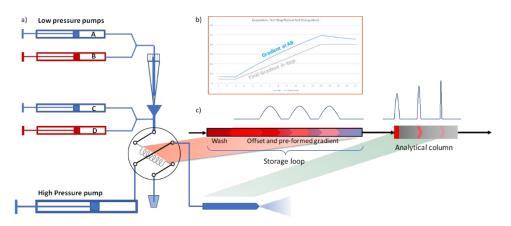


Figure 3. Evosep One a) Simplified plumbing diagram of Evosep One, b) Illustration of the A/B gradient running through the Evotip and the following C/D modified gradient resulting in an offset gradient for optimal focusing at the analytical column, and c)
 Illustration of the preformed and offset gradient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

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

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

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

Table 1. Evosep One Methods

8.2 Sample Acquisition

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

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

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

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

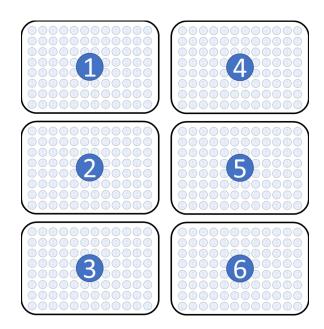
Sample	Analysis Method	2 Add Sample(s)	~	<					
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		Organize 🔻 New folde	r				8==	• 🔳	
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Save Save as Save as Print Import CSV		Evosep_MPI Frølundfoto OBH Outlook Screenshots	Generic Xcalibur Calibrate.cam Diagnose.cam Prepare.cam	2017-10-30 14:44 2017-10-30 14:44 2017-10-03 07:46 2017-10-09 11:54 2017-10-03 07:48 2017-10-24 13:30	File folder File folder Chronos Analysis Chronos Analysis Chronos Analysis	1 k 2 k 2 k	(B (B		
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		" OS (C:) V							
		File na	me: C:\Program Files (x86)\Chronos\Pl	ugins\EvosepOne\Templates\		~	Analysis methods	(*.cam)	~
							Open 💌	Cance	al

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

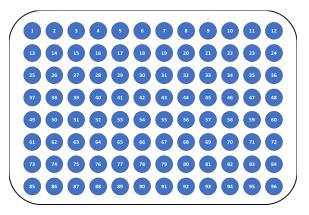


• Source Tray (Slot 1-6) must be specified using the dropdown menu.





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



- For Thermo MS: "Xcalibur Method" the MS acquisition method must be specified. The MS acquisition time must correlate with the individual LC-MS methods, as specified in Table 1.
- For Thermo MS: "Xcalibur Filename" the MS data filename(s) must be specified.
- For Thermo MS: "Xcalibur Post Acquisition Program" the MS data post-acquisition executable or batch file can be specified.
- For Thermo MS: "Xcalibur Output Dir" the MS data directory must be specified.
- When the sample list is completed, create a schedule and start the analysis. Chronos will send the sample information to Xcalibur and once the MS is in "waiting for contact closure" mode, the Evosep One sample separation will start.

Schedule
Overlapped
Priority
Infinite loop
S <u>C</u> reate

Main menu 🗧	Schedules	Run log Diagram Schedule timetable Sample status
Sample list	Deciden	Demonstration Version Schedule 1
ď	510	
Schedules and run control	Queue Echentule 1 queued	
Settings		
Activation		
		Samples -
	Selected schedule Start: 2017-11-02.161107	
	50mt 2017102241107 Prdt 2017102341107 Remaining: 00:00:01	
	S Al schedules	0.00 0.01
	Start: 2017-11-02 14:11:07	Runtime (min)
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	Cleanup	Yest action in: Buritme: 00:00:00
Main menu		Currently running:
Utilities My menu	Evcerp One: not connected	100 % VD

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

	Analysis Method	- 🔺
1	eq:c:Program Files (x86) Chronos Plugins EvosepOne Templates X calibur MS standby. On the template the template the template temp	cam

- 2. Standalone LC mode:
 - 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> А</u> dd				

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

Schedule
Priority
Infinite loop
S <u>C</u> reate

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

Hain menu 🤇	Schedules	Run log Diagram Schedule timetable Sample status
Iten more (Under	Demonstration Version Schedule 1
Varianu	Image: State	OB OB OB Ruther (min) OB Sector frage Sector
Utilities My menu	Evosep One: not connected	

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

Tips and tricks:

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

es and non control	Load Save Save Save Pont		ur 60 samples pe		(R)	an produced by	and to any	and ber out (It min) cam Metho							Overwrite device settings Show invisible taak proper
Method editor	CDS method	Columns Parameters	Token NTRAVIS	Cell type Tray with vial	Variable type	visible	Editable	Sortable	Standard value EvoStoti:1	Min. value	Max value	Vial name Source Vial	Vial increment	Vial token	Combobaxitens	File extension filter
-		Xcel bur Method Xcel bur Filename	NHETHNA NFILENA	rie	Text					>				HUNDERN		Instrument methods (*.meth)]*.meth
Activation		xcellbur Processing Xcellbor Output Dir	NORS	file Folder		N N	N N		\geq	3			8			

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

Main menu <	Sample			Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	xcalibur Processing	Xcalibur Output Dir
		Add	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 method row..." dialog. Define how many samples must be added to the sample list and press "OK".

Sample	Analysis Method C:\Pron).cam	d Tool		Source Vial	Sample Name	Xcalibur Method	Xcalibur Filename	xcalibur Processing	Xcalibur Output Dir	Volume 0
Remove					Copy meth	od with increme	nt of vial no			
	Copy r	ow.						×		
	How	man	y times sh	nould this	row be co	pied?	C	OK		
	2									

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

Sample			100		Source	viai Sample	Name Xcalibur Me	thod xcalibur Fi	iename xc	alibur Processing	xcalibur 0	Jutput Dir	volun
	C:\Pro	on).cam		EvoSlot1	1								0
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Sample		Analysis	s Meti	hod Source	Tray 5	Source Vial	Xcalibur Method	d Xcalibu	Autofi	ill with pattern.		bur Outp	ut Dir
	1	C:\Pro	n).ca	m EvoSlo	t1	1	C:\Pro\Plugins		Auton	n with pattern.		1	
<u>슈</u> dd	2	C:\Pro	.n).ca	m EvoSlo	t1 2	2	C:\Pro\Plugins	3					
Remove	3	C:\Pro	.n).ca	m EvoSlo	t1 3	3	C:\Pro\Plugins						
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Duplicate	5	C:\Pro	n).ca	m EvoSlo	t1 !	5	C:\Pro\Plugins	3					
	6	C:\Pro	n).ca	m EvoSlo	t1 (6	C:\Pro\Plugins	5					
Move up	7	C:\Pro	n).ca	m EvoSlo	t1 7	7	C:\Pro\Plugins	5					
Move down	8	C:\Pro	.n).ca	m EvoSlo	t1 (8	C:\Pro\Plugins	5					
v ····	9	C:\Pro	.n).ca	m EvoSlo	t1 9	9	C:\Pro\Plugins	5					
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🖳 Autofill with pa	ttern	×
New value with pat	tern (*)	
Test_***		
Starting number	Increment step	
1	1	
	OK Cancel	

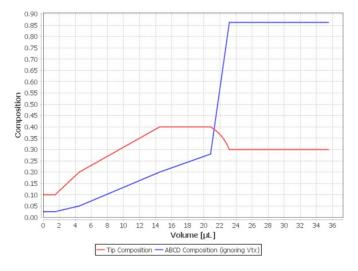
Sample			Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur Processing	Xcalibur Output Di
		1	C:\Pron).cam	EvoSlot1	1	C:\Pro\Plugins	Test_001		
÷	<u>A</u> dd	2	C:\Pron).cam	EvoSlot1	2	C:\Pro\Plugins	Test_002		
	Remove	3	C:\Pron).cam	EvoSlot1	3	C:\Pro\Plugins	Test_003		
(41113)		4	C:\Pron).cam	EvoSlot1	4	C:\Pro\Plugins	Test_004		
	Duplicate	5	C:\Pron).cam	EvoSlot1	5	C:\Pro\Plugins	Test_005		
		6	C:\Pron).cam	EvoSlot1	6	C:\Pro\Plugins	Test_006		
	Move <u>up</u>	7	C:\Pron).cam	EvoSlot1	7	C:\Pro\Plugins	Test_007		
-	Move down	8	C:\Pron).cam	EvoSlot1	8	C:\Pro\Plugins	Test_008		
V		9	C:\Pron).cam	EvoSlot1	9	C:\Pro\Plugins	Test_009		
List		10	C:\Pron).cam	EvoSlot1	10	C:\Pro\Plugins	Test_010		



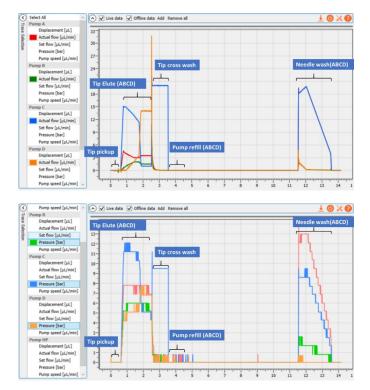
• If you often run the same analysis using the same methods, the sample list can be saved and reloaded instead of created from scratch.

