Orbitrap Mass Spectrometry with Resolving Powers above 500,000 and 1,000,000 on a Chromatographic Time Scale

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Overview

Purpose: Demonstrate ultrahigh resolving power on an Orbitrap™ mass analyzer.

Methods: 1.5-second and 3-second transients were acquired on a pre-selected compact high-field Orbitrap analyzer with advanced signal processing.

Results: Demonstrated resolving powers over 5x10⁴ and 10⁵ enable the determination of elemental composition of analytes and the identification of some important modifications.

Introduction

Typically, an Orbitrap mass spectrometer is expected to provide resolving power of not higher than 100,000 – 200,000 which corresponds to detection time of 1-2 seconds. Recent innovations in Orbitrap technology, such as the high-field Orbitrap mass analyzer and advanced signal processing method, allowed us to accelerate detection by around 3.6-fold. This allows not only an accelerated spectral acquisition rate but makes it possible to do the opposite: to use longer transients to achieve higher resolving power while still remaining on time scale compatible with chromatographic separations.

Methods

Experiments were carried out on a Thermo Scientific Orbitrap Elite hybrid mass spectrometer equipped with a compact high-field Orbitrap mass analyzer (Figure 1) specifically selected from a batch of serial assemblies. Software was modified to allow transients up to 3 seconds long to be acquired using advanced signal processing (1). Peptides, small molecules, and intact proteins were analyzed in infusion mode. The instrument was manually tuned and mass-calibrated.

Results

Instrument performance in ultrahigh resolution mode

Implementation of a compact Orbitrap mass analyzer and advanced signal processing with 0.76-second transients provides nominal resolving power of about 240,000 which became the maximum resolving power setting on Orbitrap Elite instruments. This represents a spectacular 3.5-fold improvement over the resolution of the Thermo Scientific LTD Orbitrap Velos hybrid mass spectrometer. When compared for the same transient duration, the Orbitrap Elite instrument achieved higher resolving powers than Fourier transform ion cyclotron resonance (FT-ICR) instruments with strongest commercially available super-conducting magnets starting from m/z 330 (Figure 2).

However, FT-ICR allows for much longer transients in comparison with the Orbitrap analyzer. Though physics of ion motion and limitations of electronics make it impossible for Orbitrap analyzer to detect ions for tens of seconds as possible in FT ICR, nevertheless it is possible to extend detection times several fold. A practical upper limit of detection duration is imposed by the chromatographic time scale and makes it impractical to detect longer than for a few seconds (e.g. 3 sec).

De-amidation analysis using ultrahigh resolving power

Analytic utility of ultrahigh resolving power was also demonstrated on the example of substance P and melittin peptides. Both were analyzed at resolving power above 600,000 which allowed resolution of thin isotopic structure along with de-amidation (Figures 4 and 5). For more intense isotopes, a single spectrum is sufficient for identification of this modification.

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Conclusions

• Orbitrap technology is capable of ultrahigh resolving power in excess of 1,000,000 resolving power when appropriate tolerance and tuning requirements are met.

• Ultrahigh resolving power could be achieved for 3-second detection time which makes it compatible with chromatographic separations.

• Resolving powers over 5x10⁴ allow resolution of thin isotopic structure and identify some important modifications of peptides.

References