EVOSEP ONE:

A GRADIENT OFF-SET FOCUSING UHPLC INSTRUMENT FOR ROBUST AND HIGH THROUGHPUT PROTEOMICS

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Introduction:

Mass spectrometry-based proteomics and metabolomics are fast growing and powerful technologies, with the potential to revolutionize health care and precision medicine. Evosep One has been designed specifically to address and eliminate the prevalent challenges associated with throughput and robustness of nano-flow LC-MS workflows while maintaining sufficient sensitivity and resolving power for clinical omics applications.

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

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

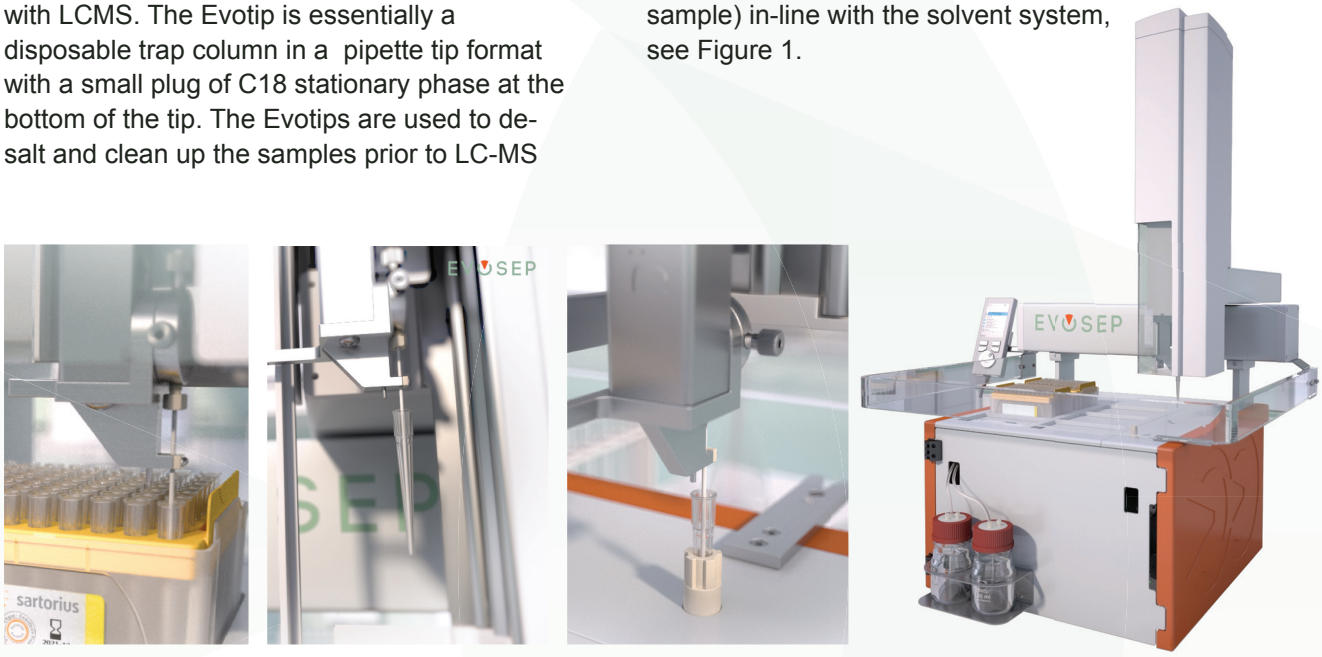

Figure 1: **Evosep One and a close-up of the pipette tip-based sample handling**

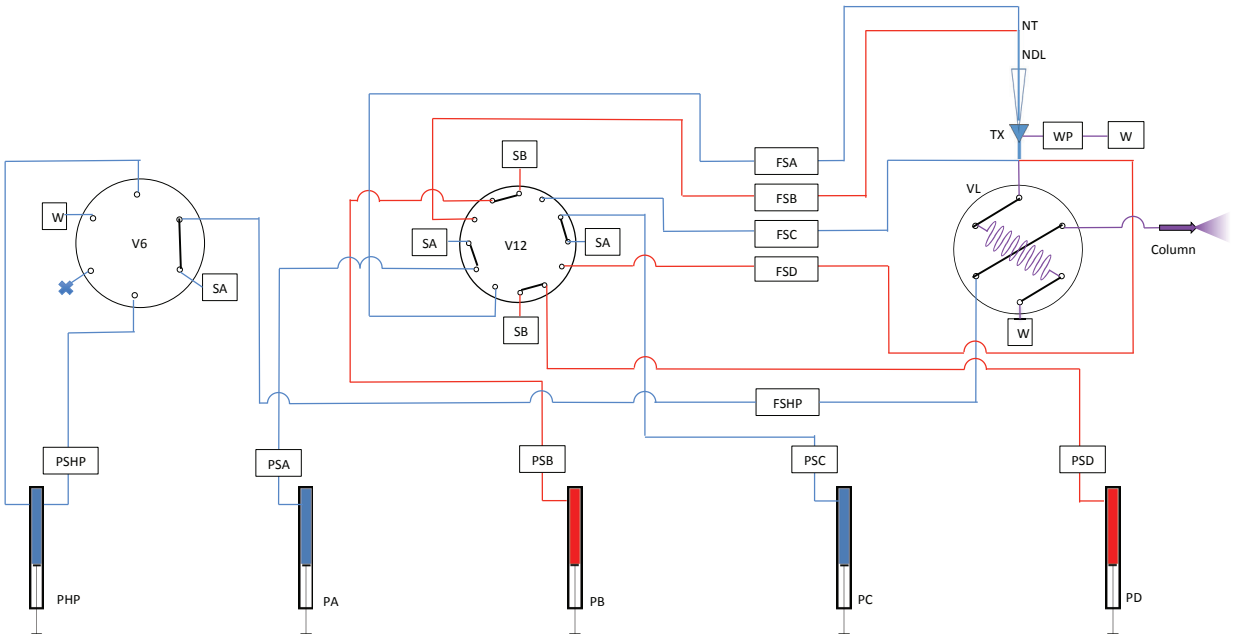

Figure 2: **Evosep One plumbing diagram. HP: High Pressure Pump, PSx: Pressure Sensor x, FSx: Flow Sensor x, WP: Waste Pump, Sx: Solvent x, W: waste, V6: 6 port high-pressure solvent valve, V12: 12 port low-pressure solvent valve, VL: Loop Valve. NT: Needle Tee, NDL: Needle, TX: Tip cross.**

Once the Evotip is sealed in-line with the solvent system, a gradient from pumps A and B runs through the Evotip and sequentially elutes the adsorbed analytes. While the gradient, with the embedded and pre-separated analytes, elute from the Evotip, a secondary gradient from pumps C and D continuously modify the composition of the initial A/B gradient to generate an offset gradient that ensures optimal chromatographic performance