Advion
10 Brown Road • Ithaca, N.Y. 14850
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Advion is a leader in mass spectrometry & synthesis solutions. The expression CMS is a high performance, compact, affordable single quad mass spectrometer. Its compact size allows it to fit in space-limited labs for direct access and immediate results for chemists requiring mass confirmation, reaction monitoring, quality control and purity analysis.

Application Note

Peptide/Protein Analysis in Less than One Minute
Using Compact Mass Spectrometry and Flow Injection Analysis (FIA)

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 available systems, the CMS brings the analytical benefits of FIA/CMS and LC/CMS within the reach of more scientists than ever before.

The CMS has a mass range of up to *m/z* 1200 and the majority of peptides fall within this range. However, larger peptides above this mass can be detected and measured by the CMS because of multiple charging produced by an electrospray ion source.

A mass spectrometer measures the mass to charge ratio (*m/z*) of an ion, not its absolute mass. A positive singly charged ion, called the molecular ion, is detected at *m/z* $(M+H)^+$ so a peptide with a mass of 2000 Da would be detected at *m/z* 2001. But large peptides generally have multiple sites that can accept a charge, the larger the peptide the more charges it can accept.

A peptide with a mass of 2000 Da is likely to have 2, 3 or even more charges so the mass spectrometer would measure ions at $(M+2H)^{2+}$ (*m/z* 1001) and $(M+3H)^{3+}$ (*m/z* 667.7) bringing them well within the mass range of the CMS. Proteins can carry even more charges, so even large proteins can be detected by the CMS also.

The final result of peptide synthesis is generally a purified sample and chromatography is often not required. Easy, fast, flow injection analysis (FIA) is all that is needed and the analysis can be made in around 30 seconds.

Materials and Methods

Mass spectrometer: expression CMS
Flow rate: 100 µL/min
Spray Solvent:
50/50 acetonitrile/water with 0.1% formic acid
Injection volume: 5 µL

Advantages

- Rapid, conclusive peptide identification
- Accurate analysis of large molecules with an easy-to-use, single quadrupole mass spectrometer

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Results

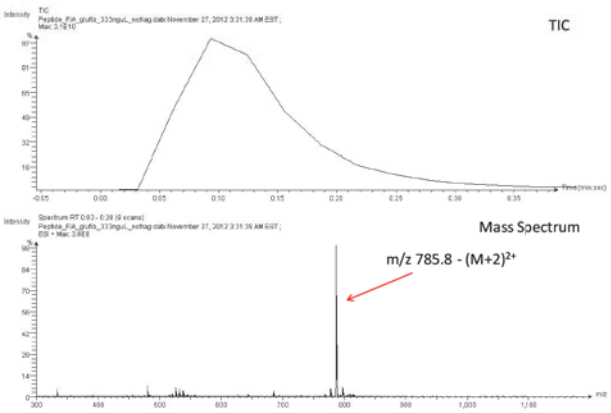

Figure 2. Flow Injection Analysis of [Glu[1]]-Fibrinogen, calculated molecular weight is (758.8 x 2) – 2 = 1569.6 (M_{iso}=1569.67; M_{ave}=1570.59)

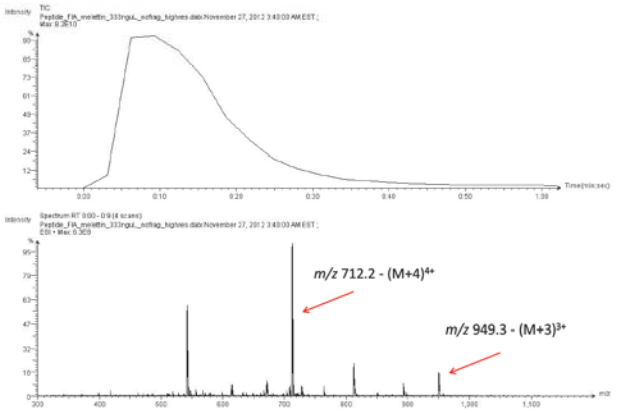

Figure 3. Flow injection analysis of Melittin peptide, calculated molecular weight is (949.3 x 3) – 3 = 2884.9 (M_{iso}=2844.75; M_{ave}=2846.50) – Honey Bee venom

The resolution and mass accuracy of the CMS allows you to distinguish between single, doubly and triply and even more highly charged ions. Singly charged ions have their isotopes spaced 1u apart, whereas doubly charged ions have their isotopes spaced 0.5 u apart, so it is easy to recognize the charge state and calculate the absolute mass. Moreover, multiple charging produces characteristic spacing in *m/z* of the ions - (M+1)/1 : (M+2)/2 : (M+3)/3… etc. yielding further information on mass and charge state.

