

# INVESTIGATING ISOTOPE RATIOS USING AN LTQ-ORBITRAP XL

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## Introduction

Mass spectrometry has traditionally been used to calculate isotopic ratios by measuring their relative abundance to the molecule ion species revealing the number of atoms of a particular element within the elemental formula. With the rise in popularity of biomarker studies and requirements of drug-metabolite screening in the pharmaceutical industry, the identification of analytes within clinical samples has become ever more important. LC/MS using an LTQ-Orbitrap has become popular for routine high throughput analysis of clinical samples with high mass accuracy generating elemental formulae information for analyte identification. Investigations of isotope ratios in Orbitrap data have been reported and used to limit the number of possible elemental formulae for an ion. Previous work<sup>[1]</sup> has been 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 settings. To fully investigate the isotopic accuracy of the LTQ-Orbitrap XL we have carried out a comprehensive review of profile and centroid data acquired using full scan and SIM modes, at high and low resolution settings for positive and negative ions of varying isotopic complexities and masses from m/z 120-1500.

## Experimental

- Selected samples submitted to the EPSRC NMSSC were dissolved in an appropriate solvent and diluted following the instrument analysis method available online<sup>[2]</sup>.
- LTQ-Orbitrap calibration mixes (CalMix) were prepared as per the manufacturer's instructions<sup>[3]</sup> 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).
- Acquisitions were performed in both a "Full-ms" analysis over a mass range m/z 120 - 2000 Da and a "SIM-ms" analysis over the mass range base-peak  $\pm 13$  Da using the run method shown in figure 1.
- Each sample was repeated in triplicate.

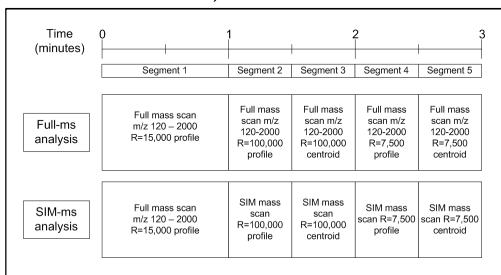


Figure 1: Experimental outline of acquisition parameters.

## Results

- For each sample, the theoretical isotope pattern was generated in both profile and centroid modes at resolutions (R) of 100,000 and 7,500 FWHM corresponding to the acquisition parameters.
- The raw data was averaged over the acquisition range and the data was analysed manually by matching the observed ions with the theoretical isotope 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% relative isotope abundance (within the compounds isotope pattern) from the theoretical isotope value were discarded.

Table 1 shows the formulae of the observed ions, m/z (of most intense ion), ppm error (of all ions in isotope series) and standard deviation (n-1) of ppm error for the compounds studied.

