

# Elemental Composition Determination of Small Molecules with High Precision using the LTQ FT Ultra Hybrid Mass Spectrometer

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## Introduction

Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) plays an important role for exact mass measurements at ultra-high resolution in both academic service labs and industry, especially pharmaceutical industry.

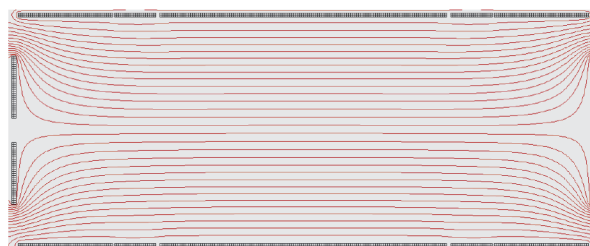
Very high accurate mass measurements at the parts-per-billion (ppb) level on precursor as well as on product ions enable high confident determination of the elemental composition as well as unambiguous assignment of fragment ions.

Since its introduction in 2003 the Thermo Scientific LTQ FT™ has rapidly built up a proven track record of delivering highly accurate mass measurements with external mass calibration even on LC-timescale on a routine basis and is therefore used in academic as well as industry labs in a wide field of applications.

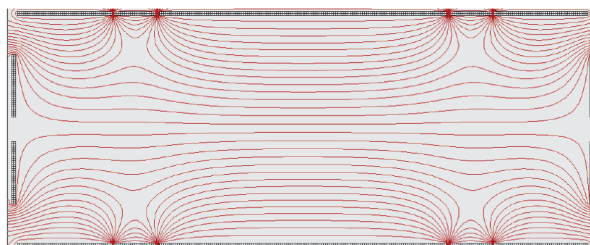
The Thermo Scientific LTQ FT Ultra is the next generation FTICR mass spectrometer from Thermo Fisher Scientific. The heart of the LTQ FT Ultra is the new ICR cell with grids as excitation electrodes<sup>[1]</sup>.

FTICR-MS uses the fact that ions are forced in a strong magnetic field into an orbital motion with a defined cyclotron frequency. A mass spectrum is obtained after Fourier-transformation of the image current induced by the ions on the two opposite detection plates in the ICR cell. Ions have to be excited into larger radii in order to move closer to the detection plates in order to attract electrons. Excitation is accomplished by applying a radio frequency field on the excitation plates of an ICR cell at the resonant (cyclotron) frequency. In conventional ICR cells the cyclotron radius is limited by axial components of the excitation field which causes ion loss due to z-axis ejection. The grid cell reduces the axial components due to a more homogeneous electric field and allows therefore higher excitation radii resulting in greater detection sensitivity and reduces unwanted space charge effects.

Figure 1 shows the isopotential contours for the new grid cell and the standard ICR cell of the LTQ FT. Further improvements are a more sensitive pre-amplifier and a larger memory to acquire transients for an extended period of time for ultra-high resolution spectra (up to 1,000,000 FWHM at  $m/z$  400).



New grid cell LTQ FT Ultra



Former LTQ FT cell

Figure 1: Isopotential contours generated by SIMION™ for the new grid cell (top) and the standard LTQ FT ICR cell (bottom).

Typically, exact mass measurements at high resolution are used to determine the elemental composition of a compound which is either unknown or the by-products or metabolites of a known compound. The second step is the structure elucidation by MS/MS or MS<sup>n</sup>.

The following study describes the improvements of the LTQ FT Ultra for unequivocal structure elucidation by means of a mixture of standard small molecule compounds (described in Table 1), in both positive and negative ion mode. The molecular weight of the compounds cover a wide range (MW 195 – MW 609) and include not only C, H, N and O atoms but also S and Cl atoms. Five compounds contain sulfur to test if high resolving power is able to separate the <sup>34</sup>S from the <sup>13</sup>C<sub>2</sub> isotopologue. Figure 2 shows the base peak chromatogram for the mixture.

