Bruker Daltonics

Technical Note # TN-25

Simple and Fast Two-Dimensional LC-MS on the Bruker EASY-nLC: EASY-2D

Abstract

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 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 systems already exist to stepelute 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.

Introduction

Here we describe the implementation of a simple 2D-LC separation strategy on the split-free EASY-nLC™. In this approach, the different salt solutions required to elute peptides from the SCX material onto the RP material are drawn from vials in the autosampler, and are injected onto the biphasic SCX/RP pre-column. This strategy eliminates the requirement for ternary or quaternary gradient systems which greatly simplifies the experimental setup.

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 concentration series of salt solutions and place these SCX elution buffers in the autosampler vial plate
3. Set up a sequence in Compass to inject the sample first, followed by each of the salt solutions and execute the sequence.

Salt plug injection with the EASY-nLC system

Well defined salt "plugs" (in terms of salt concentration and volume) are achieved in this approach, owing to the specialized and accurate injection method of the EASY-nLC. The sample (and also the salt plugs) are separated from the solvent by tiny air gaps, preventing a dilution at the boundaries. In combination with the optimized flow path and small dead volumes, this allows for precise, low carry-over, step-wise elution of peptides from the SCX material. The lower picture shows the precision of the salt plug by means of the conductivity of the fluid.

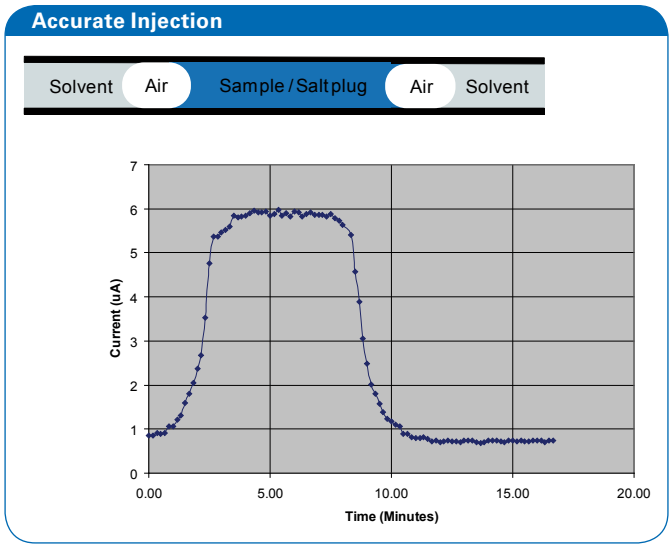


Fig. 1: Salt plug shape.

Typical settings

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

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: 0-35 %B in 60 min.
35-100 %B in 10 min.
100% in 10 min.

Flow rate: 300 nL/min
Autosampler wash: 100 µL flush volume
Pre-column re-equilibration: 10 µL, 3 µL/min
Analytical column re-equilibration: 5µL, 0.7 µL/min.

Sample/salt plugs:
Example 1: 8 salt steps ~ 15 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 ~ 6.5 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.

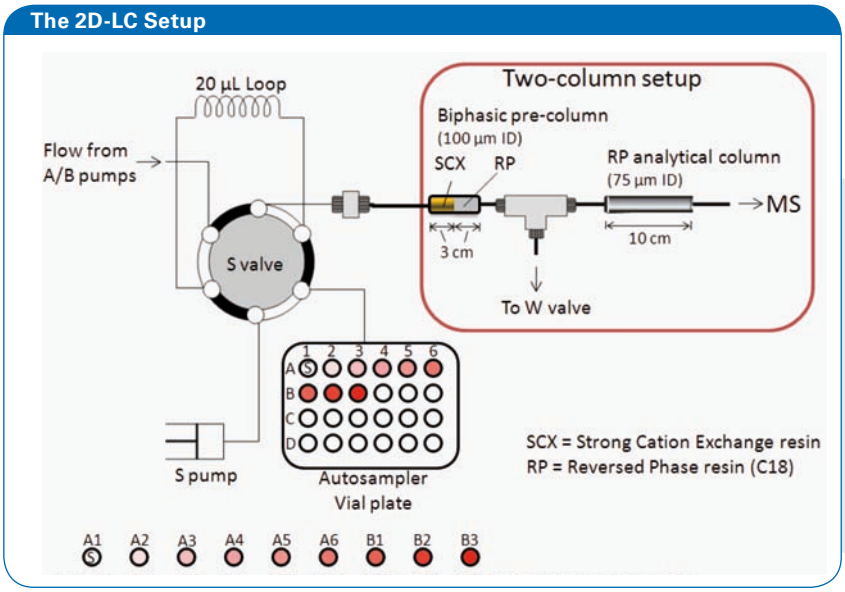
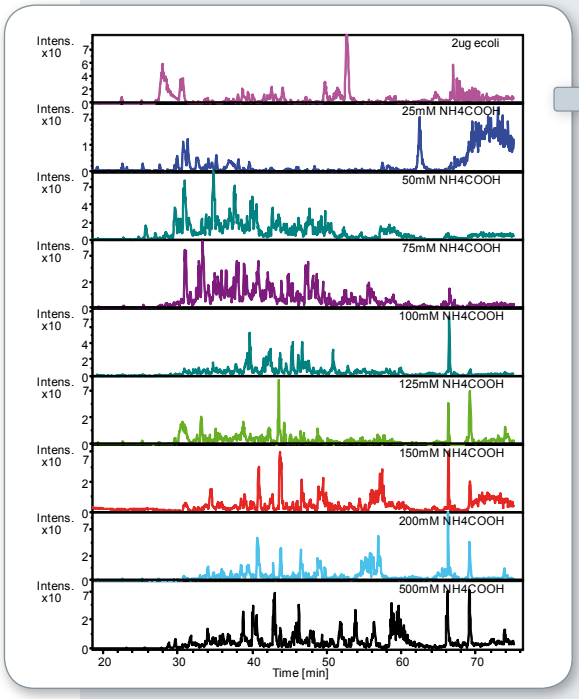