8.3 Example Pump Data

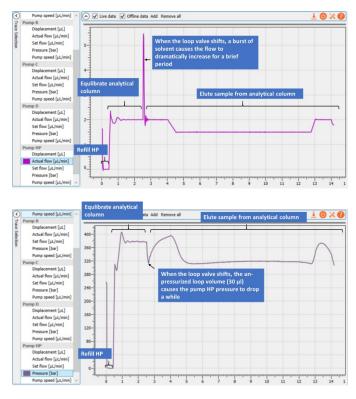
- 1. The pump graphs shown below are from a standard "100 SPD" chromatographic method on an Evosep One system using the installation analytical column (length 8 cm, ID 100 μ m, 3 μ m C18 beads).
 - Gradient



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

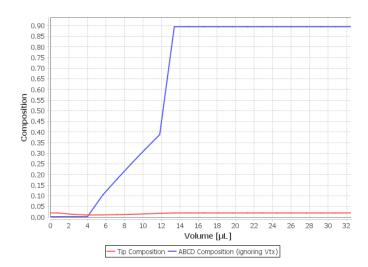


• The actual flow rate (µl/min; top) and pressure (bar; bottom) for the HP pump

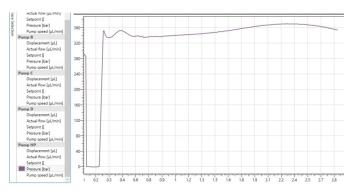


- 2. The standard "500 SPD" gradient and HP pump pressure and flow profile are shown below.
 - The 500 SPD method is optimized for speed and is unique to the other standard methods in several ways:
 - The ABCD flow rate is higher during the gradient formation.
 - \circ The pump refill speed is 200/100 µl/min for HP and A/B/C/D pumps, respectively.
 - The HP pump moves more rapidly when starting the column equilibration.
 - \circ $\;$ The tip cross wash and the needle wash is quicker.
 - \circ The HP pump is pressure controlled when the loop valve shifts to "elute" position.
 - Gradient:

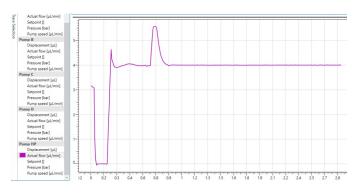




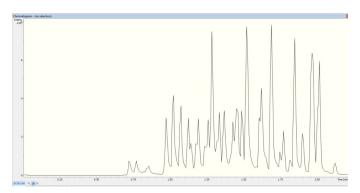
• Pump HP pressure (bar):



• Pump HP flow (μl/min):

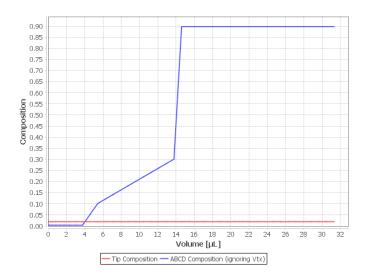


• Example base peak chromatogram of tryptic BSA digest:

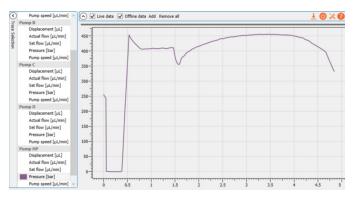




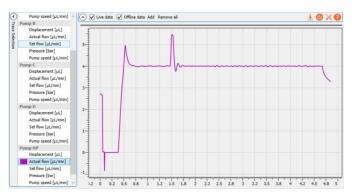
- 3. The standard "300 SPD" gradient and HP pump pressure and flow profile are shown below.
 - Gradient:



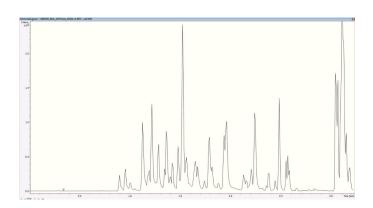
• Pump HP pressure (bar):



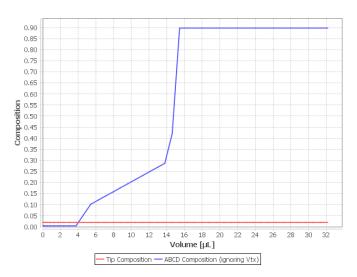
• Pump HP flow (μl/min):



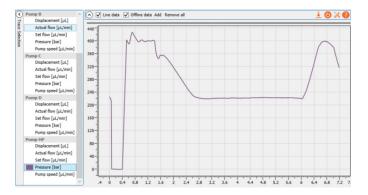
• Example base peak chromatogram of tryptic BSA digest:



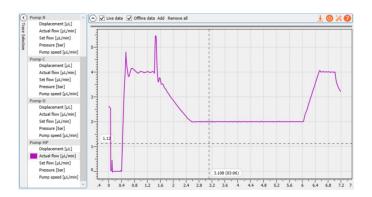
4. The standard "200 SPD" gradient and HP pump pressure and flow profile are shown below.
Gradient:



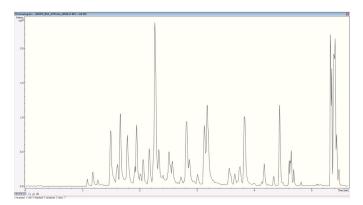
• Pump HP pressure (bar):



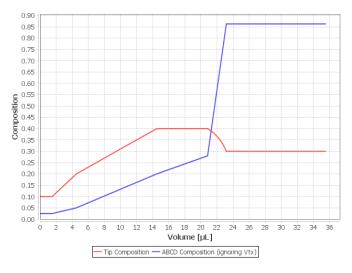
• Pump HP flow (µl/min):



• Example base peak chromatogram of tryptic BSA digest:



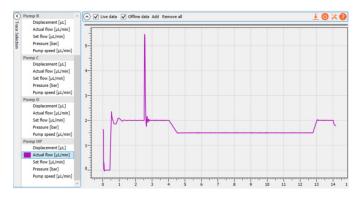
5. The standard "100 SPD" gradient and HP pump pressure and flow profile are shown below.
Gradient:



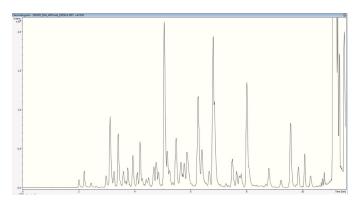
• Pump HP pressure (bar):

	data 🗹 Offline data Add Remove all	± 0 ×
Displacement [µL]		
Actual flow [µL/min]		
Set flow [µL/min] 400-	Λ	
Pressure [bar]	my /	
Pump speed [µL/min] 360-		
Pump C		
Displacement [µL] 320-		
Actual flow [µL/min]	N	
Set flow [µL/min] 280		
Pressure [bar]		
Pump speed [µL/min] 240-		
Pump D		
Displacement [µL]		
Actual flow [µL/min] 160-		
Set flow [µL/min]		
Pressure [bar]		
Pump speed [µL/min]		
Pump HP 80		
Displacement [µL]		
Actual flow [µL/min] 40		
Set flow [µL/min]		
Pressure [bar]		
Pump speed [µL/min]		