## Key Words

- LTQ FT Ultra™
- High Mass Accuracy
- High Resolution

Substance	Elem. composition	[M+H] <sup>+</sup> calc.	[M-H] <sup>-</sup> calc.
Caffeine	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>	195.08765	193.07310
Sulfadiazine	C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S	251.05972	249.04517
5,5-Diphenylhydantoin	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	253.09715	251.08260
Sulfamerazine	C <sub>11</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub> S	265.07537	263.06082
Atropin	C <sub>17</sub> H <sub>23</sub> NO <sub>3</sub>	290.17507	288.16052
Diclofenac	C <sub>14</sub> H <sub>11</sub> NO <sub>2</sub> Cl <sub>2</sub>	296.02396	294.00941
Sulfadimethoxine	C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> O <sub>4</sub> S	311.08085	309.06630
Ranitidine	C <sub>13</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S	315.14854	313.13398
Piroxicam	C <sub>15</sub> H <sub>13</sub> N <sub>3</sub> O <sub>4</sub> S	332.06995	330.05540
Buspirone	C <sub>21</sub> H <sub>31</sub> N <sub>5</sub> O <sub>2</sub>	386.25505	384.24050
Reserpine	C <sub>33</sub> H <sub>40</sub> N <sub>2</sub> O <sub>9</sub>	609.28066	607.26610

Table 1: List of elemental composition and exact masses for the molecular ions for the substances used in this study in positive and negative ion mode.

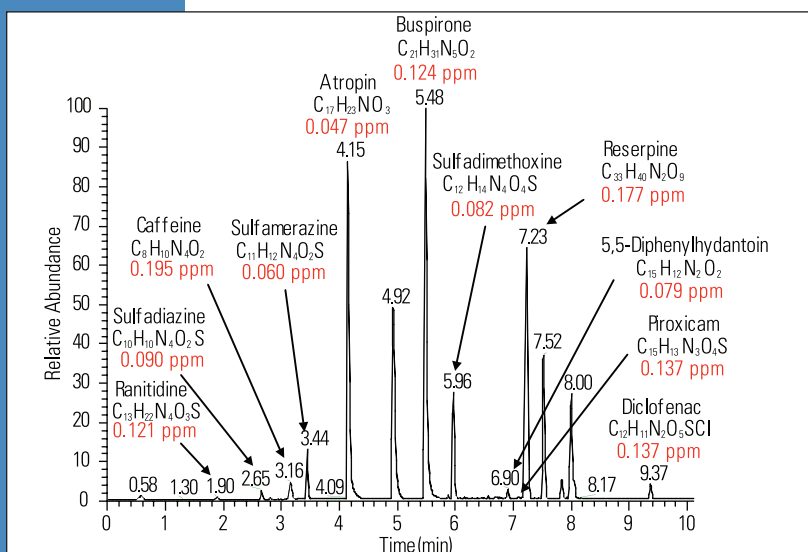


Figure 2: Base peak chromatogram of the small molecule mix (positive ion mode). The red label shows the RMS mass accuracy.

### Exact mass measurement at high resolution

The new grid cell allows higher excitation radii resulting in reduced space charge effect which leads to higher mass accuracy even at high target values. High target values are used to enhance the dynamic range for sensitive detection of by-products or metabolites.

Mass accuracy can even be pushed to routine mass accuracies in the ppb range by using Data Dependent<sup>™</sup> SIM scans<sup>[2,3]</sup>. This is a unique feature of the LTQ FT Ultra since it uses the linear ion trap for controlling the number of ions to be measured by automated gain control (AGC<sup>™</sup>). Mass accuracies heavily depend on the number of charges in the ICR cell and only the hybrid concept of the LTQ FT Ultra and LTQ Orbitrap<sup>™</sup> allows the full control over the number of ions that are collected for an FTMS mass measurement.

In this study, the instrument was programmed to acquire one full scan in FT mode with 25k resolution, followed by one SIM scan (100k resolution), one CID MS/MS and one IRMPD MS/MS scan (25k resolution) with FT detection in Data Dependent mode on the two most abundant peaks in the FT full scan. Runs were acquired in positive and in negative ion mode.

The aim of a SIM scan is to isolate one distinguished ion population at a lower target value to avoid any space charge effect. The resulting effect of the new grid cell with its higher excitation radii is three-fold:

On the one hand ultra high mass accuracies in the ppb range can be achieved with external mass calibration. On the other hand, high resolution allows distinguishing the different isotopologues of the elements in the compound of interest. Furthermore, the improved sensitivity allows the detection of the isotopologue clusters at the A+1, A+2, and even A+3 peaks.

