

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.

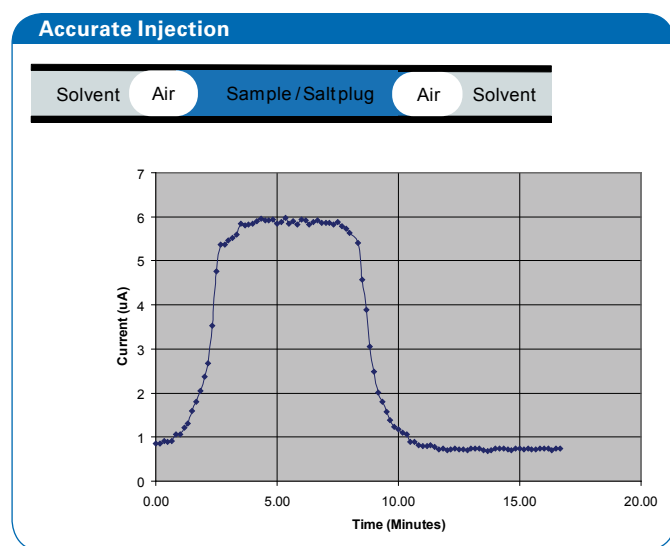


Fig. 1: Salt plug shape.

Typical settings

Biphasic Pre-column

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

Analytical Column

75 μ m 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 μ L, 20 μ L/min
Sample loading: 15 μ L, 3 μ L/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 μ L flush volume

Pre-column re-equilibration: 10 μ L, 3 μ L/min

Analytical column re-equilibration: 5 μ L, 0.7 μ L/min.

Sample/salt plugs:

Example 1: 8 salt steps ~ 15 hours analysis time

A1: Sample (5 μ L, < 20 μ g 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 μ L, < 20 μ g total protein digest)

A2-A4: 25, 100, 500 mM ammonium acetate in 5% acetonitrile, 0.1% formic acid.

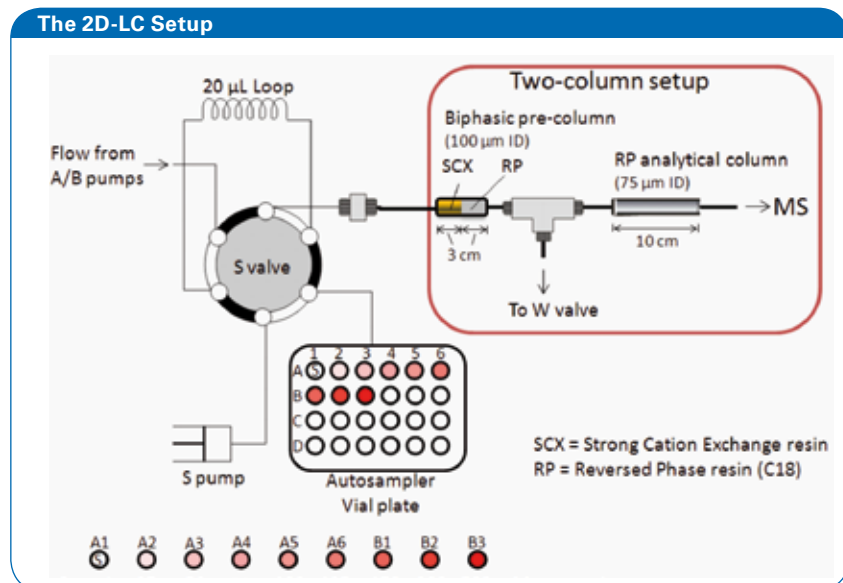
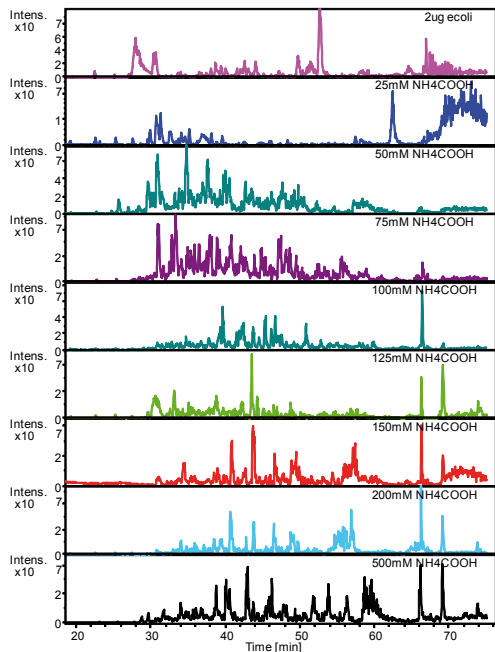


Fig. 2: Schematic of a simple and efficient 2D-LC setup on the EASY-nLC system.

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 ProteinScope 2.0 (Bruker Daltonics); Mascot searches started automatically. ProteinExtractor compiles all nine individual Mascot results into one final result.