at the analytical column, see figure 3. Pumps A+B deliver a partial gradient (0-35% ACN) which is sufficient to sequentially elute the analytes of interest but still leave all the high-molecular contaminants behind which are then discarded with the Evotip after the analysis. A high organic wash volume is introduced just after the gradient using Pump D, bypassing the Evotip, to efficiently wash the analytical column. It takes approximately one minute at 20-40 µl/min (<20 bar) to create the preformed and offset gradient with the embedded analytes and position it precisely in the storage loop (ID100µm, 30ul), see figure 3.

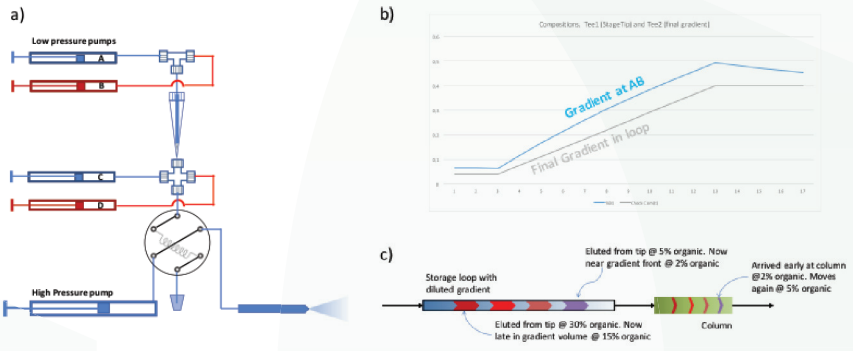

Figure 3: **Evosep One** a) Simplified plumbing diagram of Evosep One. b) Illustration of the A/B gradient running through the Evotip and the following C/D modified gradient resulting in an offset gradient for optimal focusing at the analytical column. C) Illustration of the preformed and offset gradient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

After generating the gradient, the loop-valve switches the storage-loop in-line with the high-pressure pump and analytical column and the high-pressure pump can now push the pre-formed and offset gradient with the pre-separated analytes to the analytical column. The gradient offset ensures that analytes reach the analytical column at a lower percentage of organic compared to when eluting from the Evotip. This allows each analyte to refocus on the analytical column and hereby significantly increases the capacity and chromatographic performance, see figure 2c.

