

Simplifying Analysis of Biomolecules using Two-Dimensional LC–MS

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Introduction

Standard reversed-phase LC–MS and LC–MS–MS techniques have limited effectiveness in the analysis of complex biomolecule mixtures because of co-eluting species compromising MS–MS data collection.

Components present in relatively small concentrations, such as small peptide fragments, can be neglected by the detection method or interpreted as background noise. Two-dimensional (2D) LC–MS methods increase the efficiency of protein identification as co-eluting components are separated into different fractions, in effect removing interferences from more abundant co-eluting species.

This application note compares the data obtained when using a traditional one-dimensional method with reversed-phase chromatography using a silica-based C18 column, versus a 2D technique involving initial fractionation of peptides on the basis of their charge using a cation exchange column, followed by reversed-phase chromatography of each fraction.

Instrumentation/Equipment

Columns: BioBasic® SCX 100 × 0.32 mm KAPPA® capillary,

BioBasic 18 100 × 0.18 mm KAPPA capillary

HPLC instrument: Thermo Finnigan Surveyor®

Mass spectrometer: Thermo Finnigan LCQ™ DUO

Experimental conditions

Reversed-Phase LC

Mobile phase:

A = 0.01% formic acid

B = acetonitrile + 0.1% formic acid

Gradient:

Time (min)	%B
0	0
5	0
65	60
70	80
75	80
80	0
95	0

2D Sample Loading/Fraction Eluting

A 3 µL sample (1500 fmole) of bovine serum albumin tryptic digest was loaded onto the BioBasic SCX capillary using a 0.01% formic acid mobile phase at a flow-rate of 1.8 µL/min (70:1 split flow) for a period of 20 min.

Sequential 20 µL injections of increasing concentrations of ammonium formate (0 mM, 20 mM, 90 mM and 500 mM in 0.1% formic acid) were used to elute the fractions onto the BioBasic 18 capillary for reversed-phase analysis.

Figure 1: 1D reversed-phase data-dependent MS–MS analysis of a small abundant peptide fragment (m/z 784.5). (a) base peak ion chromatogram, (b) MS–MS of m/z 784.5 and (c) 1D data.

