

# Accurate Mass Measurements Using the Finnigan LTQ FT

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## Key Words

- Finnigan™ LTQ FT™
- Accurate Mass
- FT-ICR-MS
- High Resolution
- Metabolites

## Introduction

High resolution, accurate mass spectral analysis is an essential tool for structural elucidation in the fields of chemistry and biochemistry. Use of accurate mass information facilitates the identification of known compounds and determination of elemental composition for unknowns. High resolution analysis allows isobaric compounds to be separated from one another or from chemical noise. For the identification of unknowns, it is desirable to perform the highest mass accuracy measurements possible in order to eliminate false positives. For example, Table 1 lists the number of possible elemental compositions as a function of mass accuracy for the doubly-charged ion of [Val<sup>5</sup>]-Angiotensin II. The possible chemical formulae were calculated from the list of elements and number range for each. Clearly there is an order of magnitude reduction in theoretical empirical formulae when the mass accuracy is increased from 10 to 1 ppm, (routinely achieved with Thermo Electron Corporation's Finnigan LTQ FT).

The ideal instrument for this kind of application must produce high resolution full scan accurate mass MS and MS<sup>n</sup> data without the need for internal calibration or frequent recalibration. These requirements are fulfilled by an FTMS, however, historically such instruments suffer from being complicated to use and lack high productivity and robustness.

## Results and Discussion

With the development of a new hybrid FTMS system, the Finnigan LTQ FT, we have turned high resolution/mass accuracy measurements into a routine, high productivity tool for the analytical chemist. Combining a linear ion trap with FT-MS detection, highly reproducible ion populations are maintained for each scan event. Thus the user can perform accurate mass measurements on full scan MS, MS/MS, and MS<sup>n</sup> data on a chromatographic time scale.

Example peptide: [Val <sup>5</sup> ]-Angiotensin 11, [M+2H] <sup>2+</sup> = 516.77671				
Limitation for elements:				
	Carbon:	35 – 70		
	Hydrogen:	45 – 100		
	Nitrogen:	8 – 16		
	Oxygen:	9 – 16		
	Sulfur:	0 – 2		
Instrument		TOF type MS	FT-MS	FT-MS
Mass Error	10 ppm	5 ppm	2 ppm	1 ppm
Number of Proposals for <i>m/z</i> 516.76671	49	23	10	4

Table 1: Number of possible elemental composition depending on mass tolerance

The need for high resolution is emphasized for peptide analysis due to a greater numbers of possible matches and frequent appearance of isobaric compounds. For a mixture of two isobaric peptides, it is necessary to have much higher resolution than that used for small molecules. Peptides mostly consist of C, H, O, N, and S and therefore, isobaric peptides may differ by only few millimass units. High mass resolution detection allows base line separation of the isobaric peaks which ensures

the exact determination of the peak centroid for accurate mass calculation. Figure 1 shows simulated mass spectra of isobaric peptides [Val<sup>5</sup>]-Angiotensin II and Lys-des-Arg<sup>9</sup>-Bradykinin calculated with a resolving power of 18,000 and 57,000. Due to the small mass difference between the two, data acquisition at the lower resolution (typically achieved with TOF instruments) is unable to differentiate between the two peptides causing large mass errors for both measured ions.

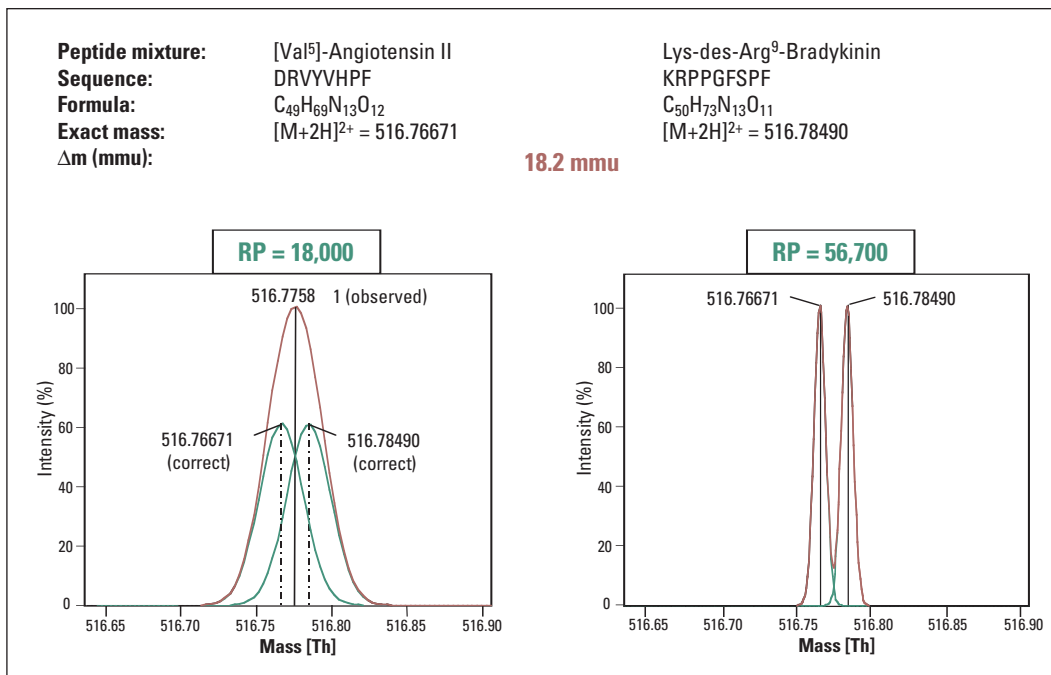


Figure 1: Simulated mass spectra for isobaric peptides at different resolution settings (here 18,000 and 57,000).

