APPLICATION NOTE

BAYSPEC
Pervasive Spectroscopy

Peptide sequencing using *Continuity*™ miniature mass spectrometer

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Peptide identification and sequencing strategies by mass spectrometry have been very well-developed during the last 25 years after the soft ionization techniques have been introduced. When sequencing peptides with tandem mass spectrometry (MS^n), peptides are cleaved at various locations off their backbones to generate fragment ions of different masses based on their amino acid sequences. The most common product ions are the *b*-, *y*-, and *a*-ions generated from the cleavage of amide bond (CO-NH) and the subsequent loss of CO from the *b*-ions to form *a*-ions. The resulting MS^n spectra can be matched with a database or computed with an algorithm to get the original sequence of known or unknown peptides.

Here we present an example of peptide sequencing with BaySpec's highly-portable (22 kg) and battery operated *Continuity*™-series portable mass spectrometer. It features a linear ion trap with collisionally induced dissociation (CID) for MS^n analysis, similar to the *Portability*™ series mass spectrometers. In addition, *Continuity*™ is equipped with a continuous atmospheric pressure sampling inlet with differential pumping, allowing the detection of a larger mass range (110 – 950 amu). BaySpec's *Continuity*™ mass spectrometer is ideal for on-site peptide sequencing.

In this application note, we show an example of peptide sequencing in a standard mixture (H2016, Sigma-Aldrich) with *Continuity*™ mass spectrometer. The mixture was dissolved in HPLC grade water, and then further diluted with an electrospray solution (50:50 methanol:water with 0.5% acetic acid). The diluted solution is directly infused into the API inlet of the *Continuity*™ mass spectrometer without any further treatment or separation.

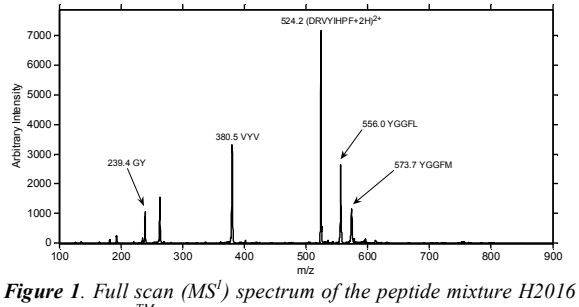

Figure 1. *Full scan (MS^1) spectrum of the peptide mixture H2016 with Continuity*™.

Figure 1 shows the full scan (MS^1) spectrum of the peptide mixture. Each peptide was isolated with stored waveform inverse Fourier transform (SWIFT), before fragmentation by CID. The resulting MS^2 spectrum consists of the sequencing *a*-, *b*- and *y*-ions. Shown in Figure 2 and Figure 3 are the SWIFT isolation and MS^2 spectra, respectively, of the largest and doubly-charged peptide, DRVYIHPF. The fragment ions in the MS^2 spectrum were successfully assigned based on the sequence.

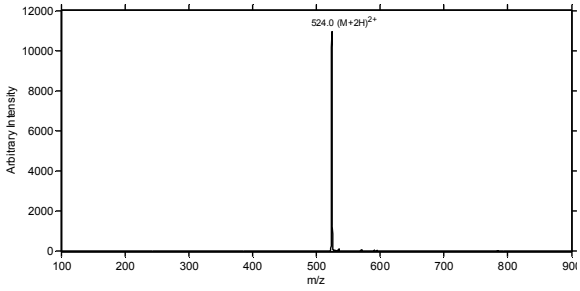

Figure 2. *SWIFT isolation of the doubly charge peptide DRVYIHPF.*

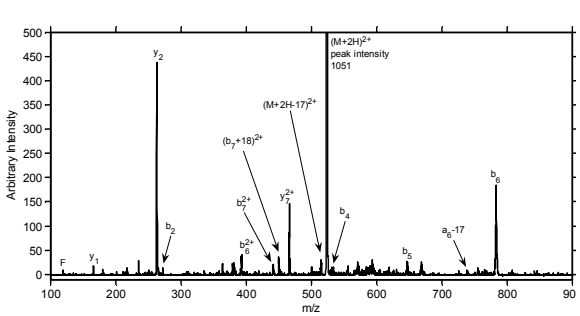

Figure 3.*MS*^{2} *spectrum of the peptide DRVYIHPF, and the assignment of the fragment a-, b-, and y -ions.*

Continuity™ Mass Spectrometer

The *Continuity*™ mass spectrometer is one of BaySpec's newest portable instruments. *Continuity*™ features a continuous sampling atmospheric pressure inlet, wide mass range and high sensitivity. Designed to bring the benefits of powerful mass to the field, the *Continuity*™ can service a variety of bulk or trace detection applications, as well as scientific studies.

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