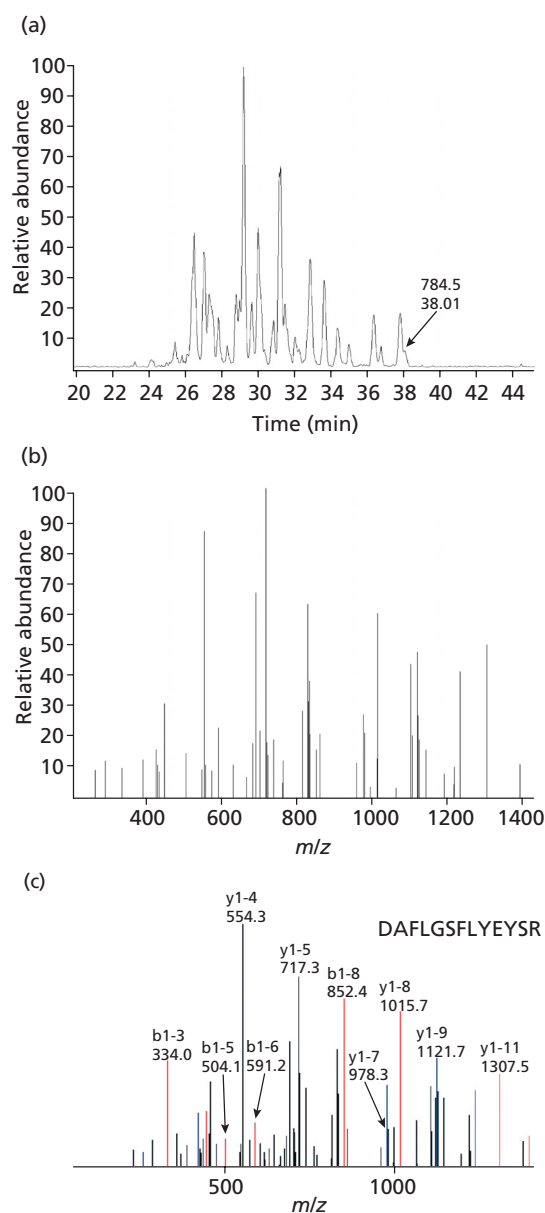
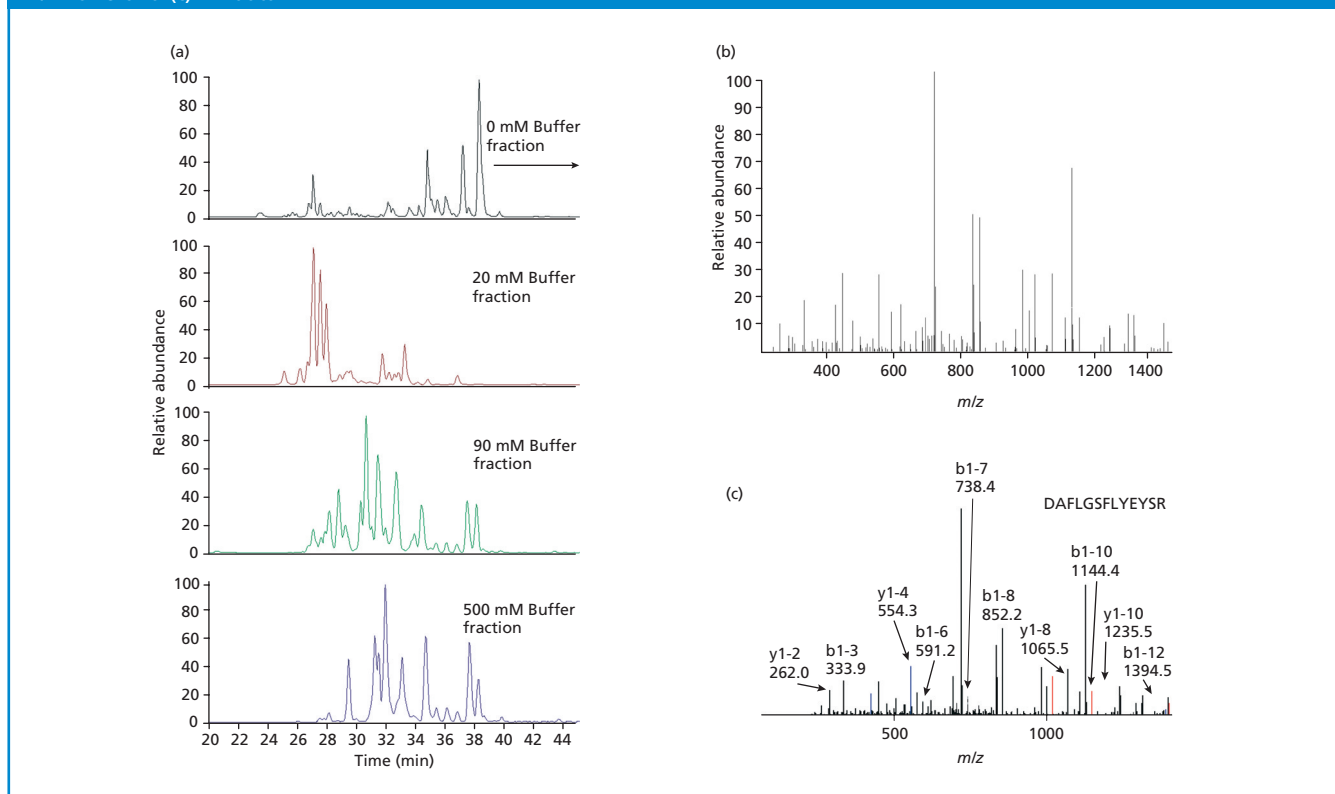


Figure 2: 2D data dependent MS–MS analysis of the same peptide fragment. (a) base peak ion chromatograms, (b) MS–MS of m/z 784.5 and (c) 2D data.



Conditions

Flow-rate: 1.8 $\mu\text{L}/\text{min}$ (70:1 split flow)
 Detector: Thermo Finnigan LCQ™ DUO
 Ionization method: + ESI
 Sheath gas: 12 arb. units
 Capillary temp: 130 °C
 Spray voltage: 2.5 kV
 Capillary voltage: 12.00 V
 Scan range: 300–2000 au

Data Dependent Settings

Default isolation width: 2
 Normalized collision energy: 35%
 Threshold: 3×10^4
 Min^m MS² signal required: 1500

Results

1 D analysis of small peptide fragment: Figure 1 shows the data collected during 1D reversed-phase data-dependent MS–MS analysis of a small abundant peptide fragment (m/z 784.5, $RT = 38.01$ min).

2 D analysis of small peptide fragment: Figure 2 shows the 2D data-dependent MS–MS analysis of the same peptide fragment (m/z 784.61).

Discussion

In 1D analysis the component of interest elutes on the shoulder of a larger peak (as shown in the base peak ion chromatogram, Figure 1). Conversely, in 2D analysis, the post ion-exchange fraction is cleaner, with significantly less co-eluting peaks in the

base peak ion chromatogram. This leads to far cleaner MS data and thus better sensitivity of the MS–MS data (3.75×10^5 versus 2.21×10^4), resulting in higher confidence as the Bioworks™ 3.0 software achieves a protein ID score of 642 versus 300 for the 1D method.

Conclusions

Multidimensional LC–MS–MS methods can increase the sensitivity and effectiveness of complicated separations:

- By separating co-eluting species (interferences) more MS–MS data can be collected which can increase the number of species identified.
- Signal intensities of low abundant species are greater allowing the exclusion threshold to be raised and reducing the size of data files.
- Automated configurations, such as the Thermo Finnigan ProteomeX™, increase the throughput of shotgun protein identification by performing both dimensions of the separation simultaneously.

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