To test the robustness in achieving highest mass accuracy of a longer period of time with external mass calibration the test sample was injected 20 times over a two day period.

The analysis of all SIM scans is summarized in Table 2. The root mean square (RMS) error of the SIM scans is 0.087 parts-per-million (ppm). Not all compounds ionize in positive as well as in negative ion mode.

Substance	[M+H] <sup>+</sup>	ppm RMS	[M-H] <sup>-</sup>	ppm
Caffeine	195.08761	0.195	-	-
Sulfadiazine	251.05974	0.090	-	-
5,5-Diphenylhydantoin	253.09718	0.079	-	-
Sulfamerazine	265.07539	0.060	263.06075	0.270
Atropin	290.17504	0.047	-	-
Diclofenac	296.02396	0.087	-	-
Sulfadimethoxine	311.08083	0.082	309.06632	0.064
Ranitidine	315.14856	0.121	313.13399	0.013
Piroxicam	332.07000	0.137	-	-
Buspirone	386.25503	0.124	-	-

Table 2: Measured SIM masses for the standard compounds in positive and negative ion mode and their mass errors. The RMS error of all SIM scans is 0.087 ppm.

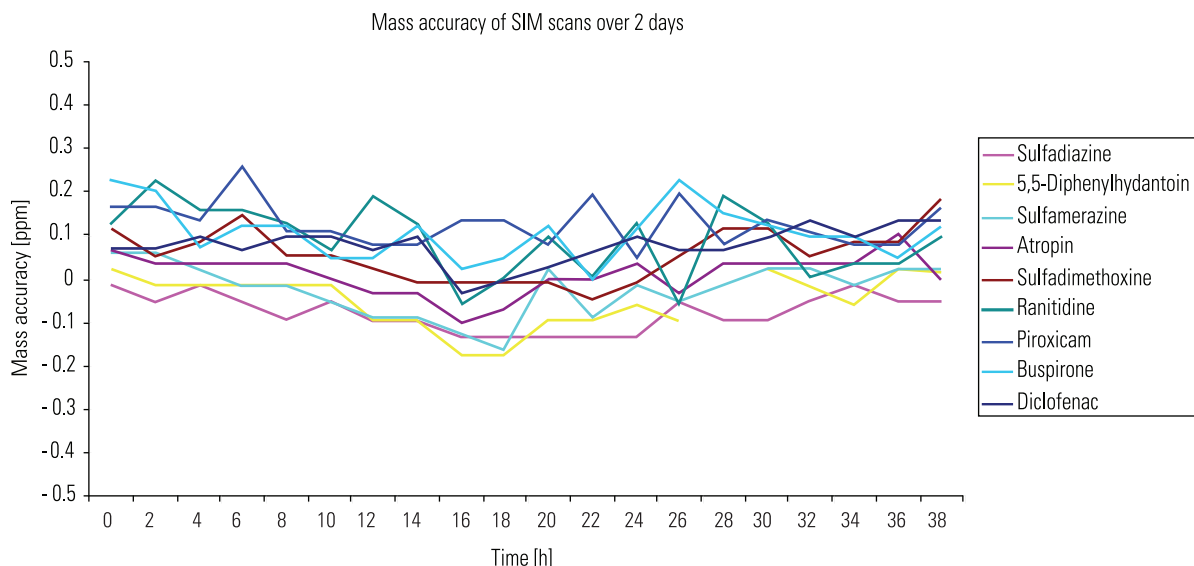


Figure 3: Plot of mass accuracies in SIM scan over two day period.

Figure 3 shows the plot of the mass accuracies achieved in the SIM scans over the period of two days. The mass accuracies of all compounds stay in this period at the ppb level. It is again noteworthy to mention that all measurements are on LC-timescale with external calibration over a period of two days.

### Exact determination of the relative isotope abundances

Another piece in the puzzle of the determination of elemental compositions are the relative isotopologue abundances of the compounds of interest, especially the relative abundance of the A+1 isotope/isotopologue cluster (nomenclature according to reference [4] where A is the monoisotopic peak and A+1 the isotope (isotopologue cluster, respectively) one mass unit heavier).