ProteinScope

Protein	Accession	Protein	MW [kDa]	pI	Score	Peptides	SC	Rank
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	1364.1 (M1094.1)	18	23.2	1
CHS6_ECOLI	CHS6_ECOLI	60 kDa chaperon...	57.3	4.7	819.9 (M618.9)	13	31.0	2
ATP8_ECOLI	ATP8_ECOLI	ATP synthase sub...	50.3	4.8	796.1 (M796.1)	13	29.8	3
EF1A_ECOLI	EF1A_ECOLI	Elongation factor...	43.3	5.2	714.2 (M714.2)	11	42.6	4
CHS6_ECOLI	CHS6_ECOLI	60 kDa chaperon...	57.3	4.7	661.4 (M661.4)	8	34.4	5

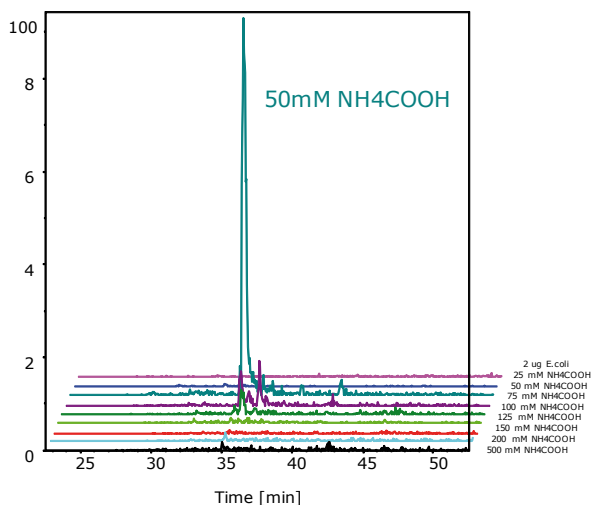
Protein	Accession	Protein	MW [kDa]	pI	Score	Peptides	SC	Rank
CHS6_ECOLI	CHS6_ECOLI	60 kDa chaperon...	57.3	4.7	2391.4 (M2391.4)	1	4.0	1
RL23_ECOLI	RL23_ECOLI	50S ribosomal pro...	11.2	10.4	112.5 (M112.5)	1	19.0	2
RPOC_ECOLI	RPOC_ECOLI	DNA-directed RNA...	135.1	6.7	110.3 (M110.3)	1	4.0	3
ALP_ECOLI	ALP_ECOLI	Phosphate-kinase...	39.1	5.5	95.8 (M95.8)	2	7.0	4
RL4B_ECOLI	RL4B_ECOLI	Protein shell - Es...	11.3	5.2	95.7 (M95.7)	1	16.8	5

Protein	Accession	Protein	MW [kDa]	pI	Score	Peptides	SC	Rank
ODF1_ECOLI	ODF1_ECOLI	Formate dehydroge...	1.1	3.0	850.7 (M850.7)	14	0.0	1
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	835.4 (M835.4)	12	24.6	2
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	575.9 (M575.9)	7	16.6	3
EF1A_ECOLI	EF1A_ECOLI	Elongation factor...	43.3	5.2	575.0 (M575.0)	10	22.0	4
EF1A_ECOLI	EF1A_ECOLI	Elongation factor...	43.3	5.2	496.6 (M496.6)	8	30.2	5
CHS6_ECOLI	CHS6_ECOLI	60 kDa chaperon...	57.3	4.7	492.9 (M492.9)	8	25.9	6
EF1A_ECOLI	EF1A_ECOLI	Elongation factor...	43.3	5.2	482.4 (M482.4)	9	32.6	7
MDH_ECOLI	MDH_ECOLI	Malate dehydroge...	32.3	5.5	424.4 (M424.4)	6	35.3	8
ODF1_ECOLI	ODF1_ECOLI	Formate dehydroge...	1.1	3.0	397.5 (M397.5)	8	37.6	9
ODF1_ECOLI	ODF1_ECOLI	Formate dehydroge...	1.1	3.0	381.4 (M381.4)	6	19.9	10
ODF1_ECOLI	ODF1_ECOLI	Formate dehydroge...	1.1	3.0	380.5 (M380.5)	6	10.1	11
PKR_ECOLI	PKR_ECOLI	Phosphorylase...	41.1	4.9	367.4 (M367.4)	7	30.8	12
GLY_ECOLI	GLY_ECOLI	Glyceraldehyde-3...	35.5	6.7	363.6 (M363.6)	5	24.8	13
ATP8_ECOLI	ATP8_ECOLI	ATP synthase sub...	50.3	4.8	357.7 (M357.7)	5	16.0	14
SUCI_ECOLI	SUCI_ECOLI	Succinyl-CoA synth...	41.4	5.2	353.9 (M353.9)	6	24.5	15
GLH_ECOLI	GLH_ECOLI	Serine hydroxyme...	45.3	6.0	350.8 (M350.8)	5	20.4	16
EF1A_ECOLI	EF1A_ECOLI	Elongation factor...	43.3	5.2	349.2 (M349.2)	7	19.2	17
CYS_ECOLI	CYS_ECOLI	Cysteine synthase...	34.5	5.8	345.3 (M345.3)	4	27.2	18
AAT_ECOLI	AAT_ECOLI	Aspartate aminot...	43.5	5.5	327.5 (M327.5)	6	21.7	19
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	305.6 (M305.6)	5	9.7	20
GLH_ECOLI	GLH_ECOLI	Serine hydroxyme...	45.3	6.0	304.7 (M304.7)	4	15.8	21
SRIC_ECOLI	SRIC_ECOLI	Phosphoserine am...	39.7	5.3	290.9 (M290.9)	3	12.2	22
TFI_ECOLI	TFI_ECOLI	Trigger factor - Es...	47.8	4.7	290.4 (M290.4)	3	13.3	23
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	288.6 (M288.6)	4	11.4	24