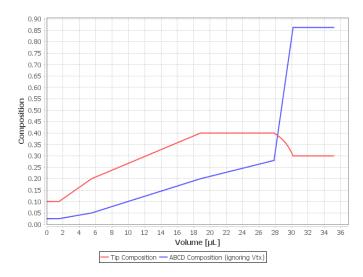
• Pump HP flow (μl/min):



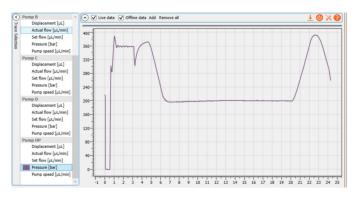
• Example base peak chromatogram of tryptic BSA digest:



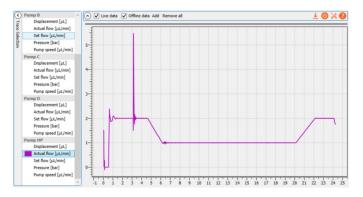
- 6. The standard "60 SPD" gradient and HP pump pressure and flow profile are shown below.
 - Gradient:



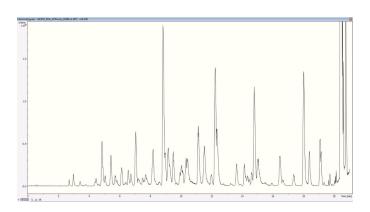
• Pump HP pressure (bar):



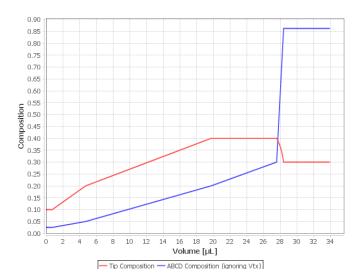
• Pump HP flow (μl/min):



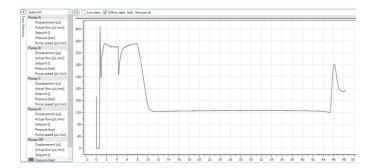
• Example base peak chromatogram of tryptic BSA digest:



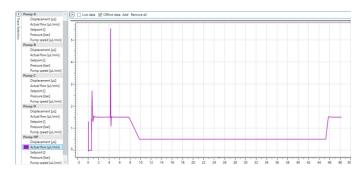
7. The standard "30 SPD" gradient and HP pump pressure and flow profile are shown below.
Gradient:



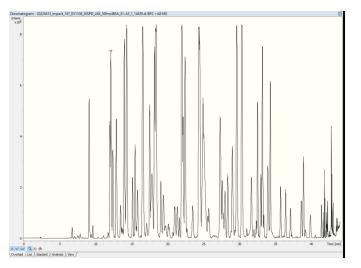
• Pump HP pressure (bar):



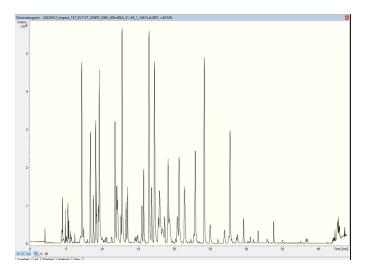
• Pump HP flow (μl/min):



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



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



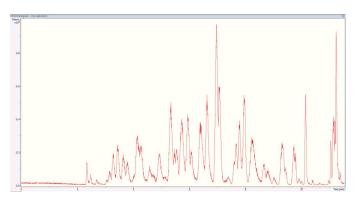
9 Troubleshooting

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

9.1 Evotip Troubleshooting

If the Sample loading onto the Evotips is not done correctly, it can have a negative influence on the results. This can happen if the chromatographic material in the Evotip dries out, either before or after loading a sample, or if the equilibration/washing protocol is not followed completely. Some examples of sample loading issues are illustrated below using 200 fmol BSA.

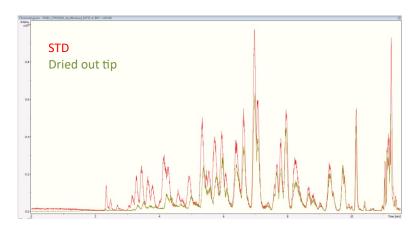
1. BSA correctly loaded on the Evotip according to the SOP.



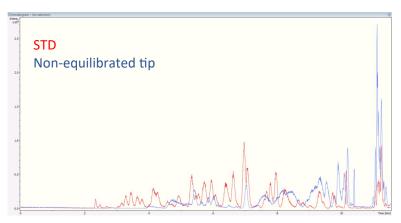
2. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out before loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



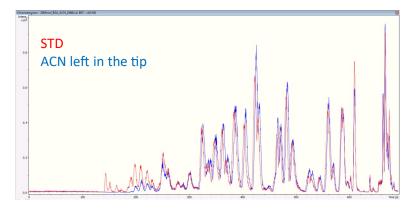
3. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which was dried out after loading (green). In the beginning of the gradient, the hydrophilic peptides are missing or weaker than normal.



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



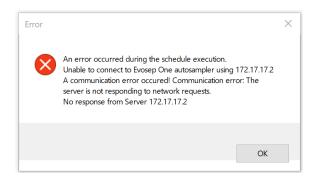
- 5. BSA correctly loaded on the Evotip according to the SOP (red) compared to an Evotip which has residual ACN from the wash step during loading the sample (blue).
 - This can happen if the centrifuge is not correctly adjusted according to the SOP (too low gforce or too short centrifugation time).





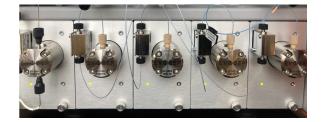
9.2 How to Troubleshoot Connection Problems Between PC and Evosep One

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



- 9.2.1 Check that Both the Pump Box and Autosampler are Powered On
 - 1. Pump box

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



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



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

9.2.2 Check LAN Connection

Scenario 1 Scenario 2 Office Evosep Office Evosep Network One Network One Switch PC PC Ethernet 1 (D) Ethernet 1 (D) MS MS Ethernet 2 (S) Ethernet 2 (S) MS link (D) Dynamically assigned IP address (DHCP) (S) Statically assigned IP address (172.17.17.14)

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

Please verify that the following:

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

9.2.3 Check Network Adapter Set Up

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

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



← → ✓ ↑ 😰 > Control Panel > Network and Internet	> Network Connections	5 V	Search Network Conne
Organize Disable this network device Diagnose this	connection Rename this connection View status of this con	nnection Change settings of this connection	8 [₽] 9 [₽]
Network 4	ments Intified network R) Ethernet I210-T1 GbE NIC	connected to Evosep One	
-	Instruments Properties X	Internet Protocol Version 4 (TCP/IPv4) Properties	×
Instruments Status	Networking Sharing	General	
General	Connect using:	You can get IP settings assigned automatically if yo this capability. Otherwise, you need to ask your ne for the appropriate IP settings.	
IPv4 Connectivity: No network access IPv6 Connectivity: No network access Media State: Enabled Duration: 01:50:46 Speed: 100.0 Mbps Details IP∨4 — Activity —	Configure This connection uses the following items:	Evosep One address	Fixed IP . 17 . 14 . 255 . 240
Sent — Received Bytes: 2,491,205,536 4,259,217,533	Install Uninstal Properties Description Transmission Control Protocol/Internet Protocol. The default wide area network protocol that provides communication across diverse interconnected networks.	Preferred DNS server:	· · · · · · · · · · · · · · · · · · ·

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

Network Connections				-		<
← → × ↑ 🔮 > Control Panel >	All Control Panel Items >	Network Connections	v 0	Search Network Conner	ctions 🔎	
Organize 👻 Disable this network de	evice Diagnose this conn	ection Rename this connection View status of this conn	ection Change settings of this connection	S: •		
HouseNET Network 4 Intel(R) Ethernet Connection (2	2) I Instrument		connected to Evosep			
Instruments Status	×	Instruments Properties	K Internet Protocol Version 4 (TCP/IPv4) Prop	erties	× Ad	dvanced TCP/IP Settings
General		Networking Sharing	General			Settings DNS WINS
PH6 Connectivity: Meda State: Duration: Speed: Detals Activity Sent — Sent — Sylfes: 1,605,671,773	No network access No network access Enabled 0:23:81:7 300.0 Megs IPv4 	Convect using:	Subnet mask: 255 Default gateway: Obtan DHS server address automatical @ Use the following DHS server addresse: Preferred DHS server:	Fixed IP 569-254-1 254-1 254-254-0 Advanced		Predess Subset mask 292.568.254.1 255.555.255.20 172.171.71.14 255.555.255.20 Ceful gatemay: Metric Catamay Metric Ck add to manually enter subnet Add Edit Remove Add Edit Remove
	Close	OK Cancel		OK Cancel		OK Cancel

9.2.4 Ping Hardware Units

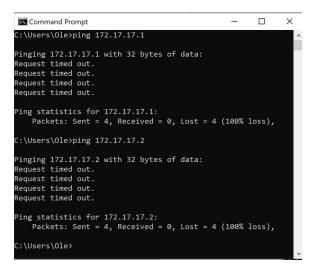
If there is still no connection to the instrument after the network adapter settings have been verified, use the "ping" command from the Windows Command Prompt to check communication with the hardware units.