Method	Sample	Observed Ion	m/z	Profile				Centroid								
				Data Points	Mean ppm	SD(n-1) ppm	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm					
Full-MS Method	A	C23H19BN03	436.0543	5.0	-1.25	0.60	5.0	0.66	0.93	5.0	-1.22	0.66	5.0	0.75	0.88	
	B	C40H28N6O2	801.1948	5.3	-1.19	0.74	5.7	-1.19	0.90	5.7	-1.19	0.77	5.7	0.64	1.04	
	C	C15H15SO5e	323.0003	11.7	0.69	1.35	9.0	1.25	1.17	12.0	0.29	2.02	9.0	3.01	1.08	
	D	C8H12BN2O25	211.0707	5.0	-0.41	0.27	4.7	-1.16	5.46	4.3	-0.28	2.28	4.0	-0.57	5.62	
	E	C8H11N4O2	195.0877	3.0	-0.96	0.40	2.0	4.42	4.67	3.0	-1.53	0.63	2.0	5.44	4.67	
	F	C23H38N7O5S	524.2856	5.0	-1.23	0.40	4.0	-1.14	1.16	5.0	-1.21	0.47	4.0	0.21	1.25	
	G	C28H39O6N3F32	1121.9970	4.7	-1.64	0.49	3.0	-0.32	1.67	3.0	-0.45	0.72	3.0	2.02	6.31	
	H	C28H39O6N3F34	1421.9779	3.0	-0.95	0.49	3.0	-0.16	0.69	3.0	-1.45	0.63	3.0	4.37	9.86	
SIM-MS Method	A	C23H19BN03	436.0543	5.0	-1.22	0.58	5.0	-1.32	1.04	5.0	-1.46	0.62	5.0	-1.34	1.01	
	B	C40H28N6O2	801.1948	5.0	-0.71	0.52	6.0	1.85	4.44	5.0	-0.70	0.62	6.0	1.91	4.45	
	C	C15H15SO5e	323.0003	11.0	0.13	1.83	9.0	-0.75	1.80	11.3	-0.16	2.11	9.0	-0.22	1.62	
	D	C8H12BN2O25	211.0707	5.0	-0.66	1.92	4.7	-2.50	5.84	4.3	-0.88	2.01	4.0	-2.50	5.84	
	E	C8H11N4O2	195.0877	3.0	-2.04	0.67	2.0	2.80	4.39	3.0	-2.27	0.63	2.0	2.46	4.38	
	F	C23H38N7O5S	524.2856	4.7	-1.64	0.49	4.0	-3.82	2.29	4.3	-2.27	0.32	4.0	-3.84	2.41	
	G	C28H39O6N3F32	1121.9970	3.0	-0.84	0.36	3.0	-2.69	2.40	3.3	-1.58	0.72	3.0	-2.88	2.41	
	H	C28H39O6N3F34	1421.9779	3.0	-1.08	0.75	2.0	-2.70	1.44	3.0	-1.70	0.57	3.0	-2.65	1.17	
Full-MS Method	Negative Mode				Profile				Centroid							
	Sample	Observed Ion	m/z	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm	
	J	C12H25O4S	265.1479	4.0	-1.78	0.78	3.0	-6.22	10.10	4.0	-2.00	0.66	3.0	-6.01	10.34	
	K	C22H40N7S	514.2844	4.0	-1.30	0.43	3.0	-1.30	0.34	4.0	-1.30	0.33	3.0	1.38	0.34	
	L	C28H21O8N3F32	1080.0100	6.3	-3.49	0.70	3.0	1.61	1.61	3.0	-3.73	0.55	3.0	2.39	3.20	
	M	C28H21O8N3F36	1479.9844	3.0	-2.99	0.53	3.0	-1.04	0.40	3.0	-3.34	0.41	3.0	-0.28	0.72	
	N	C57H97O2Br	191.9666	4.0	-0.90	1.54	4.0	2.20	1.83	4.0	-1.37	1.39	4.0	2.59	2.04	
	O	C57H97O2BrS	456.0384	3.7	-0.86	1.11	3.0	1.75	1.94	3.7	-1.31	0.95	3.3	1.40	2.55	
	P	C17H26N2O16P2	432.1592	6.3	-1.05	5.62	6.0	-1.96	2.21	6.7	-1.86	5.61	6.0	-2.40	2.56	
	Q	C17H26N2O16P2	702.8808	3.0	-6.12	0.66	3.0	-2.78	0.66	3.0	-6.19	1.07	3.0	-2.94	0.38	
	SIM-MS Method	Negative Mode				Profile				Centroid						
		Sample	Observed Ion	m/z	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm	Data Points	Mean ppm	SD(n-1) ppm
		I	C12H25O4S	265.1479	3.0	1.00	0.80	3.0	-5.18	10.15	3.0	0.80	0.81	3.0	4.89	10.09
		J	C22H40N7S	514.2844	4.0	-0.12	1.38	4.0	0.01	2.10	4.0	-0.14	1.36	4.0	0.95	2.03
		K	C28H21O8N3F32	1080.0100	3.0	-2.07	0.58	3.0	0.32	1.58	3.0	-2.26	0.52	3.0	-0.80	1.56
		L	C28H21O8N3F36	1479.9844	3.0	-1.28	0.69	3.0	0.81	0.66	3.3	-1.87	0.80	3.0	0.95	0.57
M		C57H97O2Br	191.9666	4.0	-0.94	1.39	4.0	0.48	2.13	4.0	-1.07	1.38	4.0	0.39	2.13	
N		C57H97O2BrS	456.0384	4.0	-0.11	1.09	3.0	-0.09	1.29	3.7	0.04	0.98	3.3	-1.14	4.85	
O	C17H26N2O16P2	432.1592	7.0	2.82	4.83	6.0	1.43	1.29	7.0	0.80	1.97	6.0	1.57	1.36		
P	C17H26N2O16P2	702.8808	3.0	-1.55	0.57	3.0	-1.56	1.32	3.0	-0.47	0.59	3.0	-1.61	1.35		

Table 1: List of formulae of ions observed, m/z, average number of data points (ions) per pattern, mean ppm error of ions measured in isotope pattern and standard deviation of ppm error for the triplicate experiments.

