Advion
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Advion is a leader in mass spectrometry & synthesis solutions. The expression CMS is a high performance, compact, affordable single quadrupole 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 Analysis
Using Compact Mass Spectrometry

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.

A mass spectrometer measures the mass to charge ratio (*m/z*) of an ion, not its absolute mass. A singly and positively charged ion $(M+H)^+$, called the molecular ion, is detected at a *m/z* value reflecting both its mass and charge, e.g. a peptide with 2000 Da mass would be detected at *m/z* 2001. However, a larger peptide is likely to carry additional charges, so the mass spectrometer would measure ions such as $(M+2H)^{2+}$ at *m/z* 1001; $(M+3H)^{+3}$ at *m/z* 667.7 etc. The expression CMS can be tuned to be able to resolve not only singly charged ions, but also multiply charged analytes such as a $(M+2H)^{2+}$ by increasing the *Resolution Offset* and *Resolution Span* parameters in the MS tune . In combination with its scan range of up to *m/z* 1200, the CMS is therefore very well suited for the analysis of peptides.

The final result of a peptide synthesis is usually a purified sample and chromatographic separation of the analyte is hence not required. Flow Injection Analysis can be used for easy and fast confirmation of the product.

Method

A background subtraction function is available in Data Express (V1.1) to allow easier data analysis and presentation.

As shown in Figure 1, one region of the FIA analysis is defined as the background signal and automatically substracted from the average analyte signal in the second region.

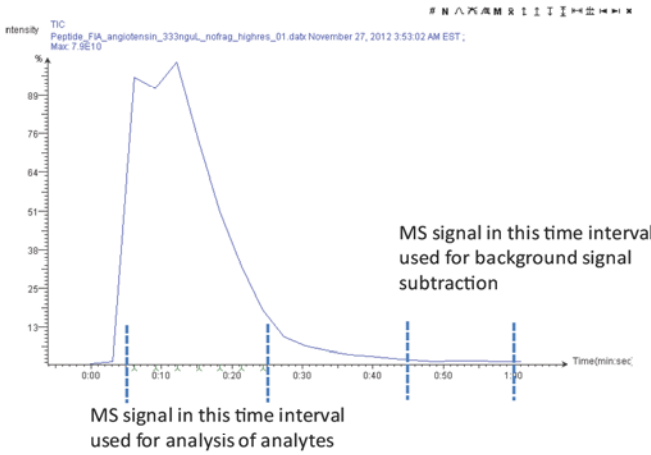

Figure 1: Typical FIA experiment showing the time ranges for analyte signal and background signal for subtraction

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Results

Figure 2 shows a typical FIA experiment analyzing the peptide angiotensin. After background subtraction, the magnified *m/z* range of the molecular ion shows a spacing of the isotopes of *m/z* 0.5 which indicates a doubly charged analyte (spacing of *m/z* 1.0 would indicate a singly charged analyte; spacing of 0.3 would indicate a triply charged analyte etc.). With the knowledge of the mass to charge ratio as well as the charge state the monoisotopic mass of the analyte can be easily calculated with M_{iso}=(Q1**m/z*)-Q1. The resulting monoisotopic mass of to 1,045.62 for angiotensin corresponds very well with its theoretical value of M_{iso}=1,045.53 (average mass of angiotensin is M_{ave}=1046.19; see comment below).

