Agilent 6540 UHD Q-TOF produces more accurate isotope ratios than Thermo LTQ Orbitrap XL

Technical Overview

Abstract

Accurate-mass measurements are useful for compound confirmation and molecular formula generation for unknown compounds, but are not sufficient by themselves. Isotope ratios provide essential orthogonal information that can greatly reduce the number of plausible molecular formulas and increase confidence in the result. The Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF LC/MS system produces isotope ratios with far greater accuracy than the Thermo Scientific LTQ Orbitrap XL. Agilent uses this isotopic information, plus exceptionally accurate monoisotopic mass, to discriminate against incorrect answers and increase confidence in correct identification and quantification of unknown compounds.

Introduction

Mass spectrometry is an essential tool for confirmation of known compounds and identification of unknowns. The end result of a typical metabolomic or non-targeted food screening analysis is a large list of spectral features which need to be identified. Without accurate information and a proper set of tools, this can be an extremely time-consuming process. Accurate relative isotope abundances are also critical for identifying and quantifying isotopically-labeled (e.g., SILAC) proteins and peptides.

A bioinformatics study showed why the accurate isotopic profile of a compound (isotopic fidelity) is critical for high-confidence molecular formula generation.[1] While researchers frequently use accurate-mass spectra to generate candidate molecular formulas, the number of candidates is often overwhelming, especially at higher masses. Fortunately, scientists can use the relative abundances of isotopes to remove more than 95% of the incorrect formulas. The conclusion of the bioinformatics study was that for molecular formula generation, it is better to use an instrument with 3 ppm mass accuracy and low 2% isotope ratio errors, than a (hypothetical) mass spectrometer with 0.1 ppm mass accuracy and no isotope ratio information.

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Results and discussion

Thermo LTQ Orbitrap XL shows poor isotope ratios in benchmarking study

A recent Agilent benchmarking study revealed serious errors in isotope ratios derived from Thermo Scientific LTQ Orbitrap XL spectra. An identical mixture of 10 compounds (comprising the Agilent calibration mix) was infused into both an Agilent UHD 6540 Accurate-Mass Q-TOF and the Orbitrap systems. Resolution modes were varied on the Orbitrap and data acquisition rates were varied on both the Q-TOF and Orbitrap. **Figure 1** shows that slower scans at higher resolution (1 spectrum/second; R=100,000) yielded very poor isotope ratios, with some ratios being less than 20% of the theoretical value. Likewise, faster scans at lower resolution (5 spectra/second: R=7500) showed unacceptably low results for the low-abundance A+3 isotope.

These results are consistent with those of another study that evaluated use of high-resolution isotope patterns for compound identification, and found that the intensity accuracy of an orthogonal acceleration-TOF instrument was much better than that of a Fourier transform-ion cyclotron resonance (FT-ICR) MS.[2]

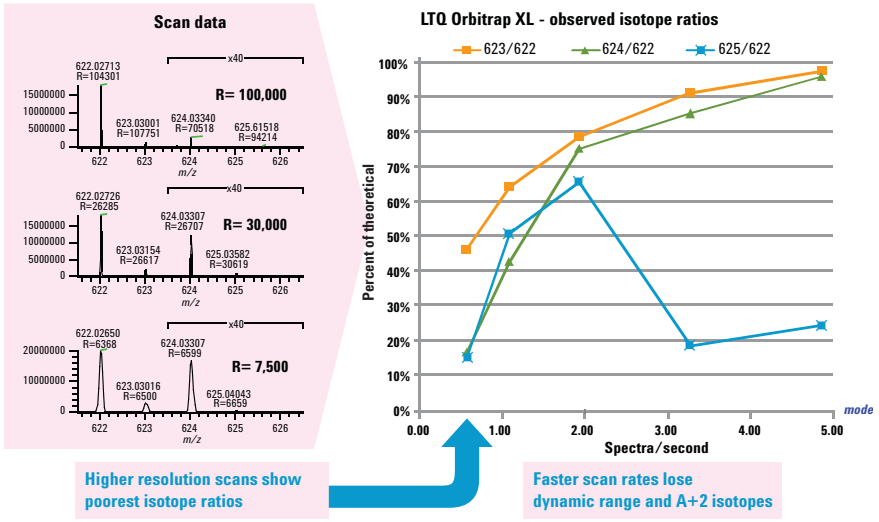

Figure 1. The Thermo Scientific LTQ Orbitrap XL exhibits large errors in relative isotope abundances.

ASMS paper notes errors with isotope ratios on the Thermo LTQ Orbitrap Discovery

A 2009 study described errors in relative isotope abundance (RIA) with the Thermo LTQ Orbitrap Discovery.[3] Researchers analyzed pure standards using flow injection analysis (FIA) in positive and negative electrospray modes. They found that about one-fourth of the Orbitrap data points had RIA errors in excess of 10%, and about one-tenth showed errors in excess of 20%. When they analyzed rat urine samples in LC/MS negative electrospray mode, more than one-half of the Orbitrap data points exhibited RIA error greater than 10%, and about one-fourth had RIA error in excess of 20%.

The bioinformatics paper mentioned earlier showed that RIA error must be less than 5% for effective molecular formula generation for unknowns.[1] The isotope ratios on the Thermo LTQ Orbitrap XL fall significantly short of the required quality to be useful for this purpose.

Agilent 6540 UHD Q-TOF demonstrates very accurate isotope ratios

A recent study with the Agilent 6540 UHD Q-TOF showed a consistently low RIA error of about 5%. The RIAs for 623/622 at 10 different acquisition rates were all within 5% of the expected value (RSD = 2.8%). **Figure 2** shows the study results.

