Development of a Novel LC Concept for Clinical Proteomics

Nicolai Bache[1], Philipp E. Geyer[2], Ole Hoerning[1], Lasse Falkenby[1], Peter Treit[2], Igor Paron[2], Florian Meier[2], Ole Vorm[1] and Matthias Mann[2]

[1] Evosep, Odense, Denmark, [2] Max-Planck Institute of Biochemistry, Proteomics and Signal Transduction, Martinsried, Germany

Introduction:

Mass spectrometry-based proteomics and metabolomics are fast growing and powerful technologies, with the potential to revolutionize precision medicine and health care. The new “Evosep One” separation system 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.

Throughput	Cycle Time	Gradient Length	Overhead	Flow rate	Column type
Samples/day	Minutes	Minutes	Minutes	µL/min	Length, ID, packing material
200	7.2	5.6	1.6	2	4 cm, ID150um, 3µm C18 Reprosil
100	14.4	11.5	2.9	1,5	8 cm, ID100um, 3µm C18 Reprosil
60	24.0	21.0	3.0	1	8 cm, ID100um, 3µm C18 Reprosil
30	48.0	44.0	4.0	0,5	15 cm, ID100um, 3µm C18 Reprosil

Table 1: **Evosep One methods.** For ease of use, four pre-configured methods have been optimized to provide the best performance to time compromise. The methods are defined by the total number of samples that can be run per day rather than referring to the length of the gradient.

The new system uses four low-pressure pumps in parallel to elute samples from a disposable trap column with a chromatographic gradient and simultaneously modifying (off-setting) the gradient immediately after the trap. The sample and off-set gradient are moved into a holding loop that subsequently is switched in-line with a single high-pressure pump and a separation column. Thus elution from the disposable trap column and gradient formation are de-coupled from the high-pressure separation.

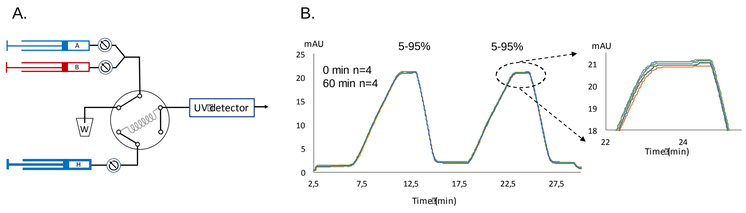

Figure 1: UV set up to test gradient storage (0 min vs 60 min). *A*, Flow diagram for testing pre-defined gradient mixing during storage in the capillary loop. *B*, Profiles of the acetonitrile and water plugs that were recorded by the UV detector (0.1% acetone) after storage for 0 minutes and 60 minutes. Profiles were almost completely superimposed, consistent with minimal mixing of the two phases during storage at room temperature.

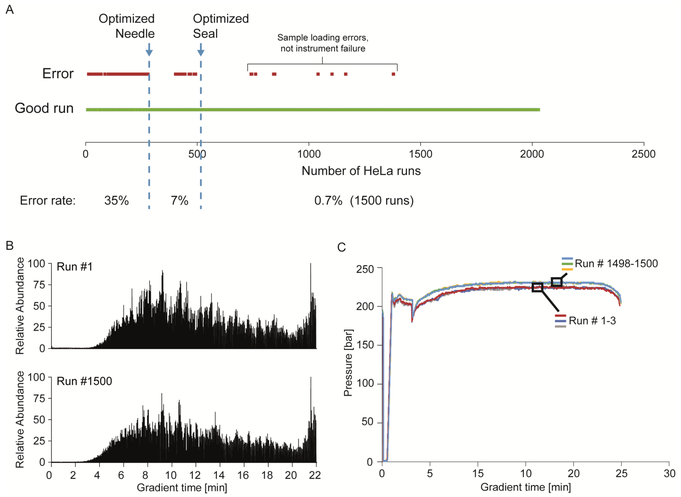

Figure 2: Systematic test of the stability of the first laboratory prototype. *A*, Error frequency during the development phase of the system assessed by consecutive measurements of HeLa digests. After 550 samples we changed the column and analyzed 1500 samples on the same column without any significant change of performance. *B*, The first and last chromatogram of a HeLa digest in a series of 1500 measurements on the same column. *C*, Pressure profiles over the gradient for the first and last three HeLa digest of the same experiment.