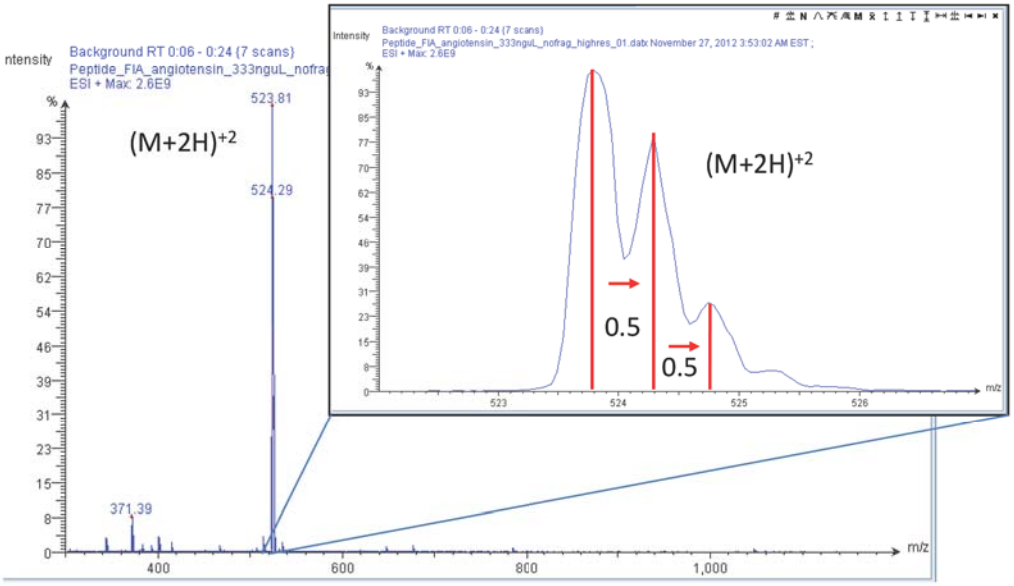

Figure 2: Typical MS data obtained for angiotensin in the mass range of *m/z* 300 to 1200; insert shows magnified area of the doubly charged molecular ion of angiotensin

Note the difference between the isotopic mass of an analyte (M_{iso}) and the average mass of an analyte (M_{ave}). In most cases the average mass of an analyte is given. However, a mass spectrometer is able to resolve the isotopic distribution of analytes and hence the isotopic mass needs to be calculated (and compared to) using the chemical formula of the analyte. Free online calculator tools such as http://rna-mdb.cas.albany.edu/RNAmods/masspec/mole.htm are available.

Figure 3 shows another example of a FIA/CMS experiment analyzing the peptide bombesine. Here the monoisotopic mass can be calculated to 1,618.9, a very good fit with the theoretical value of M_{iso}=1618.82 (M_{ave}=1619.87)

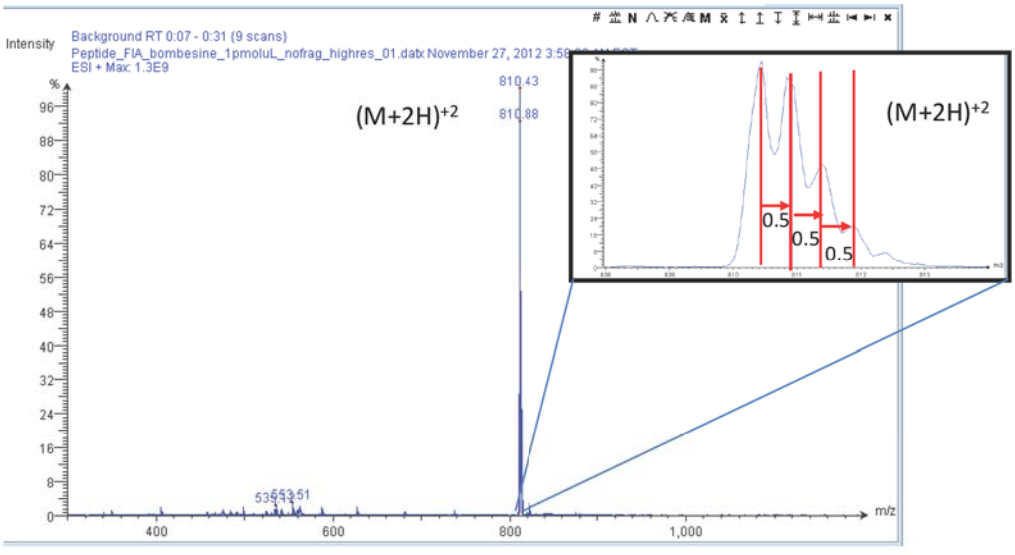

Figure 3: MS data obtained for bombesine in the mass range of *m/z* 300 to 1200; insert shows magnified area of the doubly charged molecular ion of bombesine