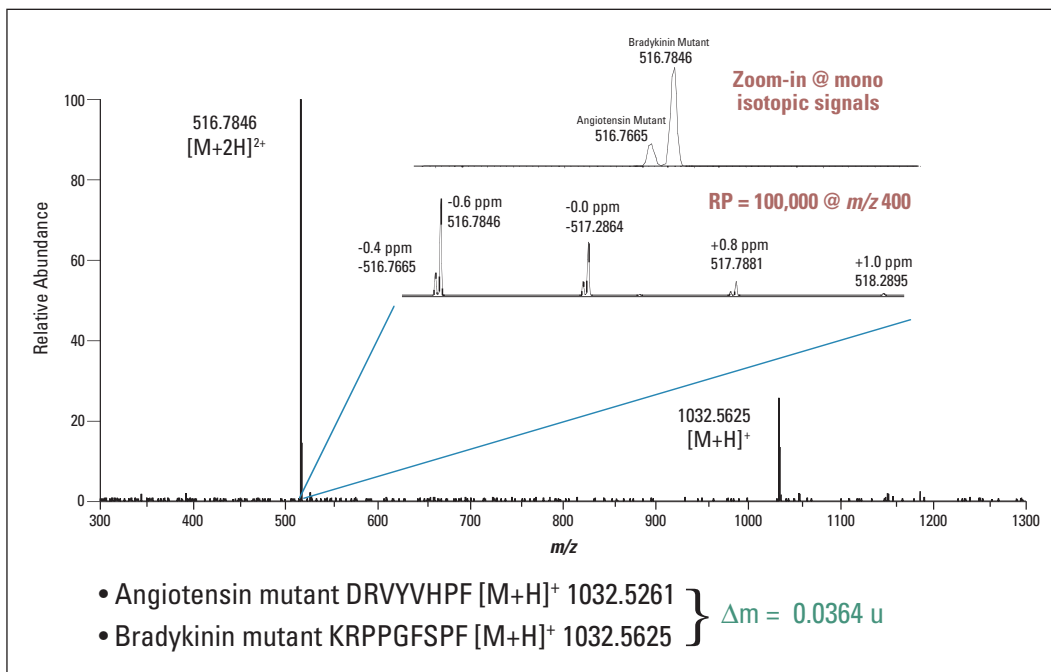


Figure 2: Mass spectrum of the isobaric peptide mixture acquired at resolving power of 100,000

Applying the greater mass resolving power provides enough resolution to detect the two peptide ions separately and therefore ensures accurate mass analysis for both. Figure 2 shows an actual mass spectrum for the two species acquired simultaneously at a resolution setting of 100,000 (at  $m/z$  400). The inset shows a magnified mass range containing the isotopes for both peptides. Clearly, the resolution setting used for data acquisition is more than sufficient for baseline separation. In addition, measured mass accuracies are less than 2 ppm for each of the bradykinin isotopes as well as for the angiotensin peptide.

In drug discovery and metabolomics, structural elucidation is greatly supported by accurate mass measurement. Figure 3 shows a full scan mass spectrum of norcotinine and nicotine acquired at a resolving power of 100,000. Despite the two compounds being separated by only 0.0365 u, the resolution at this mass is more than sufficient for baseline separation enabling mass accuracy measurements of 0.4 and 0.7 ppm for norcotinine and nicotine, respectively. In addition, accurate mass measurements can further be used to enhance structural confirmation by using ring plus double bonds (RDB) information. In this example only one of the top three closest empirical formulae, has the correct number of RDB value.