Fig. 2: Schematic of a simple and efficient 2D-LC setup on the EASY-nLC system.

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

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

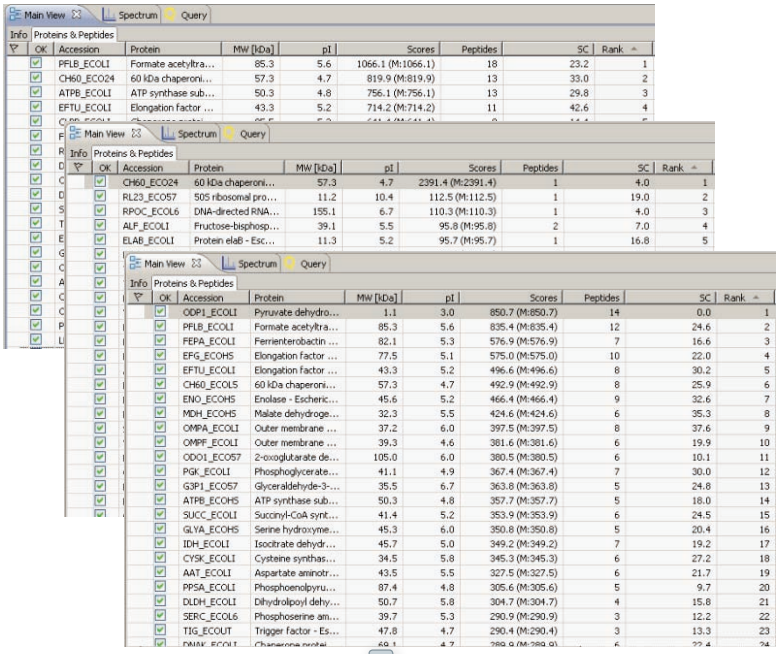
Accurate injection



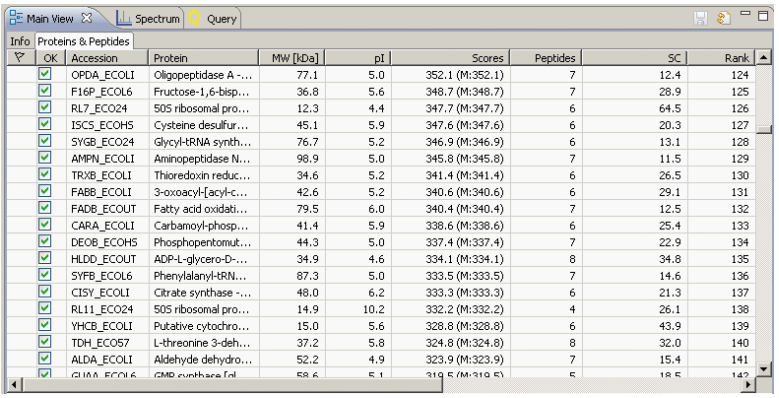
Experimental

2 µg of desalted E.coli lysate was bound to the SCX-phase of the biphasic column (Proxeon) and eluted with 8 saltsteps (25 - 500mM Ammonium acetate). RP separation was carried out using a 60 minute gradient from 1% - 35% B. Each dataset was automatically processed with DataAnalysis (Bruker Daltonics) and sent to ProteinScape 2.0 (Bruker Daltonics); Mascot searches started automatically. ProteinExtractor compiles all nine individual Mascot results into one final result.

