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..EASY-2D is easy to do..

Application Note

Simple and Fast Two-dimensional LC-MS on the EASY-nLC™

3 steps to 2D-LC on an EASY-nLC

1. Mount a biphasic pre-column (SCX/RP) instead of the normal pre-column
2. Prepare a dilution series of SCX elution buffer (salt) and place the different salt solutions in the autosampler vial plate
3. Program the EASY-nLC to first inject the sample followed by each of the salt solutions and execute the sequence.

Analysis of complex biological protein/peptide samples by tandem mass spectrometry (MS/MS) requires extensive fractionation of the sample. Automated two-dimensional Liquid Chromatography (2D-LC) is a promising separation technology which coupled on-line with MS/MS instrumentation shows great analytical potential in the field of proteomics. 2D-LC is often based on on-line strong cation-exchange (SCX) separation in the first dimension and reversed phase (RP) separation in the second dimension. Sophisticated set-ups using ternary or quaternary gradient generation systems already exist to step-elute peptides from the SCX column onto the RP column followed by gradient elution and MS/MS analysis. These methods are inherently complex and require highly skilled and experienced specialists for successful development and implementation.

Here we describe the implementation of a simple 2D-LC separation strategy on the split-free nanoflow EASY-nLC. In this approach, the salt-containing solvents required to elute peptides from the SCX material onto the RP material are drawn from vials in the autosampler, into the injection loop, and from there through the biphasic SCX/RP pre-column. This strategy eliminates the requirement for ternary or quaternary gradient systems which greatly simplifies the experimental setup. Furthermore, well defined salt "plugs" (in terms of salt concentration and volume) is achieved in this approach, owing to the specialised and accurate injection method of the EASY-nLC. This allows for precise, low carry-over, step-wise elution of peptides from the SCX material.

Conclusions

The described 2D-LC approach on the split-free nanoflow EASY-nLC features:

- Unprecedented methodological simplicity
- Analytical performance equal to regular ternary/quaternary solvent delivery systems
- Fully integrated, split-free nanoflow 2D-LC

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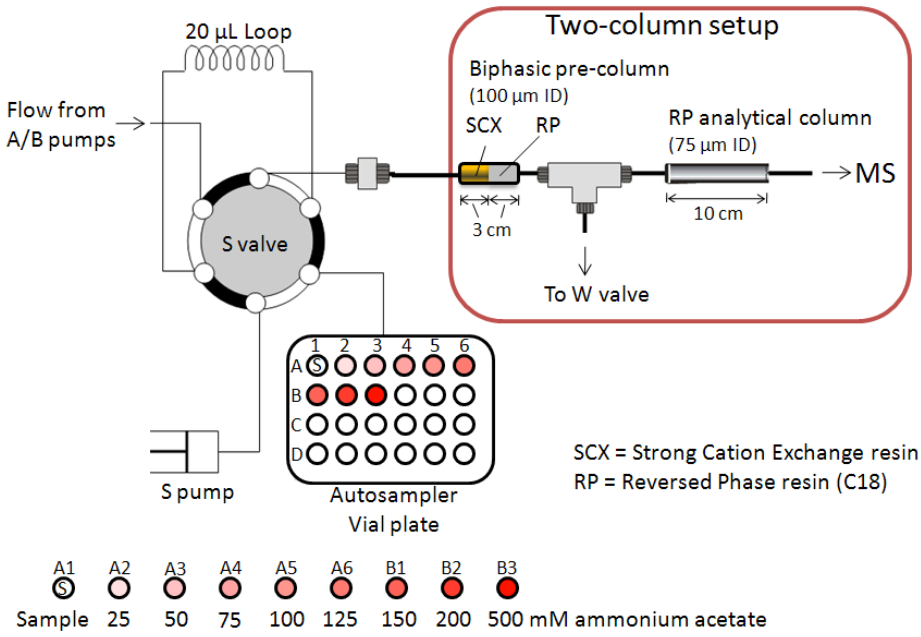
Recommended materials and methods



Schematics of a simple and efficient 2D-LC setup on an EASY-nLC system.

2D-LC experiments can be implemented on the EASY-nLC using a biphasic pre-column combined with a RP analytical column. A 2D-LC experiment consists of a batch of normal EASY-nLC injections, starting with an injection of the actual sample and followed by a number of salt plug injections of increasing ionic strength.

Biphasic Pre-column

100 µm ID fused silica column packed with:
3 cm C18 material
3 cm SCX material

Analytical Column

75µm ID fused silica column packed with:
10 cm C18 material

Note: The 2D-LC method can also be implemented as a one-column setup (recommended column specifications: 75 µm ID fused silica, 5 cm SCX material and 5 cm C18 material)

Solvents:

A: 5% acetonitrile, 0.1 % formic acid
B: 99.9 % acetonitrile, 0.1 % formic acid

EASY-nLC method parameters:

Sample pickup: 5 µL, 20 µL/min
Sample loading: 15 µL, 3 µL/min
Gradient: 300 nL/min
0-35 %B in 100 min.
35-100 %B in 10 min.
100% in 10 min.
Pre-column re-equilibration: 10 µL, 3 µL/min
Analytical column re-equilibration: 5µL, 0.7 µL/min.
Autosampler wash: 100 µL flush volume

Sample/salt plugs:

Example 1: 8 salt steps ~ 20 hours analysis time
A1: Sample (5 µL, < 20 µg total protein digest)
A2-B3: 25, 50, 75, 100, 125, 150, 200, 500 mM ammonium acetate in 5% acetonitrile, 0.1% formic acid

Example 2: 3 salt steps ~ 9 hours analysis time
A1: Sample (5 µL, < 20 µg total protein digest)
A2-A4: 25, 100, 500 mM ammonium acetate in 5% acetonitrile, 0.1% formic acid

Important: Samples need to be desalted and purified by solid phase extraction (StageTips™) prior to 2D-LC analysis.