Agilent molecular formula generation takes advantage of isotopic fidelity

As described in another technical note[4], the Agilent MassHunter Workstation software takes full advantage of accurate isotope ratios when it generates molecular formulas for spectra from the Agilent TOF and Q-TOF instruments. While the Xcalibur 2.0.5 software on the Thermo LTQ Orbitrap XL uses only the monoisotopic mass, the Agilent Molecular Formula Generator (MFG) for the Agilent 6540 UHD Q-TOF considers isotope pattern, including the accurate mass of the parent ion, isotope abundance distribution, and mass spacing between isotope peaks.

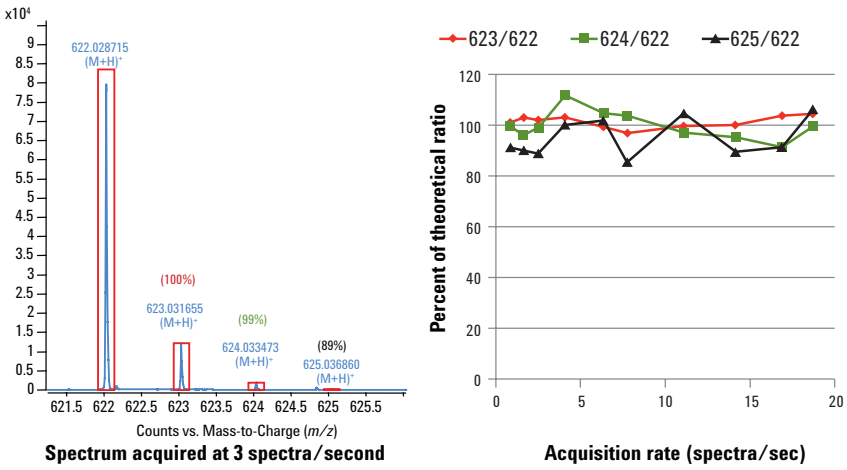

Figure 2. The 6540 UHD Accurate-Mass Q-TOF generates accurate and invariant isotope ratios, regardless of acquisition rate.

The Agilent MFG algorithm uses a unique scoring method that combines all this information into a final score for each candidate formula, as shown in **Figure 3**. In this example, isotope information is indispensible for accurate molecular formula generation. By mass error alone, the correct formula is fifth on the list, but with the MFG scoring technique that includes all the isotope information, it is the top choice with a score of 100.

m/z	Ion	Formula	Abundance
516.30019	(M+H)+	C26H46NO7S	30331

Best	Formula (M)	Ion Formula	Score	Cross Score	Mass	Calc Mass	Difference (ppm)	Abs Diff (ppm	DBE
☐	C19H37N11O6	C19H38N11O6	61.74		515.29291	515.29283	-0.16	0.16	7
☐	C27H41N5O3S	C27H42N5O3S	95.13		515.29291	515.29301	0.19	0.19	10
☐	C27H49NO2S3	C27H50NO2S3	67.12		515.29291	515.29254	-0.72	0.72	4
☐	C18H34N15O2N	C18H35N15O2N	59.1		515.29291	515.29176	-2.23	2.23	9
correct → ☑	C26H45NO7S	C26H46NO7S	100		515.29291	515.29167	-2.4	2.4	5
☐	C20H33N15O2	C20H34N15O2	54.73		515.29291	515.29417	2.43	2.43	12
☐	C30H42N3ONaS	C30H43N3ONaS	64.62		515.29291	515.29463	3.33	3.33	11
☐	C25H42N5O3Na	C25H43N5O3Na	91.06		515.29291	515.29061	-4.47	4.47	7
☐	C23H37N11OS	C23H38N11OS	91.22		515.29291	515.29033	-5.02	5.02	11
☐	C25H50NO2NaS	C25H51NO2NaS	55		515.29291	515.29014	-5.38	5.38	1
☐	C23H45N7S3	C23H46N7S3	54		515.29291	515.28986	-5.93	5.93	5
☐	C24H45N5O3S2	C24H46N5O3S2	68.69		515.29291	515.29638	6.73	6.73	5
☐	C24H46NO7NaS	C24H47NO7NaS	69.99		515.29291	515.28927	-7.07	7.07	2
☐	C22H41N7O5S	C22H42N7O5S	69.56		515.29291	515.28899	-7.61	7.61	6

Figure 3. From data achieved on an earlier-generation Q-TOF, the Agilent MFG algorithm correctly gives the correct formula the top score, even though it is fifth on the list when only the monoisotopic mass is considered. This shows the value of using the isotope information.

Conclusions

Mass accuracy is extremely important, but not sufficient, for confident compound confirmation and identification of unknowns. Accurate isotopic abundances and ratios for precursor and fragment ions are also critical. Isotope ratios can eliminate a large percentage of possible compounds, but only if the MS system produces accurate isotope ratios. This study shows that isotope ratios derived from Thermo Scientific LTQ Orbitrap XL spectra vary wildly depending on the resolution mode in which the data were acquired, with the very worst data appearing at the highest resolution (ratios were <20% of theoretical at R=100,000 and about 50% at in 60,000 Resolution mode). Results of such low quality will be of little use in the structural interpretation of Orbitrap data. The relative isotope abundances are much more accurate on the Agilent 6540 UHD Q-TOF than on the Thermo LTQ Orbitrap XL. The combination of isotopic fidelity and unmatched software algorithms makes the Agilent system a better choice for molecular formula generation and unknown compound identification.

References

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