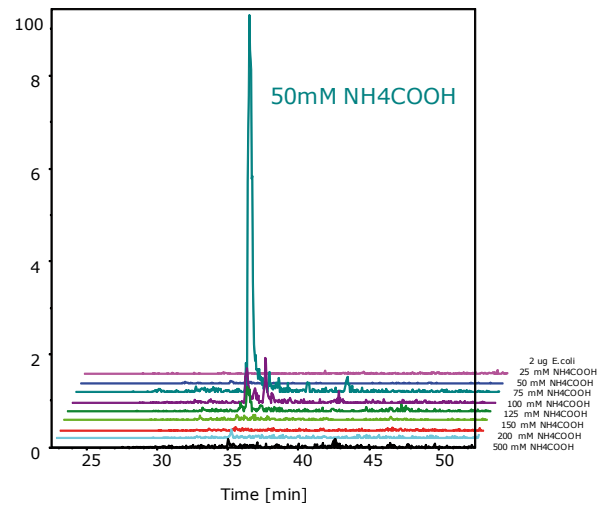
ProteinScape



ProteinExtractor



High Separation Performance



2D-LC separation

The EASY-nLC based 2D-LC set-up provides high separation performance in the first dimension. Each peptide elutes in one SCX fraction only, no distribution of peaks over multiple fractions. E.g. the peptide with m/z 683.5 is only detectable in the 50 mM ammonium acetate fraction.

The EIC traces of m/z 683.5 ± 0.1 of all other fractions do not show significant signal intensities. In summary, the 2D-LC separation efficiently reduces sample complexity without loss in sensitivity.

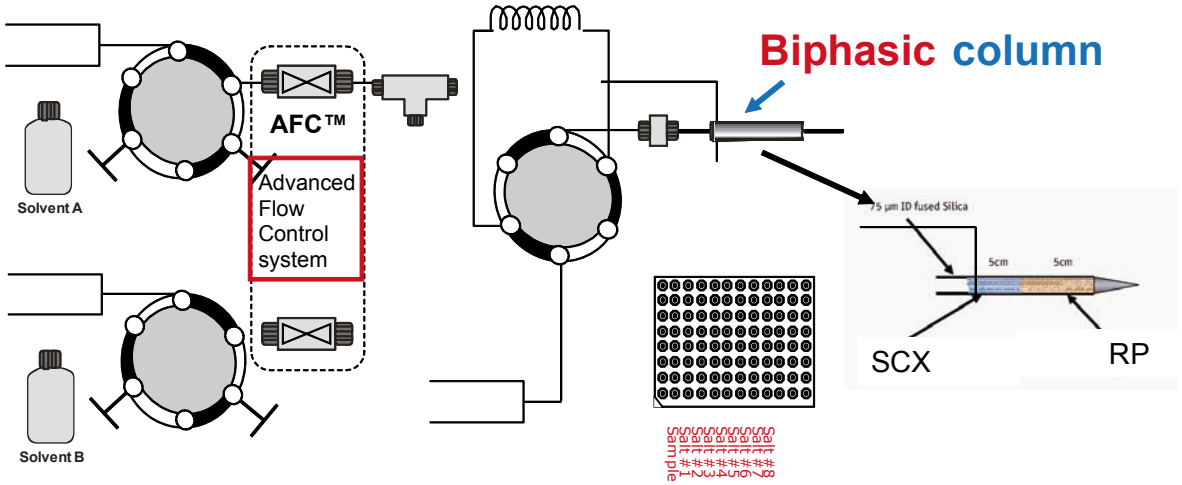
Fig. 3: EIC Traces of mass 683.5 ± 0.1 +All MS compound elutes predominantly with 50 mM ammonium.acetate.

Conclusion

The 2D-LC approach described here, using the split-free nanoflow EASY-nLC, features:

- Unprecedented methodological simplicity
- Analytical performance equal to regular ternary/ quaternary solvent delivery systems
- Sample complexity is efficiently reduced without sensitivity loss.
- Fully integrated, split-free nanoflow 2D-LC

One Column Setup



Preconfigured for 2D separations with 1 biphasic column

Fig. 4: 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.

Authors

Andrea Kiehne[1], Marina Behrens[1], Thomas Hagedorn[1], Morten Beck Trelle[2], Michael Barrett Andersen[2]
[1] Bruker Daltonik, Bremen, Germany
[2] Proxeon, Odense, Denmark

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www.bdal.com

Bruker Daltonics Inc.
Billerica, MA · USA
Phone +1 (978) 663-3660
Fax +1 (978) 667-5993
ms-sales@bdal.com

Bruker Daltonik GmbH
Bremen · Germany
Phone +49 (421) 2205-0
Fax +49 (421) 2205-103
sales@bdal.de