

Dynamic Range of Accurate Mass in FTMS

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Overview

Extend the dynamic range over which accurate masses can be determined (dynamic range of mass accuracy) for on-line experiments using a novel FTMS mass spectrometer: LTQ Orbitrap™.

Introduction

The dynamic range over which accurate measurements of mass can be made is a key analytical figure-of-merit. With accurate-mass analyzers coupled to LC devices, it is important to determine the range of intensities over which accurate masses can be determined for transient signals (e.g. when recorded at a rate of 1 spectrum/second). For all analyzers, mass accuracy is limited statistically by too few ions, or by peak position shifts due to too many ions.

This work investigates extension of the dynamic range over which accurate masses can be determined (dynamic range of mass accuracy) for on-line experiments using a novel FTMS mass spectrometer (LTQ Orbitrap).

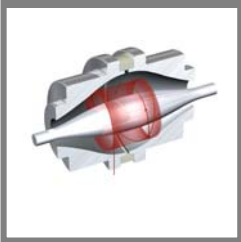
Methods

The Finnigan™ LTQ™, operating with automatic gain control and isolation capabilities, is used to selectively fill an intermediate ion storage device (C-trap), following which the ions are injected into the Orbitrap mass analyzer. Using this technique it is possible to generate ion populations with intensity ranges of 104. All measurements (ion accumulation and image current detection) were performed in less than 1 second at a resolving power 30,000.

Results

Dynamic range of mass accuracy of the Orbitrap mass analyzer reaches 5,000 (at least an order of magnitude higher than typical values for time-of-flight instruments). Due to the high resolving power of the Orbitrap, accurate mass of a signal is determined when the peak is reliably distinguished from noise ($S/N \gg 2 \dots 3$).

FIGURE 1. Electrode structure of the Orbitrap mass analyzer.



Methods

1. Instrument Operation

The Orbitrap mass analyzer is an electrostatic trap with high resolving power, mass accuracy, and high space charge capacity [1]. The electrode structure of the Orbitrap is outlined in Fig. 1, together with a typical ion trajectory.

The LTQ Orbitrap mass spectrometer, depicted in Fig. 2, is a hybrid system combining a Finnigan LTQ mass spectrometer and an Orbitrap mass analyzer. Key to operation of this system is a C-shaped storage trap, which is used to store and collisionally cool ions prior to injection into the Orbitrap. With this device ions are pulsed into the central point of the C-trap arc that coincides with

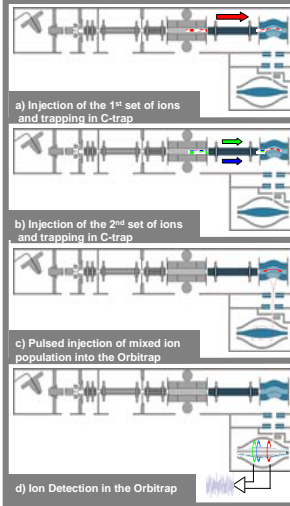
the Orbitrap entrance aperture. Ions are captured in the Orbitrap by rapidly increasing the electric field, and detection of image current from coherent ion packets takes place after voltages have stabilized [1]. Signals from each of the Orbitrap outer electrodes are amplified by a differential amplifier and transformed into frequency spectrum using fast Fourier transform. The frequency spectrum is transformed into a mass spectrum using two-point calibration and processed using Xcalibur™ software.

2. Measurement Methodology

To explore the dynamic range of mass accuracy of the Orbitrap analyzer, it is important to provide a reproducible and as wide as possible spread of signal intensities within the same spectrum without being limited by the electrospray source. This was achieved using multiple fills of the C-trap from the LTQ, as illustrated in Fig. 2. The number of ions in each fill is individually controlled over several orders of magnitude using automatic gain control (AGC™), while the set of masses in each fill is adjusted using isolation and MSⁿ capabilities of the Finnigan LTQ. This creates a flexible and versatile tool for forming desired ion populations including, if necessary, internal calibrants. Mass calibration coefficients were determined for different AGC target values and interpolated for intermediate values. No intensity-dependant corrections of m/z were made for data processing.

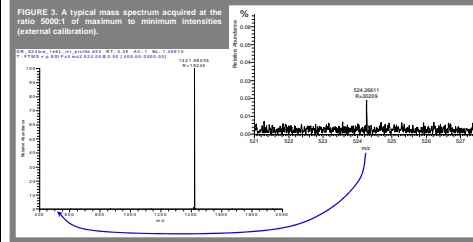
All experiments were carried out using a mixture of Ultramark 1600, MRFA peptide and caffeine in 50:50 v.v. water/acetonitrile solution. The resolving power was reduced to nominal 30,000 FWHM (at m/z 400 Th after zero-filling & apodization, 0.38 sec transient detected), so that the experiment duration of 1 second still allowed sufficient time to store millions of ions in the C-trap and detect them in the Orbitrap. To model the widest possible range of conditions, intensities of dominant and minor peaks were varied over orders of magnitude to achieve variations of ratio of intensities between 1 and 10,000. For internal calibration evaluation, the intense peak was used as the calibrant.