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

• Autosampler: "ping 172.17.17.2"

	ows\system32\cmd.exe	
C:\>ping	172.17.17.1	
Reply fr Reply fr Reply fr	172.17.17.1 with 32 bytes of data: om 172.17.17.1: bytes=32 time<1ms TTL=64 om 172.17.17.1: bytes=32 time<1ms TTL=64 om 172.17.17.1: bytes=32 time<1ms TTL=64 om 172.17.17.1: bytes=32 time<1ms TTL=64	
Pack Approxim Mini	tistics for 172.17.17.1: ets: Sent = 4, Received = 4, Lost = 0 <0% 1 ate round trip times in milli-seconds: mum = 0ms, Maximum = 0ms, Average = 0ms	.oss),
C:/>ping	172.17.17.2	
Reply fr Reply fr Reply fr	172.17.17.2 with 32 bytes of data: om 172.17.17.2: bytes=32 time(ims TTL=128 om 172.17.17.2: bytes=32 time(ims TTL=128 om 172.17.17.2: bytes=32 time(ims TTL=128 om 172.17.17.2: bytes=32 time(ims TTL=128	
Pack Approxim	tistics for 172.17.17.2: tts: Sent = 4, Received = 4, Lost = 0 <0% 1 ate round trip times in milli-seconds: num = 0ms, Maximum = 0ms, Average = 0ms	.oss),

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



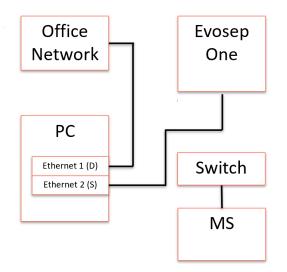
- 4. To verify that the Ping reply is coming from the Evosep One hardware and not another hardware unit with the same IP address, perform the following commands:
 - Pump box: "arp -a 172.17.17.1"
 - Verify the physical address has prefix "10-64-e2" or "00-90-e8"
 - Autosampler: "arp -a 172.17.17.2"
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

C:\Users\Quantum>arp -a	172.17.17.1	
Interface: 172.16.0.101 Internet Address 172.17.17.1	0xc Physical Address 10-64-e2 <mark>-</mark> 08-b2-5c	Type dynamic
C:\Users\Quantum>ping 17	2.17.17.2	
Pinging 172.17.17.2 with Reply from 172.17.17.2: Reply from 172.17.17.2: Reply from 172.17.17.2: Reply from 172.17.17.2:	bytes=32 time<1ms TTL: bytes=32 time<1ms TTL: bytes=32 time<1ms TTL: bytes=32 time<1ms TTL: bytes=32 time<1ms TTL:	=128 =128
Approximate round trip t	eceived = 4, Lost = 0	· · · ·
C:∖Users\Quantum≻arp -a	172.17.17.2	
	0xc Physical Address 00-14-2d-a5-1b-59	Type dynamic



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

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



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

9.3 Error Messages

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

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

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

Error messages



1.	Instrument at 172.17.17.1:2 gateway target device failed to respond
2.	Pumpa at 172.17.17.1:7 gateway target device failed to respond, or
2.	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: Th
	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 aft
	a period of time, or established connection failed because connected host has failed to respor
	(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/inject
	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 happe
	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 softwa
	package to obtain compatibility with the connected hardware!
9.	An error occurred during the schedule execution. There is already a listener on IP endpoi
	127.0.0.1:64001. This could happen if there is another application already listening on this endpoint
10.	Tip expected but not present
11.	Autosampler – Unable to access autosampler. Please check that it's powered on, the ethernet cab
	connected and that it's not locked by the handheld terminal
12.	Pumphp – Pressure overload
	Pump(a,b,c,d) – Pressure overload
14.	MoveToObject(Rack 1,1,True,True,False) (or TipCheck, Inject, Wash etc.)
	MoveTorqueMode(3,10 mm,400 mA,5 mm/s
15.	
	MoveValveDrive(Valve Drive 1, xxxdeg, xx rad/s)

Γ



	Please check the solvent bottle levels and run the Prepare – Pump preparation - Degas.
18.	Preparation – Low pressure pump(s) did not meet preparation criteria, and or
	Preparation – High pressure pump did not meet preparation criteria
19.	High pressure on pump A and/or B detected. Please wipe the needle tip and perform a Diagnose - restriction test.
20.	No Evotip was present during the analysis and the sample was aborted. Please check if the Evotip position in the autosampler matches the sample list.
21.	[Pump(hp,a,b,c,d)] fan detection fault
22.	[Pump(hp,a,b,c,d)] drive not responding
23.	Couldn't verify method script authencity
24.	Flow sensor offset
25.	Measured difference in flow between solvent A and B is x /min, please check that the solvents are correct and run Tip seal and HP system tests

9.4 Error Messages Regarding Communication Issues With Chronos and Evosep Plugin

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

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 the above
- Possible cause:
 - Not possible to establish connection to one/several or all the devices connected to the backplane (pump hp and pump a-d).
 - This could be caused by a device not fully inserted into the backplane connector, a faulty connector etc.
- Action:
 - Ensure that all pump cassettes are fully inserted and that the finger tight front screw is tightened.
 - \circ Verify that the pump LED on the front of the pump cassette is on.

Error message:

3. An error occurred the schedule execution.

A communication error occurred! Communication error: The server is not responding to network requests.

No response from Server 172.17.17.2 or 172.17.17.1

- Possible cause:
 - The data system cannot connect to the instrument, either because the instrument is not switched on, the ethernet cable is not connected, or the data system network configuration is not set up correctly.
- Action:
 - See Chapter 9.2 "How to Troubleshoot Connection Problems Between PC and Evosep One".

Error message:

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



- The error message "...An existing connection forcibly closed by the remote host" for any of the hardware devices is typically caused by the Evosep One being power cycled with Chronos previously connected to the system.
- \circ $\;$ The error message will appear when trying to connect to the system.
- Action:
 - If the Evosep One is being power cycled or moved to another data system, Chronos should always be restarted before connecting.

Error message:

- 5. Pump(hp,a,b,c,d) A connection attempt failed because the connected party did not properly respond after a period of time, or established connection failed because connected host has failed to respond (172.17.17.1:502)
 - Possible cause:
 - Typically indicates loss of communication when the instrument is connected. E.g. if the ethernet cable is disconnected.
 - Action:
 - Check ethernet cable and that all devices/pumps are fully connected/inserted.
 - Power cycle the Evosep One, restart Chronos, and connect to the system.

Error message:

- [pump(hp,a,b,c,d)] Flowmeter fault or
 [pump(hp,a,b,c,d)] Loadcell fault
 - Possible cause:
 - Indicates wrong/no signal from either the flow sensor (flow meter) or the pressure sensor (load cell).
 - Could be caused by a broken sensor or cable.
 - Action:
 - Power off the instrument and check that the relevant sensor cable is connected correctly.
 - Power back on the instrument and reconnect to instrument.
 - For flow sensor try to swap cables on the flow sensor to see if error message still is on the same sensor (see note below).