The isotope abundance of the first isotope is mainly influenced by the number of  $^{13}\text{C}$ -atoms which occurs with a natural abundance of 1.112%. Moreover, the high resolution of the LTQ FT Ultra even allows to separate the isotopologue clusters. This fine structure can deliver valuable information not only about the elements but also the relative abundances can be used to narrow down the number of the different elements in the compound of interest.

Figure 4 shows the example of the sulfonamide Sulfadimethoxine. The measured mass of the monoisotopic peak in the SIM scan matches the calculated mass by  $-0.076$  ppm. Furthermore, the high resolution does not only enable the separation of the  $^{34}\text{S}$  from the  $^{13}\text{C}_2$  isotopologue at the A+2 isotope clusters but also from the  $^{18}\text{O}$  isotopologue and the  $^{15}\text{N}$  isotopologue.

The characteristic isotope pattern proves the presence of sulfur in the elemental composition of the molecule and at the same time excludes the presence of chlorine or bromide atoms.

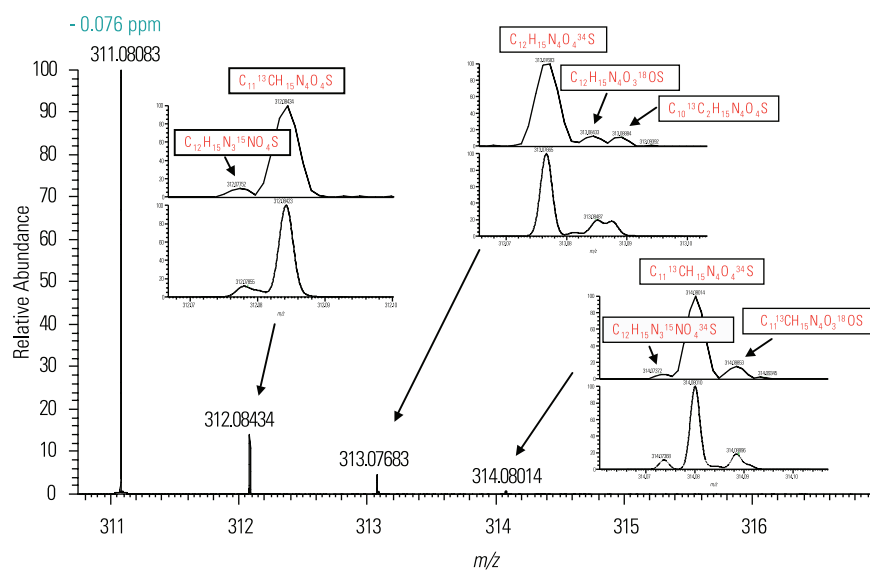


Figure 4: SIM scan of Sulfadimethoxine; the inserts show in the top trace the measured isotope pattern and in the bottom trace the simulated isotope pattern.

The elemental composition calculator in Xcalibur™ returns five possible elemental compositions within five ppm for the measured mass at  $m/z$  311.08083 in the SIM scan (with 30 C-atoms, 60 H-atoms, 15 O-atoms, 10 N-atoms, and 5 S-atoms) allowed and the presence of at least one sulfur atom and protonation is assumed (see Figure 5). In addition, even electron ions are assumed since electrospray ionization (ESI) was used for ion generation.

Simulation of the proposed elemental compositions under the measured spectrum also confirms the elemental composition of Sulfadimethoxine ( $C_{12}H_{14}N_4O_4S$ ), see Figure 4.

Furthermore, better ion statistics in the SIM scan allow exact measurement of the isotope ratios which can be used to determine the number of carbon atoms in the structure.

Table 3 compares the calculated relative isotope abundance of the A+1 isotope peaks with the measured relative abundances for the compounds used in this study. The average A+1 ratio and standard deviation was measured in 20 injections over the two day period. The relative isotope abundances can be measured with good precision and is a very good indicator for the number of C-atoms in the structure.