FIGURE 2. Operation of the LTQ Orbitrap.



Results

Figure 3 shows a typical mass spectrum used for estimation of mass accuracy at the extreme limits of dynamic range.



Observations from Fig. 4:

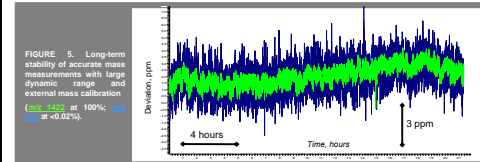
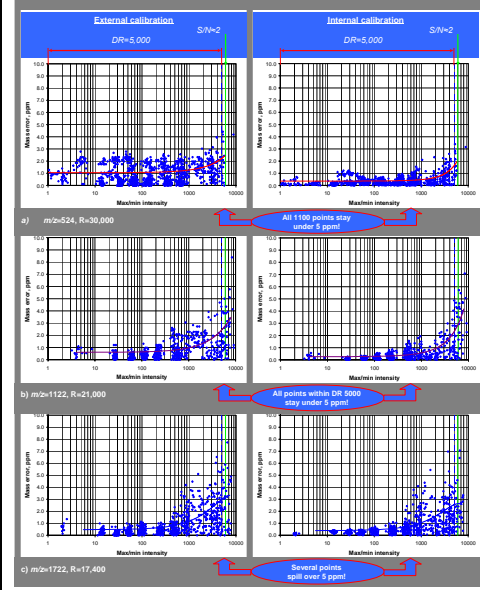
- For resolving powers 30,000 and higher, mass errors are under 5 ppm.
- For resolving powers below 20,000, several measurements show mass errors in the range 5-7 ppm.
- For ALL resolving powers above and down to $S/N=2$, r.m.s. mass errors are well within 5 ppm.
- Due to some drift of high-voltage power supply over time, longer duration of measurements (like in Fig. 4a) results in some increase of mass error for external mass accuracy, without affecting mass errors for internal mass accuracy.
- At low S/N , background is the main contributor to the error of peak centering, so there is no significant difference in mass accuracy between internal and external calibrations.
- Dynamic range of mass accuracy of LTQ-Orbitrap extends at least to 5,000.

At the upper limit of dynamic range (like in Fig. 3), a minor peak has such a low S/N that it starts to limit mass accuracy of measurement in agreement with published literature [2]. Noise is defined as the maximum peak-to-peak amplitude of thermal noise of pre-amplifier in full-profile mode over full mass range (for example, in Fig. 3 the noise is 0.008%). For low S/N , it was also found that lower resolving power results in mass errors increasing almost proportionally. Also, for shorter acquisition times, T , not only does the resolving power become proportionately lower, but also the noise increases as $1/\sqrt{T}$, thus reducing S/N and hence mass accuracy. For this reason it is important to use as long as acquisitions as feasible. Under all other conditions, mass accuracy is limited by the accuracy of the 2-point mass calibration and by the stability of the high-voltage power supply for external calibration mode. Stability of external mass calibration is demonstrated in Figure 5, as shown by two mass traces with an intensity ratio of ~5,000 which remained stable for more than 20 hours. In this plot, 1 point was acquired every 6 seconds.

Observations from Fig. 5:

- Spikes on the blue trace probably result from intermittent variations of minor component intensity, i.e. lower than usual S/N ratios.
- Mass measurement variations for minor components are much higher because of low S/N .
- Overall trend demonstrates stability and effectiveness of thermal regulation of the LTQ Orbitrap.

FIGURE 4. Measured mass errors (points) and trend line of r.m.s. mass error vs. ratio of intensities for different m/z .



Conclusions

The dynamic range of mass accuracy determines the true utility of the accurate mass capability of a mass spectrometer for real-life applications - much more than other parameters (e.g. resolving power). From this point of view, the LTQ Orbitrap enables accurate mass measurements over a dynamic range that matches or exceeds the spread of signal intensities in the electrospray ion source.

References

1. Makarov, A.A. Anal. Chem. 2000, 72, 1156-1162.
2. Marshall A.G., Verduin F.R., Fourier Transforms in NMR, Optical and Mass Spectrometry, Elsevier, Amsterdam, 1990, 150-155.

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