

Fully Automated High Throughput Accurate Mass Determination Using FT-ICR Mass Spectrometry

Jens Griep-Raming¹, Wolfgang Metelmann-Strupat¹, Stevan Horning¹, Helmut Muenster¹, Mark Baumert², and Jack Henion³
¹Thermo Electron Corporation, Barkhausenstr. 2, D-28197 Bremen, Germany; ²Advion BioSciences Ltd., 26 Queens Road, Norwich NR9 3DB, U.K.; ³Advion BioSciences Inc., 15 Catherwood Road, Ithaca, NY, U.S.A.

Overview

Purpose: To demonstrate mass accuracy achievable under fully automated conditions with external calibration on the Finnigan LTQ FT mass spectrometer.

Methods: Various samples have been analyzed using the Advion NanoMate™ 100 nano electrospray robot attached to the FT-ICR mass spectrometer in a high throughput fashion. Accurate mass information has been extracted from the files and has been evaluated statistically.

Results: Even under fully automated conditions using external calibration, mass accuracies of better than 1 ppm were routinely achieved. This is still valid when the instrument was operated in high throughput mode with sample analysis times of less than 1 minute. Excellent reproducibility of the mass measurement was demonstrated.

Introduction

Accurate mass measurement has been widely accepted as a method for confirmation of identity of substances and for identification of the elemental composition of unknown compounds. For the identification of unknowns it is important to get the best mass accuracy available, because the number of possible elemental composition increases dramatically with increased uncertainty in the mass accuracy. Most commercially available mass spectrometers achieve best mass accuracy (<5 ppm) only with internal calibration. Due to complications like ionization suppres-

sion users tend to avoid this whenever possible. The Finnigan LTQ FT provides a reliable mass accuracy of better than 2 ppm even with external calibration which is stable for several days. Thus no internal calibrant is needed. In FT-ICR mass spectrometry, mass accuracy is strongly affected by the total number of ions trapped in the ICR cell. High number of ions in an ICR cell produces space charge, which is the reason for mass shifts up to several hundreds of ppm. Therefore, it is necessary to resolve this problem in order to achieve good results. The Finnigan LTQ FT uses Automatic Gain Control™ (AGC) to regulate the number of ions for an FT-ICR-MS experiment. Figure 1 shows a scheme of the instrument, which is a hybrid linear ion trap FT-ICR mass spectrometer. Ions are generated in the electrospray ion source, then guided through transfer ion optics into the linear ion trap. With a fast prescan, the linear ion trap determines the total ion current being generated. Using this information, the optimal injection time for collecting ions is calculated. Ions are then injected for the predetermined period of time. This patented technology leads to a very reproducible ion population from scan to scan. Finally, ions are ejected from the linear trap into the FT-ICR part of the instrument, excited, and detected. With this implementation of AGC on an FT-ICR mass spectrometer, extremely good mass accuracies (usually better than 2 ppm) can be achieved even with fast scanning (1 spectrum/sec) and under full automation.