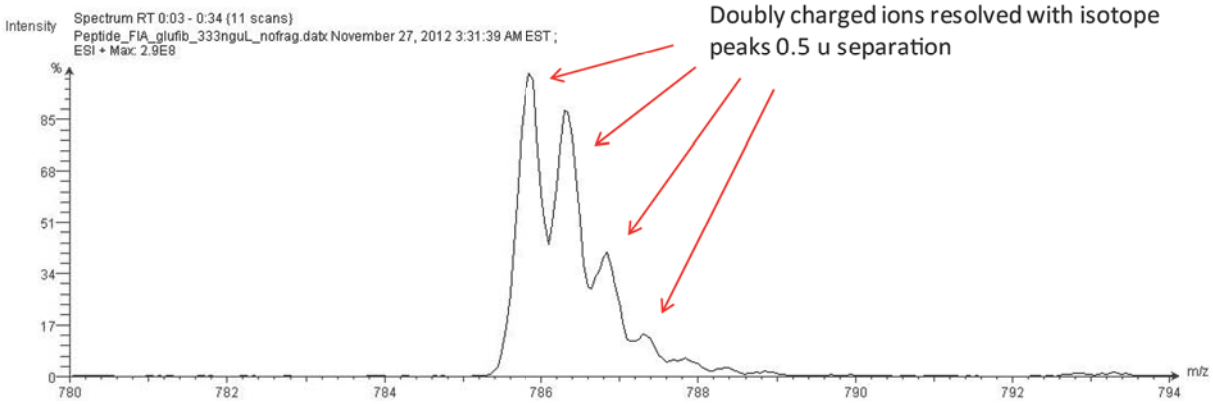

Figure 4. Spectrum of $(M+2H)^{2+}$ ions of [Glu[1]]-Fibrinogen

By fine tuning the resolution of the CMS, you can easily resolve the isotopes of doubly charged ions.

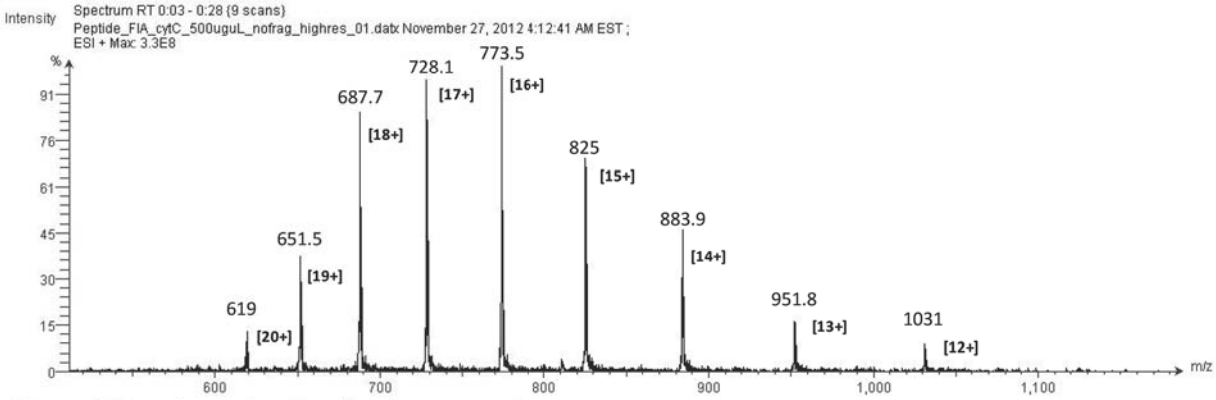

Figure 5. Spectrum showing the charge envelope of Cytochrome-C (~12 kDa Molecular weight)

The multiple charging caused by electrospray allows even large proteins to be identified using the CMS and the characteristic spacing, or charge envelope, allows calculation of charge state and the mass of the protein.

Using neighboring ions of the same mass but sequential charge states:
The number of charges on an ion can be calculated, Q1 = (*m/z*2 – 1)/(*m/z*1 – *m/z*2)
And molecular weight, M_{ave} = Q1(*m/z*1 - 1)
Where *m/z*2 is more highly charged than *m/z*1
So the charge state of the *m/z* 825 ion is (773.5 – 1) / (825 – 773.5) = 15+
And the molecular weight is 15(825 – 1) = 12,360 Da
The same mass is calculated using charge states of 12+ (12,360.4) and 13+ (12,360.0).