Please note:

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

Error message:

- 7. An error occurred during the schedule execution. Most probable an instrument or tray/agitator/injector was not defined in the setting or was forgotten to be set in a method
 - Possible cause:
 - Method stopped by user when the autosampler is active.
 - Method stopped by system due to other error state (e.g. reaching max pressure).
 - Action:
 - If method is stopped on purpose by the user, click ok and ignore message.



• If method is stopped unexpectedly, click ok to ignore message and see error message causing the method to stop unexpectedly.

Error Message:

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

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

or

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

- Possible cause:
 - Mismatch between firmware version of Evosep One and the Evosep One software plugin installed on the PC. Please note that this only refers to the Evosep One software and not Chronos.
- Action:
 - Update the Evosep One software on the PC to the latest version and connect to the Evosep One from that PC.
 - Before updating the SW, please read the release note for more information on new features, changes, etc.

Error Message:

- 9. An error occurred during the schedule execution. There is already a listener on IP endpoint 127.0.0.1:64001. This could happen if there is another application already listening on this endpoint or if.....
 - Possible cause:
 - This IP address is used for configuring the connection to the autosampler while connecting to the instrument. The error message will be shown if the address is not available. This can occur if Chronos and or the Evosep plugin has not been closed correctly.
 - Action:
 - Close Chronos and Chronos processes (using the Windows Task Manager) and try to connect again. If this does not work, please restart the computer.

Error Message:

- 10. Autosampler Unable to access autosampler. Please check that it's powered on, the ethernet cable connected and that it's not locked by the handheld terminal
 - Possible cause:
 - During maintenance, the autosampler has been controlled with the terminal and the action has not been completed.
 - Action:
 - With the terminal, check and finish pending action (e.g. change tool).
 - Check that the autosampler is powered on and all cables connected.

9.5 Error Messages Regarding Hardware

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

Error Message:

11. Tip expected but not present

- Possible cause:
 - No tip detected on needle. Typically caused by executing a sample run without having an Evotip in the correct position.
- Action:
 - Verify that a tray has been placed in correct position and that an Evotip is present.
 - For sample acquisition, any position can be chosen in the sample list. For Diagnostic runs, EvoSlot 1, pos 1 is hard coded.
 - Check that the needle is not broken.

Error Message:

12. Pumphp – Pressure overload

- Possible cause:
 - Pump HP (high-pressure pump) has reached maximum pressure during a method run.
 Typically caused by a blocked emitter or column, or using a column not suitable for the chosen method.
- Action:
 - Verify with Table 1 "Evosep One Methods" in Chapter 7 that the column being used is compatible with the chosen method.
 - Run a blank tip using the same method with and without the emitter connected a well-functioning emitter will only give a few bars added backpressure to the setup.
 - \circ Replace column with a new one and verify that backpressure drops to an acceptable level.
 - Run the Diagnose Restriction test to verify that the instrument back pressure without column connected is ok.

Error Message:

13. Pump(a,b,c,d) – Pressure overload

- Possible cause:
 - One or more of the low-pressure pumps has reached maximum pressure during method run. Typically caused by a blocked restrictor tubing or a blocked needle.
- Action:
 - Run the Diagnostic Restriction test.
 - If the backpressure is too high on both pump A and B, replace the needle (see Section 11.12) and re-run the test.
 - If only one of the four low-pressure pumps pressure is too high, disconnect the restrictor tubing for the failing pump from the 12-port valve (restrictor tubing's are labelled with a red marker). To identify the correct tubing, follow the tubing from the flow sensor down to valve 12. See below photos (flow sensors are positioned from left)



to right A,B,C,D). With the tubing disconnected from the 12-port valve, run the Prepare – Pump preparation – Degas script to flush out the valve port. Reconnect the restrictor tubing and re-do the restriction test. If this does not solve the problem, a new restrictor tubing should be installed.



Error Message:

- 14. MoveToObject(Rack 1,1,True,True,False) (or TipCheck, Inject, Wash etc.)
 - Possible cause:
 - Movement for robot X, Y or Z axis could not be completed because of a collision or error. The example given is movement to rack, but it could also be Tipcheck, Inject, Wash, etc.
 - Action:
 - Verify that nothing is physically stopping the movement of the robot arm. It could be another instrument or a wall that is obstructing the robot arm. Be aware that the Yaxis needs some room to move on the backside of the instrument.
 - Make sure that the A,B transfer line going to the needle tee has free movement, etc.

Error Message:

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

- Possible cause:
 - Movement for robot Z-axis (up-down) into Inject port, or other position, could not be completed.
- Action:
 - Verify that nothing is physically stopping Z-axis movement (up-down), including the A,B transfer tubing or a tip in a wrong position.
 - Verify that the needle is securely tightened to the needle Tee.

Error Message:

- 16. MoveValveDrive(Valve Drive 1, xxxdeg, xx rad/s)
 - Possible cause:
 - Valve could not be switched into position. This can happen if there is too much friction to move the valve or if valve drive is disconnected.
 - Action:



- Verify in the error message what valve drive has the issue (Valve Drive 1 = Valve 6, Valve Drive 2 = Valve 12, Valve Drive 3 = Valve Loop).
- Verify that the p-bus cables from robot X-axis to valve drive and between valves drives are fully connected into the connector on the X-axis and valve drives.
- Power cycle instrument and retry.

Error Message:

- 17. Xxx samples per day (x.x min) Pump HP does not contain sufficient solvent to perform the analysis. Please check the solvent bottle levels and run the Prepare Pump preparation Degas program.
 - Possible cause:
 - \circ If pump HP uses too much solvent (20 μ I) to build up pressure during column equilibration, the analysis is stopped. This is done to avoid the risk of the pump emptying completely during the gradient.
 - Action:
 - Ensure the solvent levels in the solvent bottles are adequate and run Prepare Pump preparation Degas. If the problem persists, run the Diagnose Pump HP leak test.

Error Message:

18. Preparation – Low pressure pump(s) did not meet preparation criteria and/or

Preparation – High pressure pump did not meet preparation criteria

- Possible cause:
 - During Prepare Pump Preparation Degas and Solvent exchange scripts, the solvent volume is measured to verify that pump can build up pressure (LP 50 bar, HP 200 bar). If the volume needed to build pressure is too high, the instrument will stop with above error message. The most likely cause is that the solvent bottles are empty or the tubing in the bottles are not submerged.
- Action:
 - Check the solvent levels in bottle A and B and verify the solvent lines are submerged.
 - Perform a visual inspection for obvious damage, kinks, or leaks on the flow lines between the pump(s) listed in the error message and Valve 6 and 12.

Error Message:

- 19. High pressure on pump A and/or B detected. Please wipe the needle tip and perform a Diagnose restriction test.
 - Possible cause:
 - Pump A/B sample loading pressure is above 50 bar.
 - Action:
 - Please perform a Diagnose Restriction test to verify that instrument flow paths are not blocked.

Error Message:

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



- Possible cause:
 - Pump A/B sample loading pressure is above 50 bar and a tip is not present on the needle.
 - May occur if a Tip is not present on the needle when going into the injection port, or if no Tip is detected on the needle after injection when the needle is moved to Tip eject.
- Action:
 - Verify the Evotip position in the autosampler matches the sample list (e.g., check that the same Evotip position was not chosen twice or that a wrong Evotray slot has been chosen).
 - Check that a Tip is not sitting in the Tip inject port.
 - Check needle for damage.
 - Re-run with a blank Evotip and visually inspect the Evotip pick, inject, eject, etc.

Error Message:

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

- Possible cause:
 - Pump fan is faulty or not connected.
- Action:
 - Power cycle the instrument and verify if the error is still present and reported for the same pump.
 - Contact support@evosep.com to inform about the error message.

Error Message:

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

- Possible cause:
 - Faulty pump PCB (Printed Circuit Board).
- Action:
 - Power cycle instrument and verify that the error is still present and reported for the same pump.
 - Contact support@evosep.com to inform about the error message.

Error Message:

23. Couldn't verify method script authenticity

- Possible cause:
 - The method script has been edited and is no longer in its original form.
- Action:
 - Reinstalling the Evosep plugin will overwrite the changes and return the instrument methods to their original state.

