Investigation of Isotope Patterns of Pharmaceutical Molecules by Two Independent Detectors in a LTQ/Orbitrap Instrument

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Overview

➢Spectral Accuracy is a measure of the similarity between the measured isotope pattern (mass spectrum) and the theoretical mass spectrum and plays an essential role on formula identification of unknown compounds.

➢Mass accuracy and spectral accuracy of ions measured by LTQ at different scanning modes and different AutoGain Control (AGC) target values were evaluated. The spectral accuracy of the same ions detected by LTQ and Orbitrap was compared.

➢Optimal data acquisition conditions for accurate mass accuracy and spectral accuracy are suggested and remaining challenges are discussed.

Introduction

The high mass accuracy and resolution powered by LTQ/Orbitrap instrument have proved to be effective to determine elemental composition of unknowns from complex mixtures. This mass accuracy based formula ID often relies on sufficient prior knowledge of the molecules for confident identification. When dealing with totally unknowns, however, the formula search with mass accuracy at even 2 ppm often results in too many possible molecular formulae to be useful. Current trend to resolve this problem is to use isotope patterns to further improve selectivity, which requires normal isotope distribution patterns be generated. This work will comprehensively investigate how spectral accuracy (isotope patterns) obtained inside linear ion trap and Orbitrap is affected by mass resolution, Autogain control, and signal intensities. The spectral accuracy (SA) is defined as

(1-RMSE)*100 = Spectral Accuracy(%)

RMSE = Root Mean Square Errors

Methods

LC/MS and infused mass spectra data of commercially available compounds including Probenecid, Ketoconazole, Clozapine, Quinidine, Suprofen, Tolmetin, and Erythromycin at the concentration levels of 5 mM and 20 mM were acquired in profile mode in a LTQ/Orbitrap (Thermo Fisher) instrument. Measured by LTQ detector, LTQ data were collected in normal full MS, zoom, and Ultra zoom scanning modes. Autogain control (AGC) target values of 50000 were used for full mass scans, while various AGC values at 30, 300, 3000, and 30000 were evaluated for zoom scans.
The Orbitrap data acquisition was performed with resolving power set to 7K.

For each compound, full MS spectra were recorded by both LTQ and Orbitrap detectors for the purpose of comparing their isotope patterns. Data analysis for the isotope distribution of the spectra was conducted with post-acquisition software MassWorks (Cerno Bioscience)..

Results and Discussion

Less than perfect isotope patterns often are observed in LTQ or Orbitrap spectra. With unique configuration of LTQ/Orbitrap instrument having two independent mass analyzers and detectors and quantitative evaluation of isotope profile by spectral accuracy, we can investigate whether the distortion happens inside Orbitrap or linear ion trap and what will be the best instrument conditions to produce normal isotope distribution.

For the majority of ions examined (Fig1&2), Orbitrap spectra have higher SA than LTQ full MS spectra at both concentration levels of 5 mM and 20 uM. At 20 mM, the ion counts of the compounds obviously increased and their SA decreased accordingly due probably to space charge effect. However, the SA of corresponding Orbitrap spectra remains almost the same. This suggests the Orbitrap can tolerate more space charge effect than LTQ. The exception here is the compound Probenecid negatively ionized having higher SA in LTQ spectra than Orbitrap spectra. This unusual observation is not well understood and warrants further investigation.