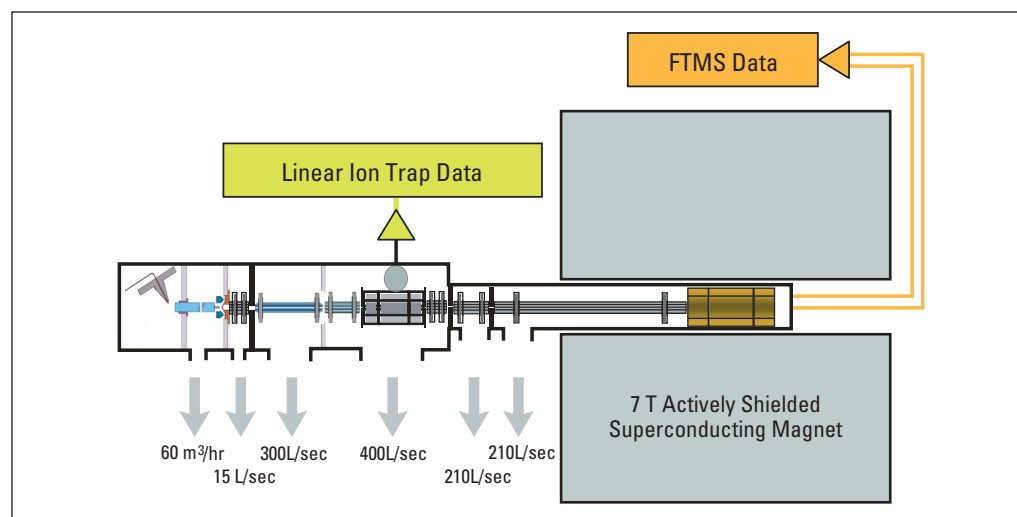


Figure 1: Diagram of the LTQ FT Hybrid Linear Ion Trap Ion Cyclotron Resonance Fourier Transform Mass Spectrometer.

Key Words

- Finnigan™ LTQ FT™
- Accurate Mass
- Automation
- Electrospray
- FT-ICR-MS
- High Throughput

Experimental

The Finnigan LTQ FT was scanned from m/z 200 to 2000 with a cycle time of slightly less than 1 sec, resulting in resolving power of 100,000 (FWHM) at m/z 400. For all experiments the same external mass calibration (generated 2 days earlier) was used. The standard ESI source was replaced by the Advion NanoMate 100 robot (see Figure 2). For each sample solution, 2 μ L were aspirated and delivered to the ESI chip with a slight gas backpressure and an electrospray voltage of about 1.5 kV. This resulted in flow rates of approximately 200 nL/min.



Figure 2: The Advion NanoMate 100 Mounted on the Finnigan LTQ FT

High Throughput Accurate Mass Measurements

In the first experiment, the NanoMate robot was filled with 48 different samples at various concentrations. Samples were from different compound classes, and included peptides, proteins, polymers, ligands, drugs, etc. Each sample was analyzed in duplicate. With a sample analysis time of ca. 1 minute, the 96 measurements were carried out in less than 100 minutes with approximately 30 spectra acquired per sample. Figure 3 presents a selection of the results achieved. The vast majority of the masses measured showed accuracies of better than 1 ppm. The total RMS error was clearly below 2 ppm. It is important to note that absolutely no carryover from measurement to measurement was observed, which is a clear advantage of the NanoMate robot over the usual flow injection technique.