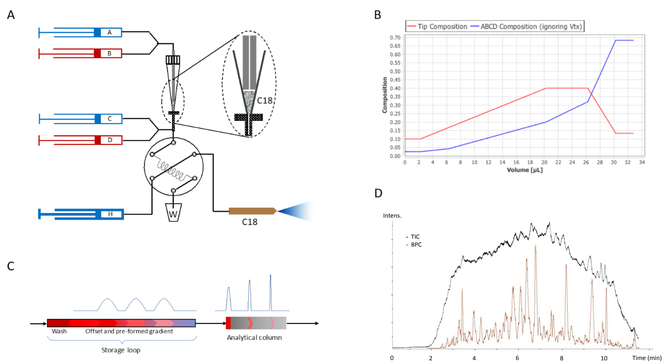

Fig. 3: Evosep One system overview and pre-formed gradient. *A*, Peptides are eluted from the C18 containing Evotip by pumps A and B. Pumps C and D, which are also low-pressure, form the final gradient, which is stored in the capillary loop together with the analytes. Subsequently, the valve switches and the high-pressure pump H pushes the gradient with its peptides over the analytical column. *B*, Composition of the gradient resulting from the confluence of the flows from pumps A, B and pumps C, D (x-axis designates the volume entering the storage loop). The proportion of acetonitrile is indicated on the y-axis. *C*, Analytes embedded in the storage loop are represented in red and as peak intensities. Due to the offset provided by pumps C and D, peptides are shortly retained at the head of the analytical column and elute with sharp peak widths. *D*, TIC and BPC of a 1 ug injection of a tryptic digest of HeLa using the 100 samples/day Evosep One method.

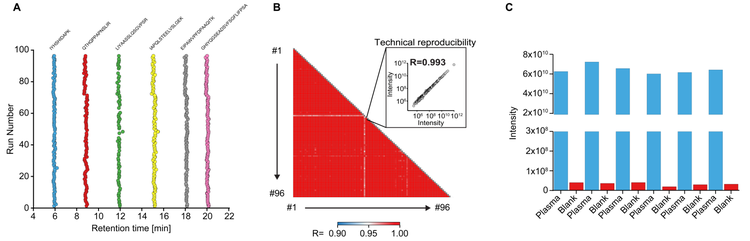

Figure 4: Clinical applicability to the plasma proteome. *A*, Retention time stability of selected peptides spanning a range of elution times over 96 plasma proteome runs. *B*, Pearson correlation matrix comparing all 96 plasma runs to each other. A single correlation graph with the median Person value is shown in the inset. *C*, Summed total peptide intensities in alternating plasma and blank runs.

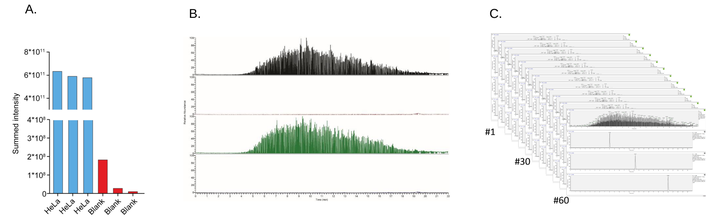

Figure 5 : Cross contamination. *A*, Cross contamination between HeLa samples (1ug) = 0.033%-0.06%. *B*, TIC of HeLa (1ug tryptic protein digest) and blank injections. *C*, TIC of HeLa (1ug tryptic protein digest) and XIC of m/z 520.25, 652.02 and 865.79 using the 60 samples/day method. Elution window of 17 min, peak capacity (4sigma) = 120, peak capacity (FWHM) = 170.

Improved Reliability & Productivity

- Partial elution leaves impurities from each sample on the disposable trap column so impurities never reach the separation column or the MS
- Low pressure elution & gradient formation cause less wear and tear

Better MS Utilization

- Fewer LC household steps
- Minimized dwell time owing to high-flow gradient formation, close to column

Higher Confidence

- 10X reduced cross-contamination through the use of disposable tips
- High flow-rates during autosampler washing steps

A novel LC system embeds analytes in pre-formed gradients for rapid, ultra-robust proteomics

Nicolai Bache, Philipp E. Geyer, Dorte B. Bekker-Jensen, Ole Hoerning, Lasse Falkenby, Peter V. Treit, Sophia Doll, Igor Paron, Florian Meier, Jesper V. Olsen, Ole Vorm, Matthias Mann

doi: https://doi.org/10.1101/323048