Error message:

24. Flow sensor offset

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



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

Error message:

- 25. Measured difference in flow between solvent A and B is x /min, please check that the solvents are correct and run Tip seal and HP system tests
 - Possible cause:
 - To find the loop volume, the system relies on being able to distinguish if solvent A or solvent B is present in the transfer line. It does so by measuring the actual flow rate at 40 bar. When solvent A is present, a lower flow rate will be measured compared to when solvent B is present in the transfer line, due to the difference in viscosity of the 2 solvents. If this difference is less than 100 nL/min, the system cannot distinguish the solvent change, and hence not calculate the exact loop volume.
 - This can happen if the solvents of the system are wrong or if a leak is present.
 - Action:
 - Verify the solvents are correct (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile).
 - Check that the system is level and free of vibration.
 - If both above are correct, then perform a Prepare Pump preparation Solvent exchange and run a new sample.
 - Run Diagnose Tip seal and HP system scripts to check for leaks.

9.6 Schedule / Sample Not Starting / Contact Closure Problems

9.6.1 Troubleshooting Tips for Xcalibur Set Up

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

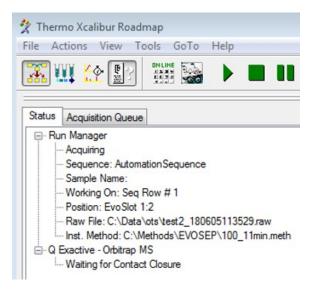
The run log for a typical schedule will look like this:

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:

- After a schedule is started it takes several minutes before the first sample acquisition is started.
 -Info: One or more pumps not referenced, referencing now.
 -Info: One or more pumps low on solvent, refilling now.
 - Possible cause:
 - If the instrument has been switched off or the low-pressure pumps has been stopped without enough solvent to complete a sample run, the instrument will automatically initiate a reference or refill followed by Prepare – Pump preparation – Degas and Align solvent before starting the first sample in the schedule. This is shown in the Run log with following message:
 - Info: One or more pumps not referenced, referencing now. Or,
 - Info: One or more pumps low on solvent, refilling now.
 - When the "degas" and "align solvents" activities are competed, the instrument will start the sample.



	2018-06-06 13:26:18	Logging to file C:\ProgramData\Evosep\EvosepOne\Jogs\Runlog Schedule 7 20	C.
++•	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, refiling now	
1	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	

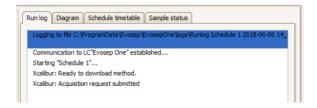
- Action:
 - o None, after successful degas and align solvents the instrument will start the sample

Typical issue 2:

- 2. Evosep One does not continue the schedule after the first sample, MS does not change status from Waiting for contact closure to Running.
 - Possible cause:
 - Contact closure cable is not connected, faulty, or not configured correctly.
 - When a schedule is started the instrument will check if MS is ready and then start the first sample. If the contact closure cable is not properly connected, the MS will not be started and will be staying in waiting for contact closure mode, and therefore not ready to receive the next sample from the Evosep One.
 - Other LC device present in MS system configuration.
 - Action:
 - Verify that contact closure cable is connected, configured correctly, and not faulty.
 - Please see "Connecting the Contact Closure" Chapter in this manual for information regarding setting up contact closure in the MS manual.
 - Other LC/autosampler device present in MS Instrument Configuration. See Chapter "How to Remove Other LC Devices from MS System Configuration".

Typical issue 3:

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

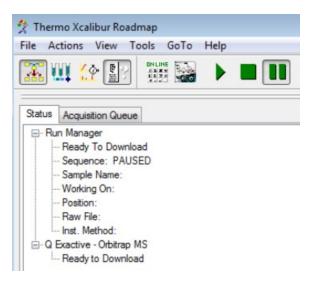


- Possible cause:
 - Most likely the MS is not ready.
 - Other LC device present in MS system configuration.
 - o Chronos Sample list not complete, e.g. Xcalibur Filename has not been filled in.

	Analysis Method	Source Tray	Source Vial	Xcalibur Method	Xcalibur Filename	Xcalibur Sample Na Xcalibur
1	C:\Program Files (x86)\Chr\Xcalibry 100 samples per day (11.5 min).cam	EvoSlot 1	1	C:\Thermo\Instruments\TSQ\Methods\11.5minLCMS_030717.meth		C:\Xcalit
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		⊂ı\Xcalit
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:
 - In the status view for MS, verify that Sequence is not set to "PAUSED" and that MS status is "Ready to Download".
 - Other LC/autosampler device present in MS Instrument Configuration. See Chapter "How to Remove Other LC Devices from MS System Configuration".
 - Check that Chronos Sample list is filled in correctly.



Typical issue 4:

4. Evosep One is still in the middle of a sample but the MS has stopped the acquisition, or

The MS is still acquiring sample 1 while Evosep One has finalized sample 1 and does not proceed to sample to the next sample in the sample list.

- Possible cause:
 - Most likely the MS method is not set to the same length as the Evosep method.
- Action:
 - Verify that the correct MS method length is the same as the Evosep method.

9.7 Hardware Troubleshooting

The Evosep One software is preconfigured with several diagnostic programs that enable the user to do basic system leakage and restriction tests. The programs run automated procedures to pinpoint failing hardware parts and provide information on how to fix the most common errors. This Section describes the diagnostic programs and provides additional background information on the error causes and how to repair the issue.

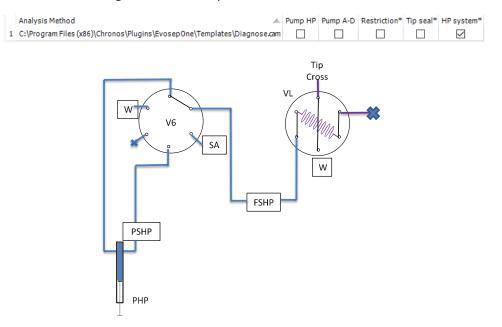
9.7.1 Leak in the HP System

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



When asked to blind the transfer line, please use the stainless-steel cap (P/N EV1062).

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



1. 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 tubing connections and re-run the script.
- If the script fails, blind the line FSHP to valve loop with the cap and re-run the script.
- If the script passed, it indicates that the leak is in the valve. Exchange the rotor seal and re-run the script.
- If the script fails, call for assistance.
- 2. The flow is too high, 0.10 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.
- 3. The flow is too high and is observed on the pump speed, >0.50 μl/min, and no flow is observed on the flow sensor. A leak measured as pump speed is a sum of a leak before and after the FSHP. If both are failing fix the leak after the flow sensor first.
 - This indicates a leak before the FSHP. It could be in the pump, valve, or connecting lines.
 - Check all lines and re-run the script.
 - If the script fails, please run the Diagnose Pump HP script.

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

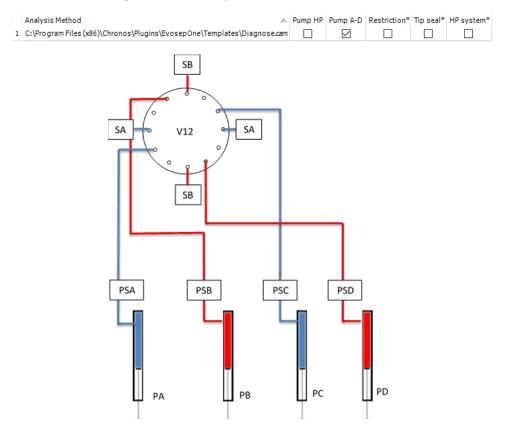


- If the script fails, exchange the rotor seal, and re-run the script.
- If the script fails, call for assistance.

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 script Diagnose – Pump A-D.

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



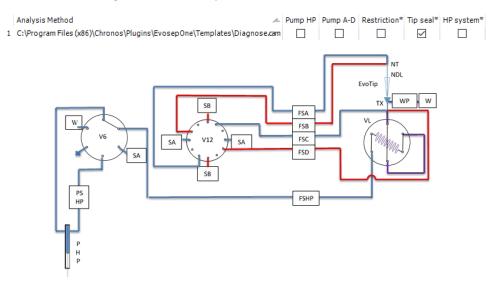
- 1. A leak is observed as a flow that is too high on pump speed for PSA, PSB, PSC or PSD, pump speed > 1.0 μ l/min.
 - Tighten the connections in the subsystem displaying a leak and re-run the script.
 - If the script fails, block the line going from the pump pressure sensor to the V12 at the V12 end with the 1/32" cap, and re-run the script.
 - If the script passes, exchange the rotor seal and re-run the script.
 - If the script fails, call for assistance.