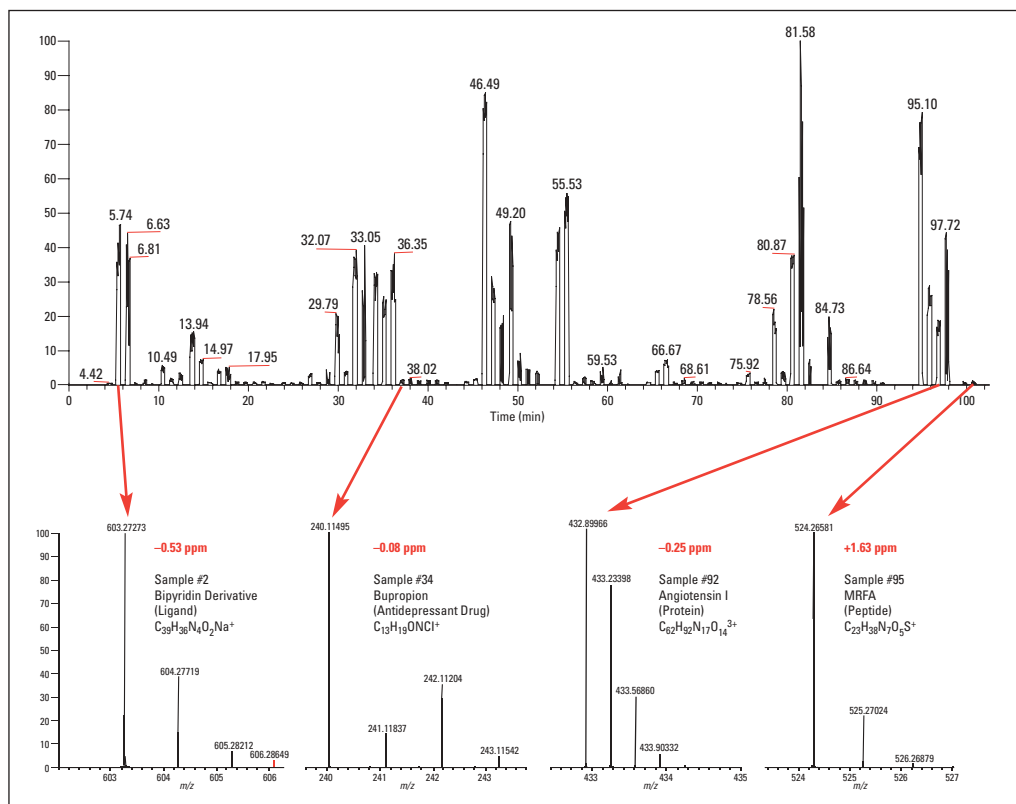


Figure 3: TIC Chromatogram and Mass Accuracies Achieved during a 96 Sample 100 Minute Run

Reproducibility of Accurate Mass Measurements

To get an estimate of the reproducibility of the mass measurements, one sample was analyzed repeatedly. Bupropion, an antidepressant drug was randomly selected from the set of samples from the previous experiment. Over a period of 45 minutes, the sample was sprayed 13 times with sample turnover times around 3 minutes. This resulted in ca. 2400 spectra which were evaluated. Figure 4 shows the ca. 2400 data points for the MH^+ peak of the molecule and its deviations from the theoretical mass.

The average error of the accurate mass was only -0.2 ppm with a standard deviation of 0.07 ppm. The evaluation was also carried out for the +1, +2, and +3 isotope peaks of the protonated molecule. Results are summarized in Figure 5. Here the results are averaged for each of the 13 injections. All averaged results showed mass accuracies of better than 0.4 ppm. In 2400 spectra not a single measurement exceeded 2 ppm error.

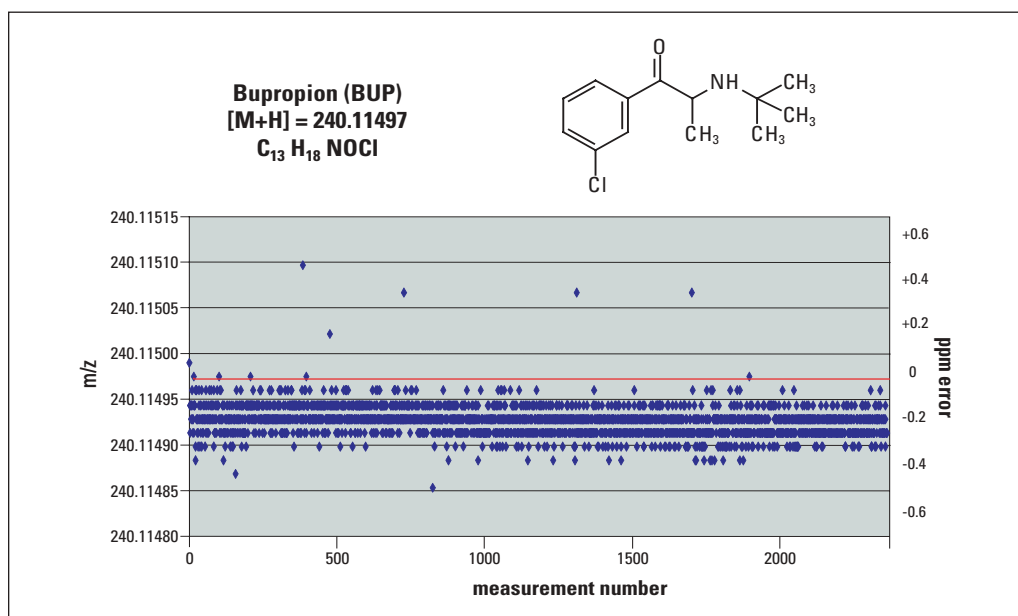


Figure 4: Mass Accuracy for Repetitive Measurements of the MH^+ Peak of Bupropion. The red line indicates the theoretical mass. The average error is -0.20 ppm