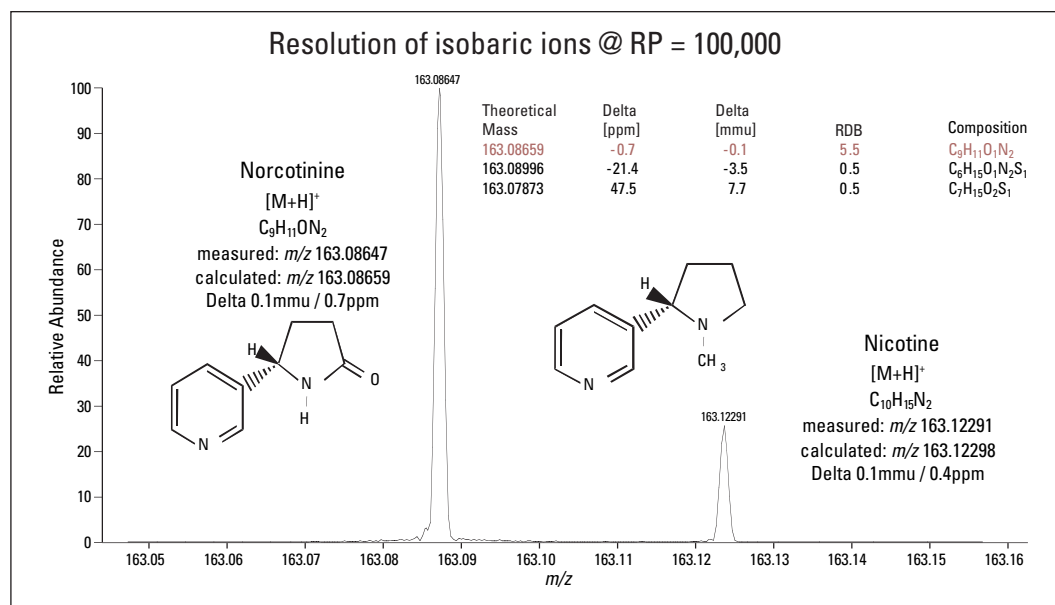


Figure 3: Full-scan mass spectrum of nicotine and its metabolite norcotinine acquired at resolving power of 100,000 (at  $m/z$  400)

To underscore the utility of accurate mass analysis for determination of metabolic pathways, a full scan MS/MS spectrum is displayed for the drug FK228 with a molecular weight of 540 u. (Figure 4) The tandem mass spectrum shows two primary fragment ions at  $m/z$  272 and  $m/z$  244 together with the two best empirical formula matches (according to search criteria outlined above). Note that the top match for each is definitive as evident by 0.74 and 0.85 ppm mass measurement deviations and therefore, unequivocally determines the correct fragmentation pathway.

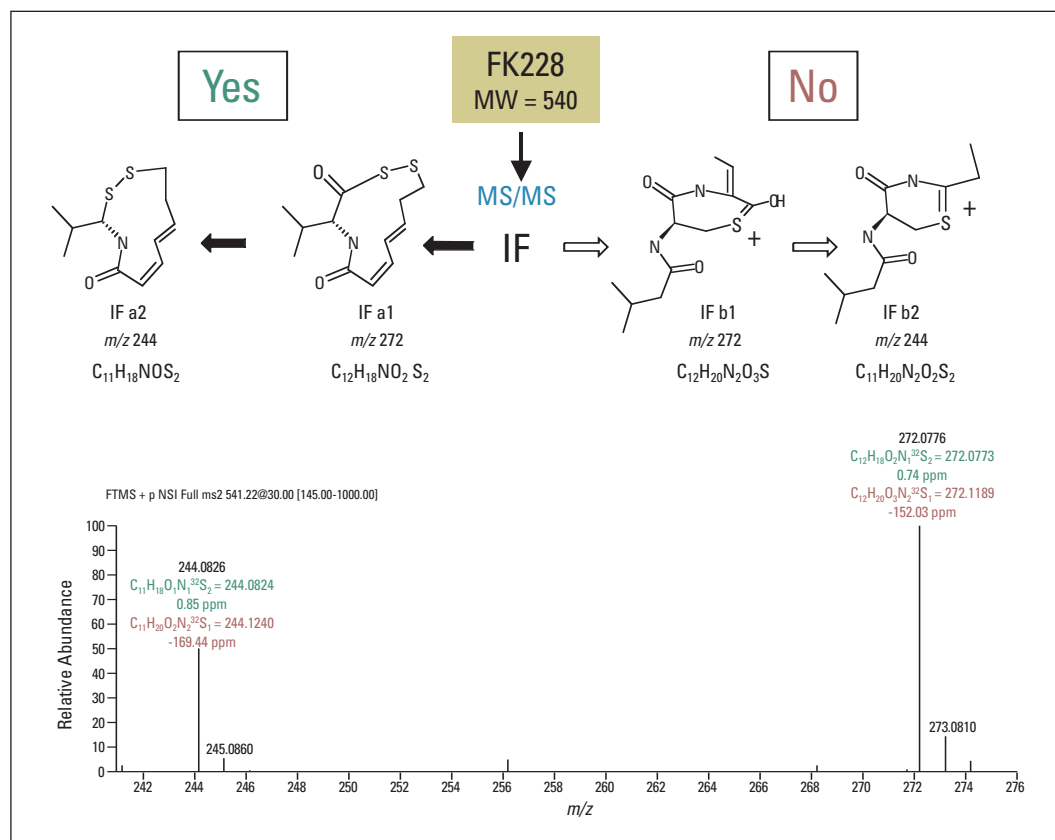


Figure 4: Metabolic pathway proven by high resolution accurate mass MS/MS measurement.

## Conclusion

The Finnigan LTQ FT achieves sub-ppm mass accuracy measurements without the need for internal mass calibration:

- High mass resolution allows separation of isobaric ions
- High mass accuracy provides confident assignment of empirical formulae
- Wide mass range allows detection of small molecules as well as peptides
- Structural elucidation is supported by a combination of accurate mass analysis with compound characteristics (e.g. RDB or nitrogen rule)

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