

Peptide sequencing using *Continuity*TM 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 (MSⁿ), 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 MSⁿ 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*TM-series portable mass spectrometer. It features a linear ion trap with collisionally induced dissociation (CID) for MSⁿ analysis, similar to the *Portability*TM series mass spectrometers. In addition, *Continuity*TM 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*TM 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*TM 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*TM mass spectrometer without any further treatment or separation.

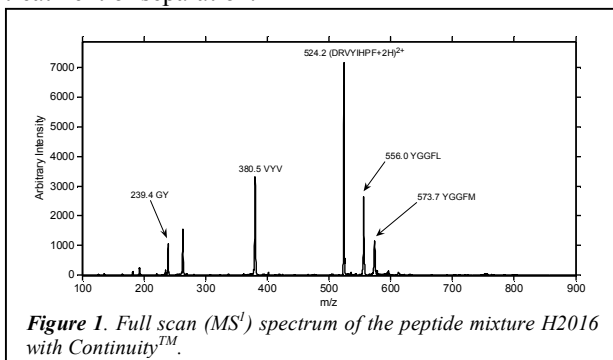


Figure 1 shows the full scan (MS¹) spectrum of the peptide mixture. Each peptide was isolated with stored

waveform inverse Fourier transform (SWIFT), before fragmentation by CID. The resulting MS² spectrum consists of the sequencing *a*-, *b*- and *y*-ions. Shown in Figure 2 and Figure 3 are the SWIFT isolation and MS² spectra, respectively, of the largest and doubly-charged peptide, DRVYIHPF. The fragment ions in the MS² spectrum were successfully assigned based on the sequence.

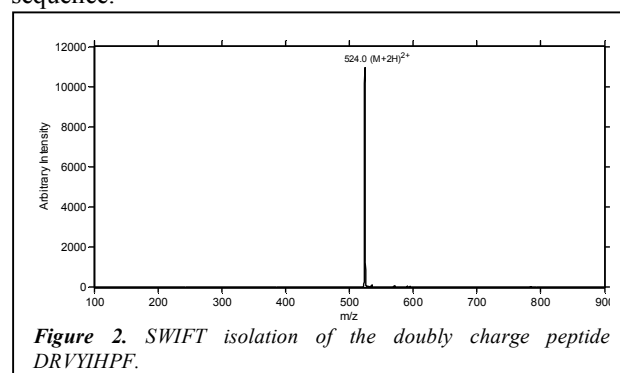


Figure 2. SWIFT isolation of the doubly charge peptide DRVYIHPF.

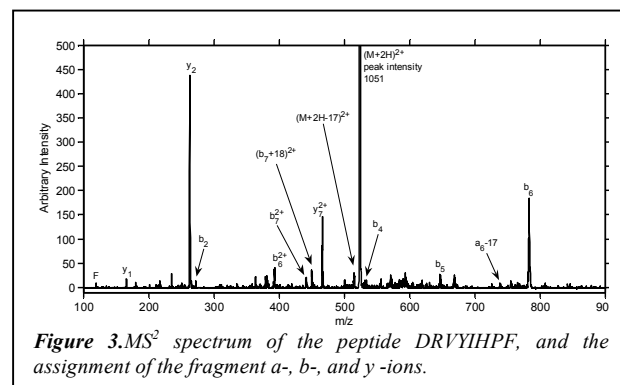


Figure 3. MS² spectrum of the peptide DRVYIHPF, and the assignment of the fragment *a*-, *b*-, and *y*-ions.

*Continuity*TM Mass Spectrometer

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