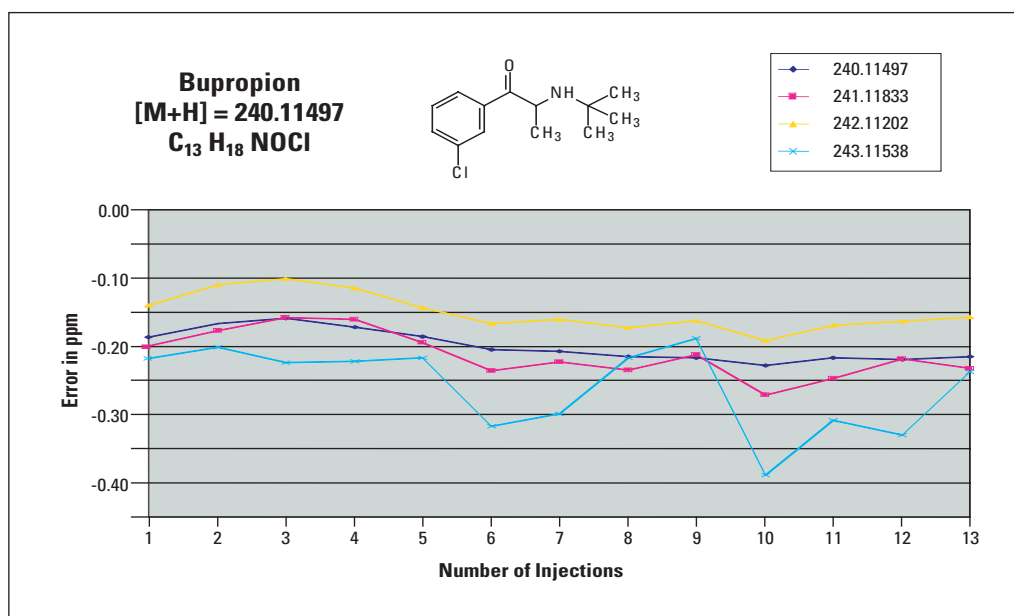


Figure 5: Mass Accuracy on 4 Isotope Peaks for Repetitive Injections of Bupropion

Variation of Mass Accuracy with Sample Concentration

In order to minimize sample preparation time, it is desirable to have reliable mass assignment independent on the signal intensity, i.e. sample concentration. To show the dependency of the mass measurement on the sample concentration, the previous experiment was carried out again for different sample concentrations and different analytes. As an example, the results achieved with Threohydrobupropion, a metabolite of Bupropion, are presented. It was sprayed in concentrations of 10, 5, 1, 0.2, and 0.1 mg/mL.

The acquired spectra were analyzed for the accurate masses of the four isotope peaks on the protonated molecule. In Figure 6 the mass chromatogram for the MH^+ ion of Threohydrobupropion is shown as well as one exemplary spectrum. Again, the first four isotope peaks on the MH^+ ion were evaluated. Figure 7 shows the ppm mass accuracy for the different concentrations. As expected, there is no trend visible with regard to mass accuracy. The errors are randomly distributed across the measurements with all results better than 0.5 ppm.

Conclusions

Fully automated high throughput accurate mass measurement has been demonstrated using the Finnigan LTQ FT. Extremely stable mass calibration and excellent mass measurement accuracy has been shown for a wide variety of compound types at different concentrations. The mass accuracies obtained averaged only -0.2 ppm with a standard deviation of 0.07 ppm. The use of AGC to control the number of ions used in ICR detection has proven essential in order to achieve accurate mass measurement results independent of the type of analyte, its concentration, spraying conditions, etc.

Acknowledgement

The authors thank Dr. Arne Lützen, Dr. Marko Hapke, Oliver Hass and Frank Thiemann (University of Oldenburg, Oldenburg, Germany) for providing the majority of the samples.

