Analysis of peptides using CE/MS/MS

Application Note

Proteomics

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Abstract

Capillary electrophoresis (CE) is a powerful complementary tool to liquid chromatography (LC) for the analysis of peptides. Different selectivity and higher resolution is the biggest advantage in the analysis of clean samples such as synthetic peptides. When analyzing ppm-levels of peptides that are present in complex sample matrices, minimal sample preparation together with short analysis times enable high throughput. CE coupled to a fast-scanning ion trap MS with automated MS/MS provides the structural information needed to unambiguously confirm the peptide structure. The peptide data shown here were acquired on a fully integrated CE-ion trap system.

Experimental

An Agilent CE system with CE/MS capillary cassette was connected to an Agilent LC/MSD Trap with electrospray ionization and the orthogonal sprayer for CE/MS. An Agilent binary pump equipped with a 1:100 flow splitter was used to deliver sheath liquid for the sprayer. The LC/MSD Trap software provided complete control of all instrumentation. The test sample was a standard peptide mixture (Sigma). A new fused silica capillary was prepared by sequential 10-minutes flushes with acetonitrile, 0.1 N HCl, 1 N NaOH, and water followed by a 20-minutes flush with running buffer.

Results

The automated data-dependent scan capability of the LC/MSD Trap can trigger MS/MS on the most intense ion (or ions) from a single full scan mass spectrum. The number of ions to be selected for MS/MS is specified in the method. For this example, MS/MS was done for just the most abundant ion in each mass spectrum. Figure 1 shows an overlay of the total ion electropherogram (TIE) and the base peak electropherogram (BPE). Drops in the TIE intensity indicate where MS/MS spectra are taken automatically. The averaged mass spectrum of peak 4 (figure 2A) shows a singly-charged molecule with *m/z* of 574.3. The presence of Met-enkephalin (molecular weight of 573.7) is then unambiguously confirmed by examining the MS/MS mass spectrum (figure 2B). The MS/MS of Met-enkephalin shows all b- and y-series fragments within the scan range as well as fragments from the other series. Analysis of peaks 1 to 3 revealed angiotensin II, Val-Tyr-Val, and Leu-enkephalin, respectively. The data shown here demonstrate that even with the relatively narrow peak width of the CE peaks, automated MS/MS can generate high quality CID spectra for confirmation of peptide structure.

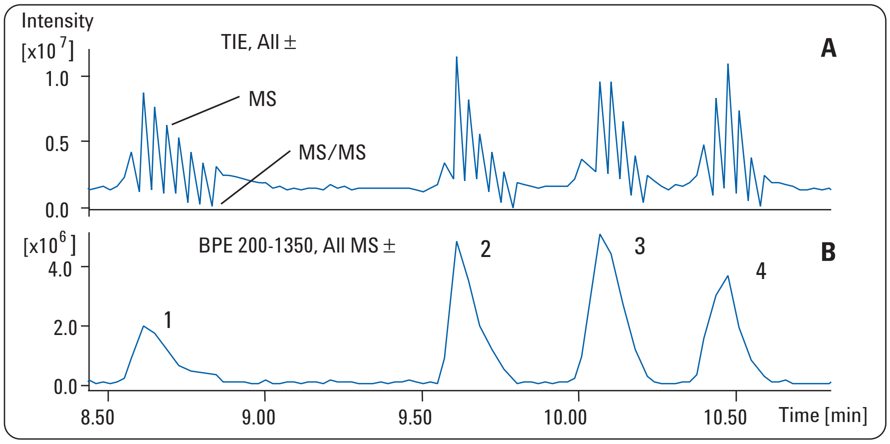

Figure 1
Total ion electropherogram A and base peak electropherogram B.

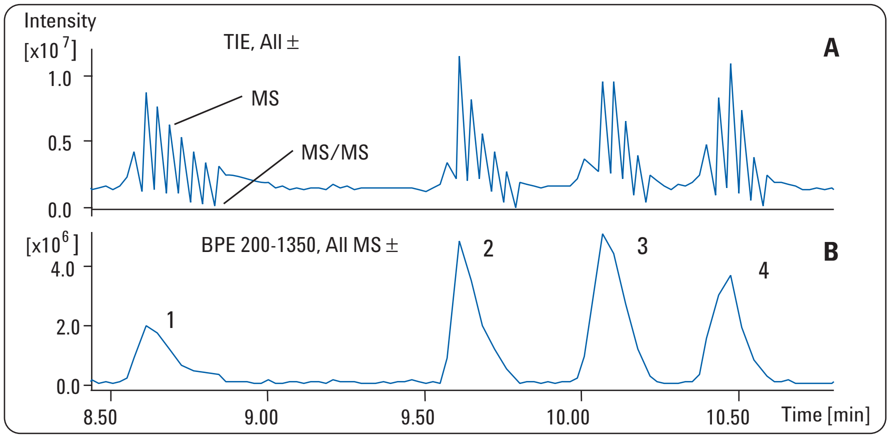

Figure 2
A. Average mass spectrum from peak 4,
B. Average MS/MS spectrum from peak 4.

Experimental conditions

CE

Sample:	peptide standard (Sigma), 0.1 mg/mL
Injection:	6 sec @ 50 mbar
Capillary:	bare fused silica, total length, 85 cm, 50 µm id

Buffer:	10 mM acetic acid
Voltage:	27 kV (0.3 minutes ramp)
Temperature:	25°C
Preconditioning:	5 minutes flush with buffer at 1 bar

MS

Ionization mode:	ESI
Sheath liquid:	0.5 % acetic acid in 50 % methanol, 4 µL/minutes
Drying gas flow:	10 L/min
Nebulizer:	10 psi
Drying gas temp:	150 °C
Skim:	1: 25 V
Skim:	2: 6 V
Cap exit offset:	40 V

Averages:	2
ICC:	On
Max accu time:	200 ms
Target:	50000
MS/MS:	automatic, no masses excluded
Threshold:	500000
No. of parents:	1
Isolation width:	3.0
Fragmentor amplitude:	1

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