- Previous work<sup>[1]</sup> 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 isotope patterns (Sample C has 30 isotopes at R=100,000 and 16 isotopes at R=7,500). CalMix was chosen to provide intra-analysis isotope information for both positive and negative ionisation modes. For each isotope series the relationships, Isotope Profile Accuracy (IPA) and Isotope Profile Error (IPE), were defined as

$$\text{Isotope Profile Accuracy (\%)} = \left( 1 - \sqrt{\frac{\sum x_o^2 - \sum x_t^2}{\sum x_o^2 + \sum x_t^2}} \right) \times 100$$

Equation 1

$$\text{Isotope Profile Error (\%)} = \sqrt{\frac{\sum x_o^2 - \sum x_t^2}{\sum x_o^2 + \sum x_t^2}} \times 100$$

Equation 2

where  $x_o$  = observed ion intensity,  $x_t$  = theoretical ion intensity and  $x_{t_{100\%}}$  = intensity of the principal ion<sup>[4]</sup> in the theoretical isotope pattern.

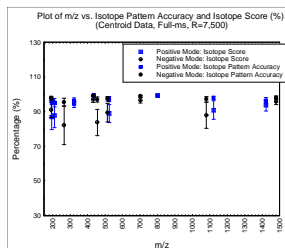


Figure 2: Centroid mode data, Full-MS scan, R=7,500.

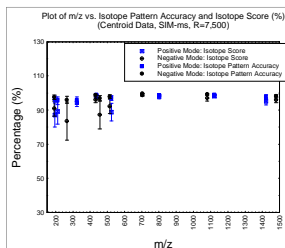


Figure 3: Centroid mode data, SIM-MS scan, R=7,500.

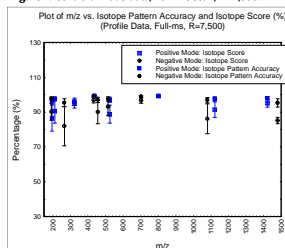


Figure 4: Profile mode data, Full-MS scan, R=7,500.

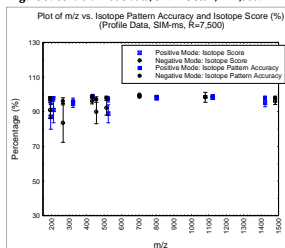


Figure 5: Profile mode data, SIM-MS scan, R=7,500.

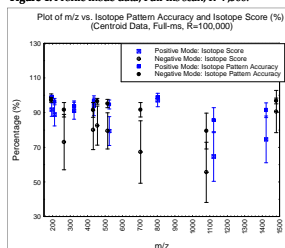


Figure 6: Centroid mode data, Full-MS scan, R=100,000.

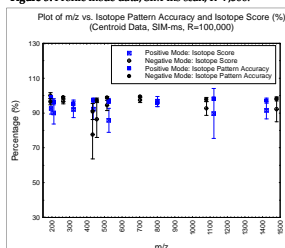


Figure 7: Centroid mode data, SIM-MS scan, R=100,000.

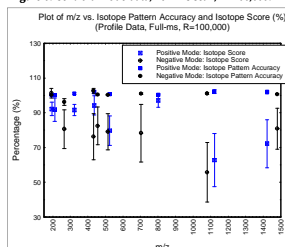


Figure 8: Profile mode data, Full-MS scan, R=100,000.

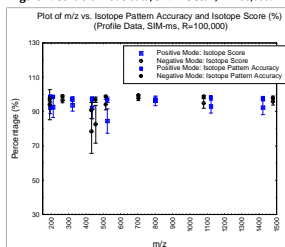


Figure 9: Profile mode data, SIM-MS scan, R=100,000.

- The plots shown in figures 2-9 show the IPA across the mass range for both positive and negative data.
- Figures 2-9 also show 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 isotope, calculated by

$$\text{Isotope Score} = \frac{\sum x_o \times 100}{n}$$

Equation 3

## Conclusions

- Generally, there is a trend that the IPA is closer to 100% and the IPE is closer to 0% 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, especially 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 isotope 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.
- Background, "instrument noise" and FT effects may have a contributing factor to the isotopes, especially for lower intensity ions.

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## References

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