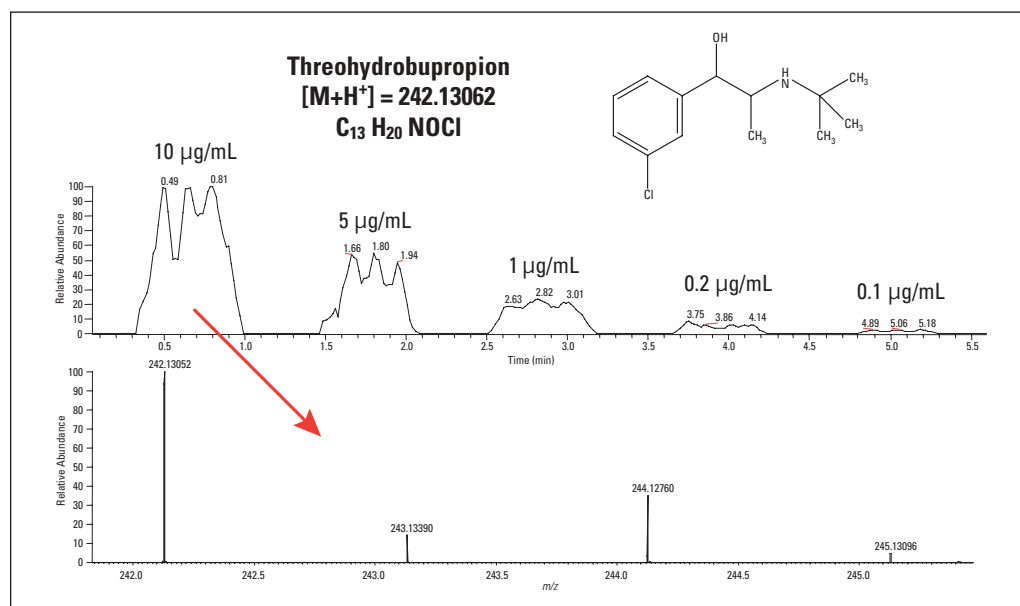


Figure 6: Variation of Sample Concentration for the Measurement of Threohydrobupropion

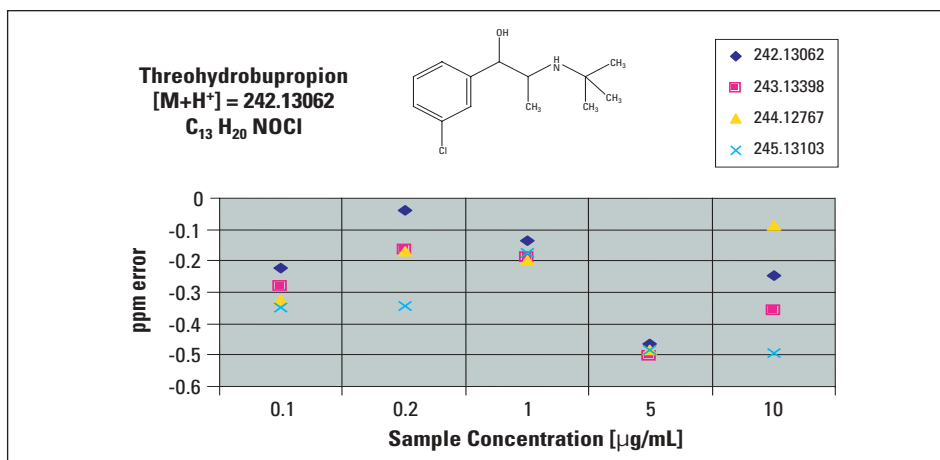


Figure 7: Variation of Mass Accuracy with Varying Sample Concentration in the Measurement of Threohydrobupropion

In addition to these offices, Thermo Electron Corporation maintains a network of representative organizations throughout the world.

Australia
+61 2 9898 1244

Austria
+43 1 333 50340

Belgium
+32 2 482 30 30

Canada
+1 800 532 4752

China
+86 10 5850 3588

France
+33 1 60 92 48 00

Germany
+49 6103 4080

Italy
+39 02 950 591

Japan
+81 45 453 9100

Netherlands
+31 76 587 98 88

Nordic
+46 8 556 468 00

South Africa
+27 11 570 1840

Spain
+34 91 657 4930

Switzerland
+41 61 48784 00

UK
+44 1442 233555

USA
+1 800 532 4752

www.thermo.com



Thermo Electron (Bremen) GmbH is certified DIN EN ISO 9001:2000

©2004 Thermo Electron Corporation. All rights reserved. NanoMate is a trademark of Advion Biosciences. All other trademarks are the property of Thermo Electron Corporation and its subsidiaries.

Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

AN30023_E_06/04S

Thermo
ELECTRON CORPORATION