Substance	Number of carbon atoms	A+1 ratio calc.	A+1 ratio measured (average)	A+1 ratio Std-Dev.
Caffeine	8	8.90 %	10.15 %	0.47 %
Sulfadiazine	10	11.12 %	11.46 %	0.62 %
5,5-Diphenylhydantoin	15	16.68 %	16.87 %	0.67 %
Sulfamerazine	11	12.23 %	13.09 %	0.52 %
Atropin	17	18.90 %	19.39 %	0.68 %
Diclofenac	14	15.57 %	15.69 %	1.02 %
Sulfadimethoxine	12	13.34 %	13.45 %	0.65 %
Ranitidine	13	14.46 %	14.97 %	0.74 %
Piroxicam	15	16.68 %	18.00 %	1.06 %
Buspirone	21	23.35 %	23.58 %	0.54 %

Table 3: Relative isotope abundances of the A+1 isotopes.

The isotope ratios can be used to elucidate the elemental composition of unknown compounds. High mass accuracy alone might not be sufficient to determine the elemental composition of a compound, especially at higher mass due to the increase of permutations for complex elemental compositions<sup>[5]</sup>.

This can be exemplified by the elemental composition proposals for Reserpine. The exact mass for the molecular ions of Reserpine ( $C_{33}H_{40}N_2O_9$ ) is 609.28066 and it contains only C, H, N and O atoms. Figure 6 shows the proposed elemental compositions for the measured mass in the SIM scan. Sulfur, chlorine and bromine were not used because the intensity and the peak shape of the A+2 peak in the SIM scan did not show any evidence for their presence. Interestingly, the proposed elemental compositions ID 1 and 3 have the same number of O and N atoms if fluorine is allowed.

Therefore, it is not possible to draw any conclusion from the isotopologue pattern at high resolution. ID 1 contains 25 C atoms and the calculated intensity ratio for the A+1 peak would be 28.22 %. Figure 6 compares the isotope intensity of the A+1 peaks of the measured intensity in the SIM scan with the simulated intensities. Fluorine is a monoisotopic element and contributes only to the intensity of the monoisotopic peak. Many pharmaceutical active compounds contain fluorine and thus fluorine should be considered if the compound administered contains fluorine or when dealing with unknown compounds.

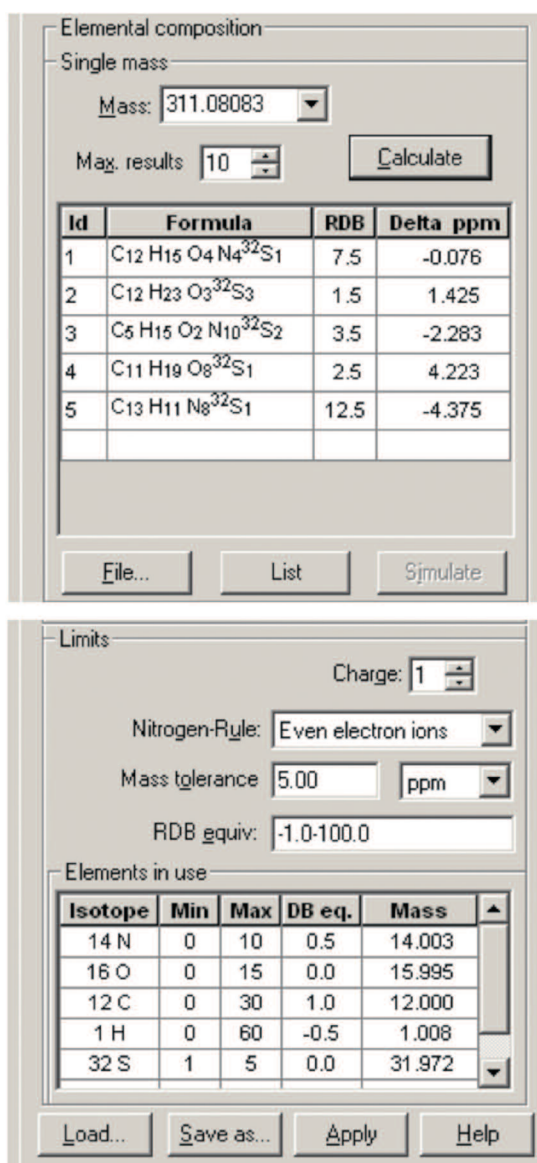


Figure 5: Proposed elemental compositions for Sulfadimethoxine.