9.7.3 Leak in the LP System, Tip Seal Area

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



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

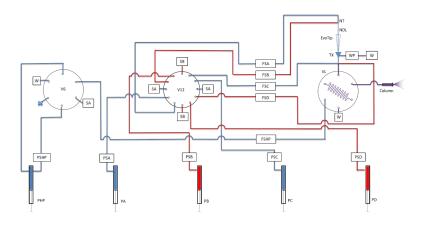


- 1. A leak is observed with a flow that is too high on FSHP, flow > 0.10 μ l/min and negative flow on FSA, FSB, FSC, or FSD.
 - Tighten the line connecting the FS to the V12 and re-run the script.
 - If the script fails, place the endcap on the line connecting to the V12 and re-run the script.
 - If 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.
- 2. 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 Diagnose – Restriction.

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



The restriction test is divided in 3 parts:

1. Low pressure system

- A pressure above 70/50/70/50 bar for pump A/B/C/D respectively is a sign of a partially blocked subsystem.
- If both pump A and pump B are restricted:
 - This indicates a blocked needle.
 - Please try to rinse the tip of the ceramic needle with Kimwipe or similar, soaked in methanol.
 - Exchange the needle.
 - Re-run the script and if the restriction still is too high, call for assistance.
- If either pump A/B/C/D are restricted:
 - This indicates a blocked line from V12 to the respective flow sensor.
 - Disconnect the tubing on valve V12, flush the valve port with ethanol to remove residual material, and reconnect the tubing.
 - Exchange the tubing.
 - Re-run the script. If the restriction is still too high, call for assistance.

2. High pressure system:

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

3. Tip interface and loop:

- A pressure above 50 bar for either pump A/B/C/D indicates a partial restriction of the tip interface or the loop.
 - Disconnect the loop on the loop valve, flush the valve ports with ethanol to remove residual material, and reconnect the loop.



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



10 Routine Maintenance

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

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

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

10.1 Recommended Maintenance Schedule

10.1.1 Daily Maintenance

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

10.1.2 Weekly Maintenance

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

10.1.3 Prepare Instrument for Storage

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

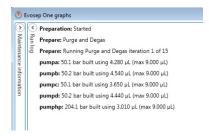
- 1. To prevent bacterial growth, it is recommended to switch to organic solvents.
- 2. To minimize risk of instrument contamination, always wear gloves when handling the tubing going to the solvent bottles.



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

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

- 1. As acetonitrile is a much better solvent for degassing the pumps, start out with a few "Solvent exchange" cycles with acetonitrile on both channel A and B to ensure there is no air trapped inside of the pumps.
- 2. Empty, rinse, and refill solvent bottles A and B.
- 3. Place both A and B solvent lines in Solvent B bottle as illustrated above.
- 4. Empty waste bottle.
- 5. Run 3-4 cycles of the "Solvent exchange" script and ensure that values in the run log are within specification.
 - Pump HP is less than 6 μ l.
 - Pump A-D is less than 7 μ l.



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



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

11 Replacing Spare and Wear Parts

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



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

11.1 Recommended Wear Part List

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

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

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

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

Please find pictures of all parts in our web shop.

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

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

11.2 Power Off the Instrument

- 1. Stop any running procedures, including idle flow.
- 2. Go to the Graph viewer window.
- 3. Click the orange power icon and click "OK" in the pop-up window to park the autosampler in the lock-position.

۲	Evosep One graphs		
2	[Run log is empty]	Elive data Offline data Add Remove all	<u>⊾ @ × @</u>
lainten	un log	20 0.04	Park autosampler Park autosampler in lock-position
ance inforr		lection 0.03	Do you want to park the autosampler in the lock-position?
nation		0.02	OK Cancel

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

Evos	ep One graphs
Run log	Prepare for power off: Parking the autosampler in the lock-position Prepare for power off: Done, please close the software and disconnect the power supplies

Note: if for some reason it is not necessary to switch off the instrument after moving the autosampler Z-axis to the lock position, a new procedure can be started.

11.3 Replacing the HP Pump Cassette

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

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H.	
•	•
	Pump H
-	

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



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

11.4 Replacing the LP Pump Cassette

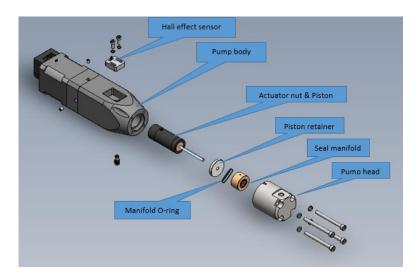
- 1. Power off the instrument.
- 2. Disconnect the peek tubing from the bottom port on the LP pressure sensor.
- 3. Loosen the knurled nut in the lower right corner of the pump cassette.



- 4. Gently pull out the pump cassette from the instrument by pulling the knurled nut. Be careful not to kink or break the surrounding tubing.
- 5. Insert the pump cassette in reverse order.
- 6. Run Preparation Pump preparation Solvent exchange to ensure the new pump is fully purged and degassed.
- 7. Run the Diagnose Pump A-D to ensure that no leaks are present after the replacement of the pump cassette.

11.5 Replacing HP/LP Pressure Sensor

- 1. Remove the pump cassette from the instrument as described in Sections 11.5 and/or 11.6.
- 2. Disconnect the tubing from the top port on the LP pressure sensor (for the HP pressure sensor this is already done when removing the cassette).
- 3. Using a T10, remove the 2 screws that hold the pressure sensor.
- 4. Gently slide the pressure sensor away from the cassette and disconnect the pressure sensor cable.
- 5. Install pressure sensor in reverse order. Make sure the pressure sensor cable connects securely into the pressure sensor.



11.6 Replacing Pump Piston Seals Including Seal Manifold

- 1. Run the Service script to fill pumps. This will fully retract the piston within the pump housing.
- 2. Disconnect the tubing from the pump head.
- 3. Use a 3 mm hex key to loosen and remove the 4 pump head screws. Loosen the screws diagonally to evenly loosen the pump head.
- 4. Carefully, and in a straight line from the pump block, slide the pump head away from the pump.
 - WARNING: sapphire pistons are very shock sensitive. Use extreme caution, and do NOT shock or side-load the piston in any way!
- 5. Remove the seal manifold with its two piston seals and O-ring by sliding it off the piston.

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



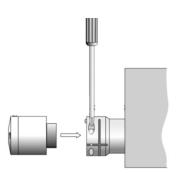
1. To install, first insert the seal manifold in the pump head with the O-ring pointing outwards and make sure to align the manifold drain slots vertically.



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



- 5. Run the degas script to remove air from the pump.
- 6. Run leak test for that pump to ensure that the new seal is sealing correctly.
- 11.7 Replacing a Valve Stack (Field Service)

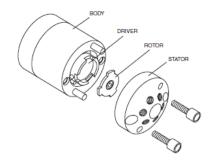


- 1. Disconnect all tubing from the stator port.
- 2. For replacement of Valve 6, run the service script called "Set valve 6 pos 2-3"
- 3. Unscrew the Torx screw at the valve adapter, which holds the valve to the valve drive.
- 4. Install the replacement valve and reassemble the above steps in reverse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

11.8 Replacing Valve Drive Actuator (Field Service)



11.9 Replacing a Valve Rotor and/or Valve Stator





- 1. Use a 9/64" hex driver to remove the socket head screws which secure the stator on the valve. Start by alternating between the two screws, loosening them in quarter-turn (90°) increments until all the load is removed.
- 2. Slide the stator off the two guiding rods, . Be careful not to stress any tubing connected to the stator.
- 3. Visually inspect the stator surface within the inner ring.
 - If stator surface is not 100% clean, remove residues with a lint free tissue soaked in appropriate solvent.
 - If the stator surface cannot be cleaned or is scratched, it should be replaced.
- 4. With your fingers or a small tool, gently pry the rotor away from the driver.
- 5. Replace the rotor in the driver. Make sure that the rotor sealing surface with its engraved flow passages is facing out. The tab pattern is asymmetrical to prevent improper placement.
- 6. Slide the stator back onto the guiding rods with port one pointing upwards. Be careful that rotor sits securely in valve drive and that no tubing is caught between stator and valve driver.
- 7. Insert the two socket head screws and tighten them gently until they start to get snug. Then alternate between the two screws, tightening them in quarter turn (90°) increments until the stator is flush against the valve body. Do not overtighten the screws they simply hold the assembly together and do not affect the sealing.
- 8. If a new stator has been installed, please refer to tubing diagram in Section 11.10 for connecting all tubing to the correct stator ports.