Results and Discussion

Fig 1. LTQ and Orbitrap Spectral Accuracy
Conc. = 5 µM, AGC= 50000

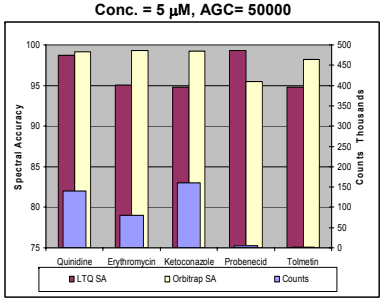


Fig2. LTQ and Orbitrap Spectral Accuracy
Conc. = 20 µM, AGC= 50000

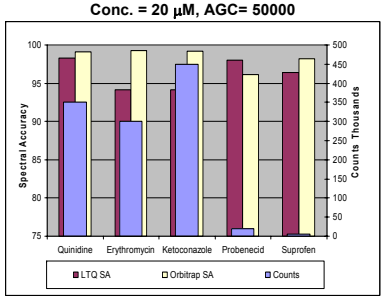
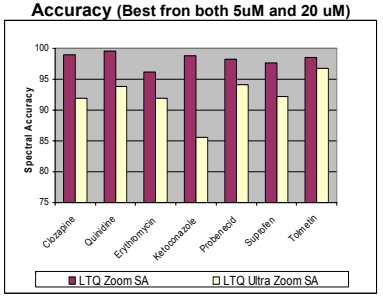


Fig 3. LTQ Zoom and Ultra Zoom Spectral Accuracy (Best fron both 5uM and 20 uM)



SA of Zoom scan and Ultra Zoom scan spectra also were evaluated (Fig 3&4). ALL zoom scan spectra have better SA than Ultra zoom scan spectra probably due to higher S/N of zoom scan spectra than Ultra zoom scan spectra. The best AGC target values for zoom scan were found to be 3000.

Experimentally measured spectra with high SA above 98% has excellent match against theoretical spectra and helps identify unknown compounds. This is demonstrated by LC/MS of zoom scan for microsome incubation of Quinidine (Fig. 5). The entire LC/MS data file was calibrated by parent drug molecules of Quinidine at m/z 325. At least four oxygenated metabolites were found at m/z 341. Mass accuracy for all the metabolites was found to be within 10 mD and their spectral accuracy varies

Fig 4. Zoom Scan SA vs. AGC

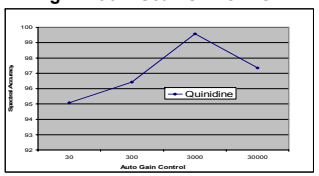


Fig 5. Formula ID for a Metabolite by Mass Accuracy and Spectral Accuracy

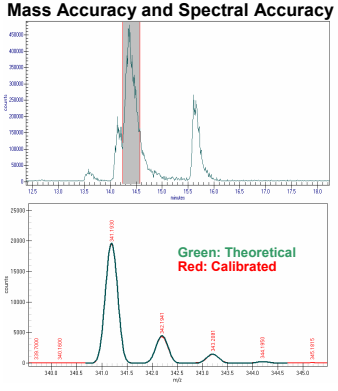


Table 1. First Five Match of CLIPS Search for the Metabolite at m/z 341

Formula	Mono Isotope	Mass Error (mDa)	Spectral Accuracy
C20H25N2O3	341.1865	-6.4823	98.9381
C19H33OS2	341.1973	4.2821	98.4971
C17H29N2O3S	341.1899	-3.1115	98.3293
C18H29O6	341.1964	3.4137	98.1612
C16H37OS3	341.2007	7.6529	97.5739

from 96.5% to 98.2%. Poor SA for some metabolites were likely due to low S/N and possible background interference. All the metabolites were identified as top 1 or top 2 hit with mass tolerance of 10 mD and elements of $C_{0\text{-}40}$ $H_{0\text{-}100}$ $N_{0\text{-}2}$ $O_{0\text{-}20}$, and $S_{0\text{-}5}$.

Conclusions

- Due to space charge effect, isotope pattern distortion of positive ions begins to appear in the LTQ spectra at 20 µM. The same ions detected by Orbitrap show almost perfect isotope pattern indicating Orbitrap has high tolerance of space charge effect.
- With 10 mDa mass accuracy and normal isotope pattern, it is possible to identify unknowns with zoom scan spectra by CLIPS.
- Among full MS, zoom, and ultra zoom scan mode, the zoom scan with AGC target values of 3000 produced the spectra with the best spectral accuracy.
- Future work will focus on investigation of isotope pattern of negative charged ions detected by both LTQ and Orbitrap.