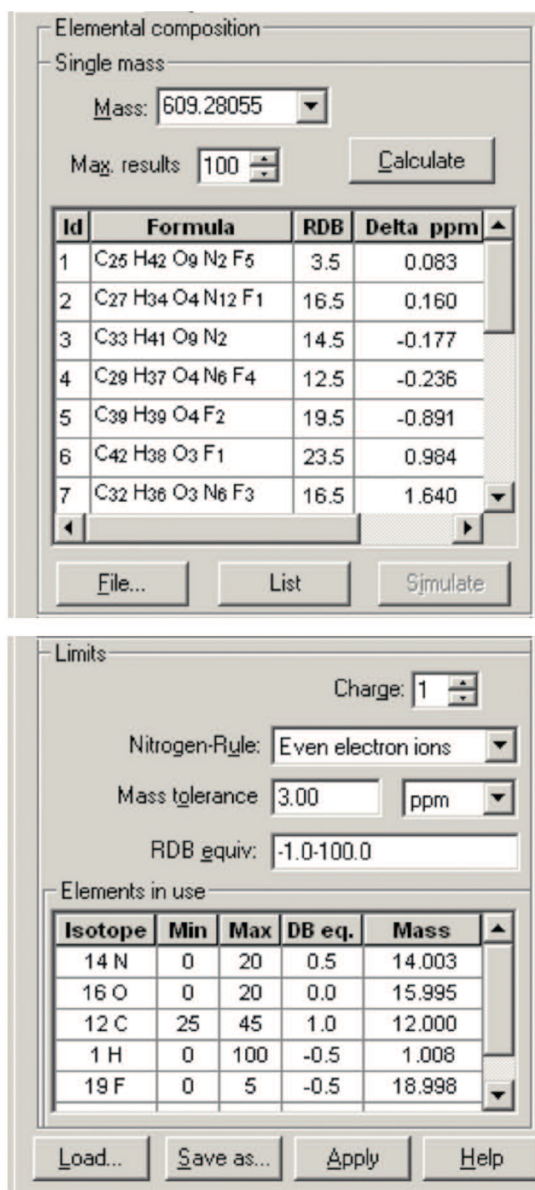


Figure 6: List of proposed elemental compositions for Reserpine.

Figure 7 top trace shows the measured isotope pattern of Reserpine. The middle trace shows the simulated isotope pattern for Reserpine and the calculated intensity ratio of the A+1 peak (37.10 %) matches the measured isotope ratio (37.05 %). The good precision of the isotope ratio measurement that can be achieved on the LTQ FT Ultra facilitates the rejection of the elemental proposal containing fluorine.

### Nitrogen rule and Ring and Double Bond equivalent

Other analytical tools to determine the correct elemental composition are nitrogen rule and the Ring and Double Bond equivalent (RDB).

The nitrogen rule says that an organic molecule containing the elements C, H, O, S, P, or halogen has an odd nominal mass if it contains an odd number of nitrogen atoms.

The nitrogen rule states that if the molecular ion is odd then the compound will have an odd number of nitrogen atoms. Since electrospray produces protonated species and not molecular ions this rule can be adapted and restated as, "If the protonated pseudo molecular ion ( $[M+H]^+$ ) gives an odd mass then the number of nitrogen atom is even."

McLafferty states the Nitrogen Rule as follows: "If a compound contains no (or an even number of) nitrogen atoms, its molecular ion will be at an even mass number... [Similarly,] an odd-electron ion will be at an even mass number if it contains an even number of nitrogen atoms."

If for example the class of the compound of interest is known then also the RDB is a valuable analytical tool to rule out elemental compositions with unrealistic RDB.

RDB is a measure of the number of unsaturated bonds in a compound and limits the calculated formulas to only those that make sense chemically.