11.10 Tubing and Fittings

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

Viper and nanoViper tubing:



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

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



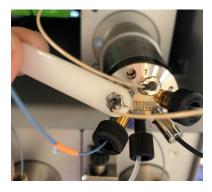
5. If the fitting is leaking, turn the screw up to an additional 45 degrees. Do not turn the screw beyond 90 degrees from where the initial resistance was felt.

NanoConnect tubing:



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

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



Peek tubing:

Peek tubing is used for low pressure connections on the system with various fittings. These are all finger tight connections. No tools should be used for tightening.

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



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

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



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

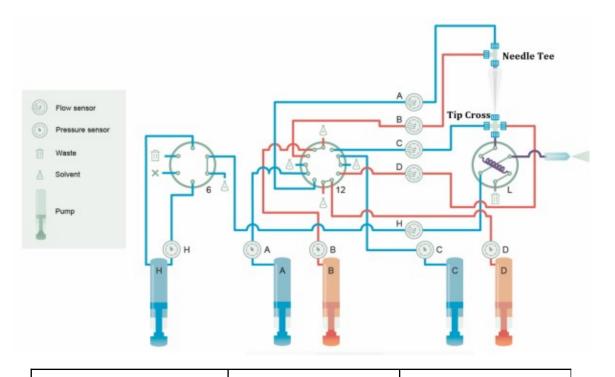
Tubing for Viper compatible ports on LP pressure sensors, comes with a 10-32 coned fitting for 1/32" OD tubing.



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



Tubing diagram and ports valve port positions:



Valve Drive 1 Valve 6 tubing position		Valve Drive 2 Valve 12 tubing position		Valve Drive 3 Valve Loop tubing position	
P 1	Pump HP	P1	Solvent B Tee	P1	TipCross
Р 2	Waste	P2	Pressure sensor B	P2	Loop
Р 3	Blank	Р3	Flow sensor B	Р3	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		

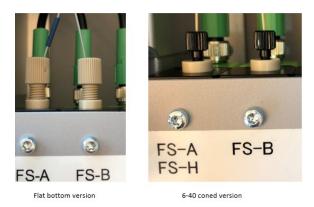


P1 0	Solvent C Cross	
P1 1	Pressure sensor C	
P1 2	Flow sensor C	

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 <u>Evosep Support</u> or contact <u>support@evosep.com</u>.



To remove the tubing:

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





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



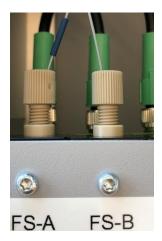


7. With a T10, loosen the screw from the tubing holder in the back-left corner of the cabinet and gently remove the holder.





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



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

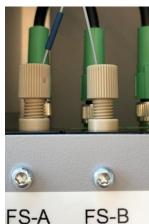


To connect new tubing:

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







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







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





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





11.12 Replacing the Needle

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

PAL	RSI	0 11:22	RobotArmLeft	😑 11:3
			Press 'Enter' to edit a p	arameter.
	Valve Drive 1		Plunger Drive 1	>
10	SelectorValve; Valve6		Tool Control 1	>
	Valve Drive 2 SelectorValve; Valve 12		X Drive 1	>
D	Valve Drive 3 SelectorValve; Valve Loop		Y Drive 1	>
	TipInject		Z Drive 1	>
	RobotArmLeft			
)	Input Output 1			
Ī	•			
oti	ions		Options	

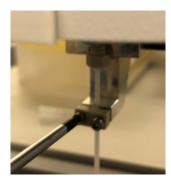
2. Select "Options" and "Change Syringe".

RobotArmLeft Press 'Enter' to edit a pa	😁 11:36 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. Select "Move" to move the needle to the exchange position.



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



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



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



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

11.13 Replacing the Needle Tee

- 1. Remove the needle as described in Section 11.12 "Replacing the Needle".
- 2. Disconnect the A and B NanoConnect tubing from the needle tee. Be careful not to kink or stress the tubing.
- 3. Loosen the lock screw from the needle tee holder and remove the needle tee.
- 4. Install needle tee in reverse order.

11.14 Replacing the Tool

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

PAL	RSI 🥌 11:22	RobotArmLeft	😑 11:2
		Press 'Enter' to edit a p	arameter.
_	Valve Drive 1	Plunger Drive 1	>
0	SelectorValve; Valve6	Tool Control 1	>
b	Valve Drive 2 SelectorValve; Valve 12	X Drive 1	>
	Valve Drive 3 SelectorValve; Valve Loop	Y Drive 1	>
-	TipInject	Z Drive 1	>
	RobotArmLeft		
)	Input Output 1		
	•		
ti	ions	Options	

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

RobotArmLeft	📵 11:40			
Press 'Enter' to edit a parameter.				
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. Select "Move" to move the tool to the exchange position.



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



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

Change Tool	😝 11:43						
Step 2 of 2: Assign Tool parameters							
Press 'Enter' to edit a parameter.							
Syringe Type	GT Syr1						
Ndl Guide Type	NonMagn2mL						
Please note: The Needle Guide Type has to correspond with the vial cap type.							
Options	Ok						

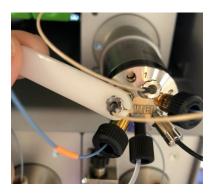
11. Press "Ok" to move robot arm to home position.

11.15 Replacing the Loop

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



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

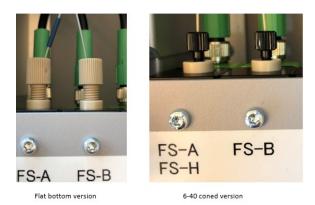


- 4. Install the new loop in reverse order using the small torque wrench to ensure proper tightening of the loop fittings.
- 5. After installation, run the Service Loop flush script to flush the new loop with solvent.
- 6. Run the Diagnose HP system script to verify that there are no leaks around the loop.
- 7. Run the Calibrate Loop volume script to measure the volume of the loop.
 - The loop volume calibration script measures the exact volume of the sample loop. The calculated volume is used in the sample runs to ensure higher analyte retention time accuracy. If the loop is replaced, the Calibrate Loop volume script must be executed.

11.16 Replacing a Flow Sensor

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

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



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

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



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



5. Install the new flow sensor in reverse order making sure to orientate the cable correctly.



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

		🖉 Add Sample(s)			×	
		Method Number of samples	Calibrate	•		
⊂] A			Add Close			
1	Analysis Method	Chronos\Plugins\Evo	ے sepOne\Templates\Calibrate.cam	Flow sensor ABCD	Flow sensor HP	Loop volume*

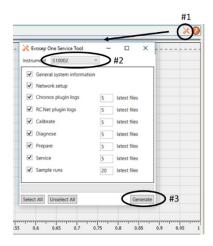
9. If a low-pressure sensor (FS-A-D) has been replaced, schedule and run the Diagnose – Tip seal script to verify that the tubing connections around the flow sensor is leak free. If the high-pressure sensor (FS-HP) has been replaced, schedule and run the Diagnose – HP system script.

12 Support, Service, and Warranty

12.1 How to Request Technical Support

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

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

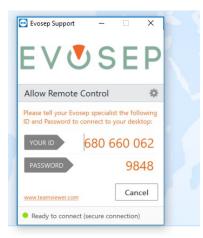


• For remote support please supply TeamViewer ID and Password as described below.

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

12.2 Remote Support via TeamViewer

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



12.3 How to Arrange for a Service Visit

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

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

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

12.4 Product Warranty

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

The warranty covers defects or failures of the Evosep One system and its major hardware parts occurring due to normal use or manufacturing defects.

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

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