Investigation of isotope ratios using an LTQ-Orbitrap XL

Christopher Williams1; Ruth Godfrey2; Gareth Brenton1;2

1 EPSRC NMSSC, Swansea University, Swansea, UNITED KINGDOM; 2 Institute of Mass Spectrometry, Swansea University, Swansea, UNITED KINGDOM

email: christopher.matthew.williams@swansea.ac.uk

Overview
- This study was instigated to determine the accuracy of the isotope profiles observed on a LTQ Orbitrap XL, compared with theoretically generated isotope profiles.
- A variety of samples differing elemental complexities were analysed at two resolutions in both profile and centroid acquisition modes.
- The results suggest that the intensity of the isotopes and the resolution used to acquire the data are important factors.

Introduction
- The Orbitrap mass analyser has become popular for routine high throughput analysis.
- It is capable of high mass accuracy for generating elemental formulae information for analyte identification. Investigations of isotope ratios have been reported10 and used to limit the number of possible elemental formulae for an ion.
- Previous work was limited to data acquired in selected-ion-monitoring (SIM) mode as a profile signal only for both positive and negative ions over a range of mass resolution settings10.
- We have carried out a comprehensive review of profile and centroid data acquired using normal scan (Full ms) and SIM (SIM-ms) modes, at resolutions (R) of 100,000 and 7,500, for positive and negative ions.
- Singly charged ions of m/z 190-1450 were examined.

Methods
- Selected samples submitted to the EPSRC NMSSC were dissolved in an appropriate solvent and diluted following the instrument analysis method available online25.
- LTQ-Orbitrap calibration mix (CalMix) were prepared as per the manufacturer’s instructions10 for both positive and negative modes.
- Samples were infused using an Advion TriVersa Nanomate (Advion Biosciences Inc., USA) into a LTQ Orbitrap XL (Thermo Fisher Scientific Inc., USA), using the experimental outline in Figure 1 for the acquisition.

Results
- For each sample, the theoretical isotopic pattern was generated in both profile and centroid modes at resolutions equal to the measured resolution of the most intense (base peak or 100%) ion in the observed isotope profile.
- The raw data was averaged over the acquisition range and the data was analysed manually by matching the observed ions with the theoretical isotopic pattern.
- A spread sheet was generated to hold all the observed ions over the range of interest.
- Ions of m/z greater than 20 ppm and less than 1.5% relative isotope abundance (within the compounds isotopic pattern) from the theoretical isotope value were discarded.
- Table 1 shows the forms of the observed ions, m/z (of most intense ion, or base peak), base peak intensity (arbitrary units, a.u.), mean ppm error (of all ions in isotopic series) and standard deviation (n-1) of ppm error for the compounds studied.
- Previous work10 used compounds comprising of CHNO, CHNOS and CHNOPS; the most complex atom is S with 4 isotopes.
- Samples were chosen with a variety of elements to provide more complex isotopic patterns (Sample C has 30 theoretical isotopes at R=100,000 and 16 theoretical isotopes at R=7,500). CalMix was chosen to provide intra-analysis isotopic information for both positive and negative ionisation modes.
- For each isotone series the relationships, Isotope Profile Accuracy (IPA) and Isotope Profile Error (IPE), were defined as

\[
\text{Isotope Profile Accuracy} = \left(\frac{100}{\text{number of ions in isotopic series}} \right) \times \text{theoretical formula} \times 100
\]

\[
\text{Isotope Profile Error} = \sqrt{\left(\frac{100}{\text{number of ions in isotopic series}} \right) \times \text{theoretical formula} \times 100} \times \text{ppm error}
\]

\[
\text{Isotope Score} = \sum_{x} \frac{x_{n}}{n}
\]

Where x_n = observed ion intensity, x_n = theoretical ion intensity, x_n = total intensities of observed ions, x_n = total intensities of theoretical ions and x_n = intensity of the principal ion in the theoretical isotopic pattern.

- Figures 2-5 show the base peak intensity plotted against an Isotope Score, based upon the relationship between the ions of the same m/z of the observed data with the corresponding ions in the theoretical isotopic pattern, calculated by

Isotope Score

Conclusions
- Generally, there is a trend that the IPA is closer to 100% with lower m/z in all acquisition types.
- More complex elements scored lower, especially at R=7,500.
- Higher resolution allows for more ions to be separated, improving the IPA for complex elements.
- Profile mode data shows better results than Centroid mode data.
- The Isotope Score shows that the variation between the observed isotope intensity and the theoretical isotopic intensity is less using SIM scanning.
- Whilst the IPA and IPE give a measure of the isotope profile as a whole, the Isotope Score indicates the variation between the observed and the theoretical isotopes.
- The isotope profile accuracy is best when the base peak intensity is in the 10x range, which agrees with previous reports regarding the number of ions stored in the FT11.
- Background, “instrument noise” and FT effects may have a contributing factor to the isotopes, especially for lower intensity ions.

References
2. EPSRC NMSSC, http://www.nmssc.ac.uk/ftp.html

Acknowledgments
The authors would like to thank EPSRC for funding this work (grant no. EP/F014341/1).