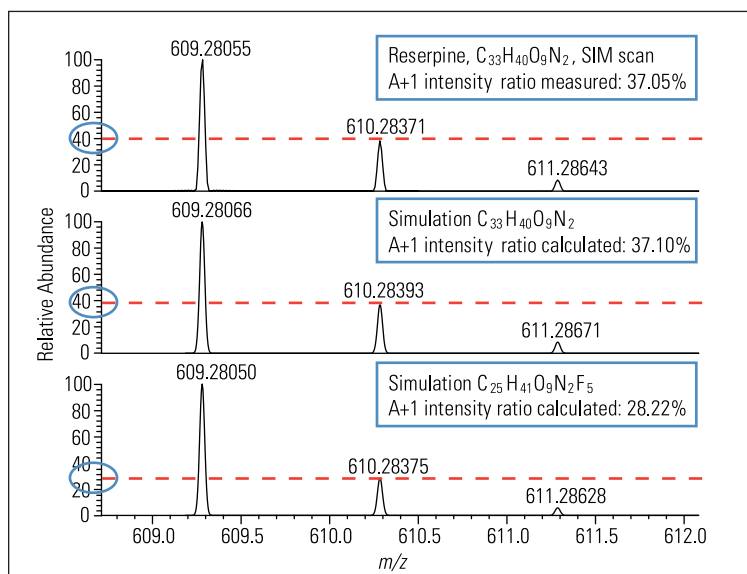


Figure 7: Measured and calculated isotope patterns.

## Summary

This study shows that the Thermo Scientific LTQ FT Ultra is an extremely efficient tool for highly exact mass measurements on a routine basis. Moreover, ppb mass accuracy is achieved on LC conditions and with external calibration over an extended period of time. Ultra high mass accuracy results in high confident assignments of elemental compositions.

The RMS error of all SIM scans was 0.087 ppm. In addition, the high precision of the isotope ratio measurements in the SIM scans is used to confirm or to reject elemental composition proposals.

The basis of ultra-high mass accuracy is the automated gain control in the linear trap which allows exact control over the ion population. The new grid ICR cell allows the accumulation of even higher ion populations to gain dynamic range without sacrificing mass accuracy. A high dynamic range is a prerequisite for the detection of by-products and metabolites at very low levels.

The higher excitation radii and the new pre-amplifier allow more sensitive detection of even very low intense ions without increasing the noise band.

The possibility to generate low as well as high-energy fragment ions by CID and IRMPD at LC-timescale makes the LTQ FT Ultra a versatile instrument for high confident compound characterization.

## Experimental

All spectra were acquired on a Thermo Scientific LTQ FT Ultra, a 7 Tesla hybrid linear ion trap-FTICR mass spectrometer, equipped with an indirectly heated dispenser cathode (Heatwave, Watsonville, CA, USA) and a 20 Watt continuous CO<sub>2</sub> infra-red laser (Synrad, Mukilteo, WA, USA).

HPLC separation was achieved with a Thermo Scientific Surveyor™ MS plus pump and MicroAS autosampler using a Hypersil GOLD™ 2.1 x 50 mm C18 reversed phase column with 1.9 μm particles. The method used for all experiments starts with an FT full scan (25k resolution, 1e6 target value), followed by one data-dependent SIM scan (100k resolution, 5e4 target value), one CID MS/MS in the linear trap with FT detection (25k resolution, 2e5 target) and one IRMPD scan (25k resolution 2e5 target) on the two most abundant ions in the FT full scan. The discussion of MS/MS results is not covered in this paper.

All reference compounds were purchased from Sigma-Aldrich (Taufkirchen, Germany).

The instrument was operated with LTQ FT Ultra Tune Version 2.2. Data analysis was done with Xcalibur 2.0 and Mass Frontier™ 5.0.

## Abbreviations

AGC	automated gain control
ESI	electrospray ionization
FWHM	full width at half maximum
CID	collision induced dissociation
FT	Fourier-transform
HPLC	high performance liquid chromatography
ICR	ion cyclotron resonance
IRMPD	infra-red multiphoton dissociation
LC	liquid chromatography
MS	mass spectrometry
MS/MS	tandem MS
<i>m/z</i>	mass-to-charge ratio
ppb	parts-per-billion
ppm	parts-per-million
RDB	ring double bond equivalents
RMS	root mean square
SIM	selected ion monitoring
Std-Dev.	Standard deviation

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