Throughput	Cycle Time	Gradient Length	Overhead	Flow rate
Samples/day	Minutes	Minutes	Minutes	µL/min
300	4,8	3	1,8	2,0
200	7,2	5	2,2	1,5
100	14,4	12	2,4	1,2
60	24,0	21	3,0	1,0
30	48,0	45	3,0	0,6

Table 1: Evosep One Methods. *Methods not final

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

Robustness comes from:

1. Partial elution from disposable trap columns (Evotip) ensures that all high-molecular weight contaminants are discarded with the Evotip and will never be transferred to the analytical column and into the mass spectrometer.
    a. This will dramatically increase the lifetime of the analytical column for crude samples to thousands of injections per column.
    b. This also reduces the contamination of the mass spectrometer, increasing the up-time.
2. All gradient generation and modification steps are performed at high flow-rate but low pressure which results in far fewer leakage problems and component maintenance.
3. The simple high-pressure system with only one high-pressure pump.
4. Using analytical flow rates from 1-2 ul/min for better spray stability
5. Intelligent system preparation and diagnostics

Throughput comes from:

1. Evotips are used for de-salting off-line and act as disposable trap columns to significantly reduce the overhead time between samples
2. Elution from the Evotip and gradient formation is done at low pressure (<20bars) and high flow rates (20-40µl/min)

Performance comes from:

1. Using disposable Evotips with extremely low levels of carry over.
2. Offsetting the gradients for optimum focusing performance of the analytical column and also allowing the use of relatively short columns, see figure 4.
3. Integrated and reduced sample handling leading to less sample loss and variation, see figure 4.

Most of the system functionality is contained in the low-pressure sub-system of the instrument, see figure 2. This ensures long lifetime of the mechanical components, ultra-precise flow manipulation, and a low risk of critical leaks and malfunction.

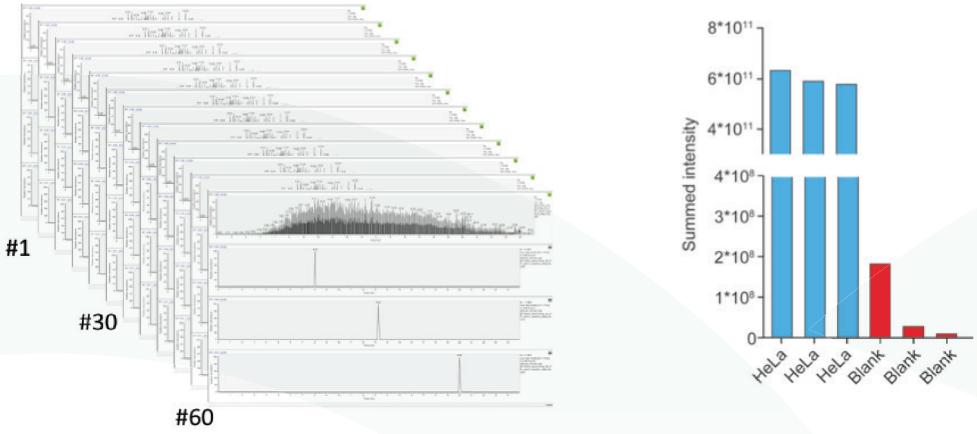

Figure 4: **Evosep One data: a) 60 Hela samples/day, 21minute gradient, elution window of 17 min, peak capacity (4sigma) =120, peak capacity (FWHM) = 170. b) Cross contamination between HeLa samples (1ug) = 0.03%-0.06%. Data courtesy: Dr. Philipp Geyer, Max Planck inst., Martinsried, DE.**

Conclusion:

Evosep One is designed for sensitivity, throughput, and robustness - tailored for large clinical studies. Using an Evotip as a desalting and sample clean up cartridge and also a disposable trap column integrates the sample preparation workflow with the Evosep One analysis. This significantly reduces sample loss and variation associated with these steps. Using the Evotip as a disposable and single use trap column reduces the sample cross contamination to <0.05% for HeLa and plasma samples while ensuring stable and uninterrupted operation for thousands of injections.

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