ProteinExtractor

Protein	Accession	Protein	MW [kDa]	pI	Score	Peptides	SC	Rank
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	1364.1 (M1094.1)	18	23.2	1
CHS6_ECOLI	CHS6_ECOLI	60 kDa chaperon...	57.3	4.7	819.9 (M618.9)	13	31.0	2
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ODF1_ECOLI	ODF1_ECOLI	Formate dehydroge...	1.1	3.0	850.7 (M850.7)	14	0.0	1
PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	835.4 (M835.4)	12	24.6	2
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PF18_ECOLI	PF18_ECOLI	Formate acetylase...	88.3	5.6	288.6 (M288.6)	4	11.4	24

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

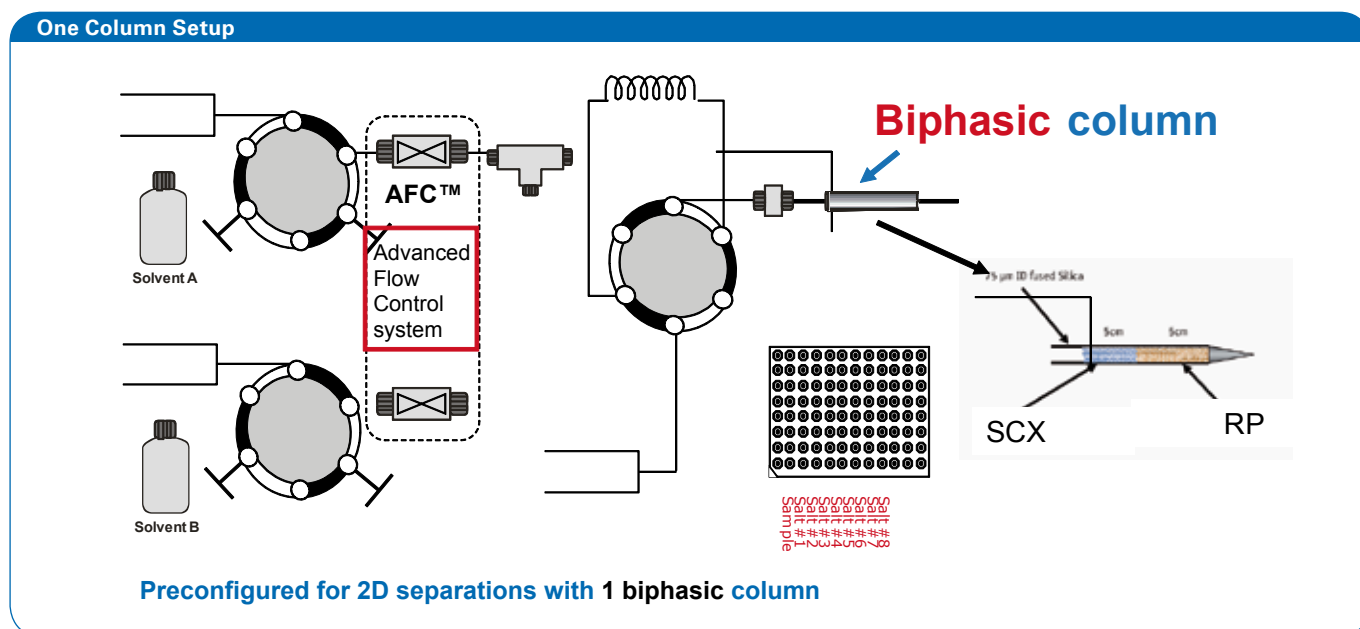


Fig. 4: The 2D-LC method can also be implemented as a one-column setup. Recommended column specifications: 75 µm ID fused silica, 5 cm SCX material and 5 cm C18 material.

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Keywords

Complex Samples
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Instrumentation & Software

EASY-nLC
HCTultra
Compass
ProteinScape

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