Advion
10 Brown Road • Ithaca, N.Y. 14850
www.advion.com

Application Note

Peptide Analysis

Using Compact Mass Spectrometry with In-Source Fragmentation Capability

Introduction

The expression compact mass spectrometer (CMS) is a high performance, easy to use single quadrupole mass spectrometer with a small footprint to fit in space restricted labs. Priced substantially lower than other systems, the CMS brings the analytical benefits of mass spectrometry to more scientists than ever before.

The expression CMS can be tuned to induce in-source fragmentation of analytes. This fragmentation function provides valuable additional information about the analyte and can assist in the identification of an unknown peptide or the confirmation of a known peptide and its amino acid sequence.

Peptides predominantly fragment along their amide backbone (although side chain fragmentation and re-arrangements can also occur). Fragmentation patterns and nomenclature was described by Roepstorff and Fohlman [1] and later refined by Johnson et al. [2].

Method

Figure 1 shows the simple change to typical source tune settings for the Mass Express software. An increase in the source voltage span by 30 volts causes sufficient energy to be placed in the molecule to induce fragmentation in the source region of the mass spectrometer. The subsequently formed ions can be mass analyzed and interpreted using the above nomenclature. Since the final result of a peptide synthesis is usually a purified sample chromatographic separation of the analyte is not required and a simple and fast Flow Injection Analysis can be done (FIA/CMS).

Source Type *ESI*

	Setpoints	Readbacks
Polarity	+	
Capillary Temperature	200	200.1 Deg C
Capillary Voltage	150	155.7 Volts
Source Voltage Offset	30	
Source Voltage Span	30	
Source Gas Temperature	200	200.1 Deg C
ESI Voltage	3,000	3094.7 Volts

Figure 1: Screenshot showing the change to a typical tune setting that will induce analyte fragmentation in the source region of the MS (increased *Source Voltage Span* parameter from 0 to 30 V)

Advion
10 Brown Road • Ithaca, N.Y. 14850
www.advion.com

Results

Analyzing [Glu[1]]-Fibrinogen with FIA/CMS one can first determine the *m/z* ratio of the analyte to be *m/z* 785.85 with a charge state analysis showing its a doubly charged analyte (Figure 2a and insert). This results in an isotopic analyte mass of 1569.7, which is in line with the theoretical M_{iso}=1569.67 of [Glu[1]]-Fibrinogen. A second FIA/CMS run utilizing in source fragmentation shows a fragment pattern with predominantly y-ion formation. Pattern analysis shows the partial amino acid sequence of Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser (Figure 2b). The combined information of peptide mass AND partial peptide sequence confirms [Glu[1]]-Fibrinogen as the analyte in this sample.

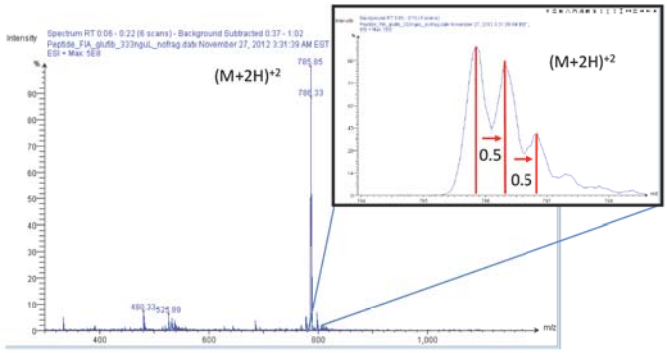

Figure 2a: MS Data obtained from FIA/CMS analysis of [Glu[1]]-Fibrinogen

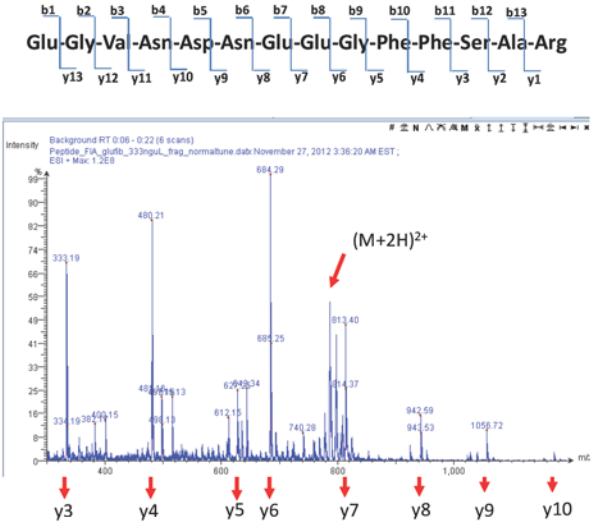

Figure 2b: MS data obtained from a FIA/CMS experiment using in source fragmentation analyzing [Glu[1]]-Fibrinogen

Another example of this analysis approach is shown in Figure 3 using Angiotensin. The *m/z* ratio is determined to be *m/z* 523.81 with a charge state of 2+. The resulting isotopic mass is calculated to 1045.6, which corresponds well with the theoretical M_{iso}=1045.53 of Angiotensin. The fragmentation experiment shows a fragment pattern of predominantly a- and b-series ions, which the partial amino acid sequence Val-Tyr-Ile/Leu-His-(Pro-Phe/Phe-Pro). Both information combined confirms Angiotensin as the peptide in this sample.

In-source fragmentation greatly increases the information that can be obtained from an analyte sample. In the case of peptides, the fragmentation pattern can result in the direct assignment of the amino acid sequence.

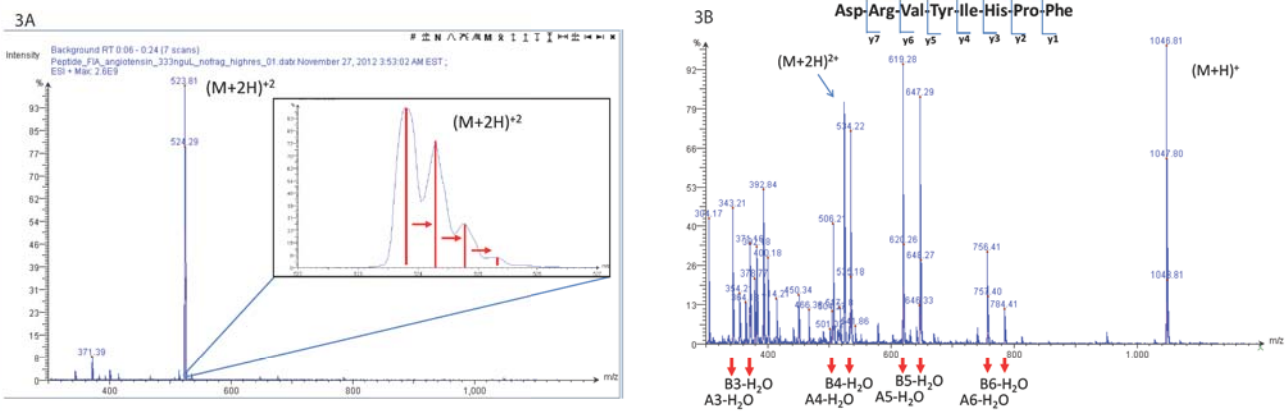

Figure 3: FIA/CMS analysis of Angiotensin without fragmentation (A) and with in-source fragmentation (B)

Literature

[1] Roepstorff P and Fohlman J: Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomedical Mass Spectrometry 1984 11(11) 601.

[2] Johnson RS, Martin SA, Biemann K, Stults JT and Watson JT: Novel fragmentation process of peptides by collision-induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine. Analytical Chemistry 1987 59 (21) 2621–2625