A NOVEL METHOD OF ISOTOPE PREDICTION APPLIED TO ELEMENTAL COMPOSITION ANALYSIS

Kirsten Hobby

Waters Corporation, Micromass MS Technologies, Floats Road, Manchester, M23 9LZ, UK.

OVERVIEW

Exact mass measurement and elemental composition analysis is a core methodology for small molecular weight compounds on oa-TOF instrumentation. Elemental composition analysis with a wide range and number of elements can produce a list of hundreds or even thousands of proposed combinations within the exact mass tolerance of the instrument.

Isotope predictive filtering is a strategy to reduce the number of proposed elemental compositions using algorithms to estimate the number of Carbon, Sulphur, Chlorine or Bromine atoms in an unknown molecule based the mass of the molecular ion and the relative intensity of the 1st and 2nd isotopes.

^{13}C is the predominate contribution to the first isotope but there is also a contribution from isotopes of hydrogen, oxygen, nitrogen and sulphur. Taking into account an estimate of these additional contributions to the intensity of the first isotope allows the number of carbons to be estimated to typically plus/minus 1 carbon.

The intensity of the second isotope is influenced by the presence of Sulphur, Chlorine or Bromine but there is also a contribution from isotopes of carbon, hydrogen, oxygen and nitrogen. The key to the success of second isotope prediction is a novel algorithm to predict the contribution of the isotopes of carbon, hydrogen, oxygen and nitrogen to the relative intensity of the second isotope. Application of a second isotope prediction algorithm to the measurement of an unknown reveals the contribution of Sulphur, Chlorine or Bromine to the second isotope. The second isotope prediction algorithm can be successfully applied to singly charged ions in excess of 1000m/z.

Application of the estimated absolute values for sulphur, chlorine or bromine and restriction of the number of carbons to a range of values results in a significantly reduced number of proposed elemental compositions.

Experimental evidence from a variety of organic molecules acquired on Q-Tof Premier and LCT Premier instruments is presented showing a dramatic reduction in the number of proposed elemental compositions, typically by one to two orders of magnitude.

INTRODUCTION

Three example sample sets are presented consisting of 12 pharmaceuticals, 10 pesticides and four peptides. Tabulated information show the measured mass and relative intensities of the first and second isotopes for each compound which were used in the isotope prediction methodology. The absolute values from the prediction algorithms for each estimated element are shown in blue. The number of elemental compositions within 5ppm are shown before and after restriction of elemental parameters following estimation of the number of Sulphur, Chlorine, Bromine and Carbon (green and orange colours respectively). The elemental compositions were restricted to absolute estimated values of Sulphur, Chlorine and Bromine. The number of Carbons were restricted to a range of values i.e. Carbon estimate plus/minus 3 carbons.

METHODS

LC Conditions

HPLC System: Waters Alliance 2695 or Waters ACQUITY UPLC. Column: Waters Symmetry C_8 2.1mm ´ 50mm 3.5mm. Flow Rate: 300ml/min

Mobile phase: Mixture of isocratic and gradient conditions using mixtures of Water, Acetonitrile or Methanol all with 0.1% (v/v) formic acid. Optimised Ad Hoc per compound. Direct infusion used for some compounds. Standards were prepared in either Methanol or Water.

Data was acquired in continuum mode and processed with an automatic peak detection algorithm which performed simultaneous background subtraction, dead-time and lockmass correction.

MS Conditions

Mass Spectrometer: Waters Micromass Q-Tof Premier and LCT Premier

Ionisation Modes: ESI +ve and -ve. Sample Cone voltage: 35V typical, tuned for best sensitivity/least fragmentation for some compounds. Reference mass: Leucine Enkephalin 556.2771 in +ve, 554.2615 in –ve.

Acquisition parameters. Mixture of scan rates (1 and 10 spectra per second), Inter-scan delay 20ms for Q-Tof, 10ms for LCT. 100-1000m/z or 100-2000m/z.

DISCUSSION

The isotope prediction algorithms applied the spectra generated for the 26 test compounds have unequivocally identified the presence (or absence) of Sulphur, Chorine or Bromine in every case. In some examples simultaneous estimation of the number of Chlorines and Sulphurs has been shown.

The isotope prediction algorithms have also been very successful in estimation of the number of Carbons. The number of Carbons in 12 example compounds were estimated exactly, 11 compounds were estimated to ±1 Carbon and 3 compounds to ±2 Carbons. The application of a tolerance of plus/minus 3 Carbons around the estimated number of Carbons to create a range of Carbons was successful in reducing the number of formulae particularly at higher mass.

The first sample set shows a typical decrease of one order in the number of proposed formulae. The most significant reduction in the number of proposed formulae was for Actinomycin D which showed approximately 2.5 orders decrease in the number of proposed formulae.

The second sample set of pesticides was a difficult analysis because of the presence of Phosphorus and Fluorine in some of the compounds. Phosphorus and Fluorine do not have isotopes and their presence in a molecule will not significantly alter the observed isotope distribution. The addition of Phosphorus or Fluorine to a calculation of elemental compositions will result in significantly more proposed formulae. This effect is apparent by comparison of the number of proposed elemental compositions (without filtering) for similar mass molecules in table 2 verses table 1. There is an approximate one order of magnitude increase in the number of elemental compositions as a result of inclusion of Phosphorus and Fluorine.

The third set of samples has two examples at higher mass and shows that the absolute number of Sulphurs can be estimated successfully at elevated mass. The data for the highest mass peptide at 1531.8m/z shows a 2.3 order decrease in the number of proposed formula after application of Carbon and Sulphur estimates.

The Elemental Composition calculator can be set to order the list of proposed elemental compositions according to the fit of the experimental data to the theoretical isotope distribution (i-FIT). At lower mass and with a limited range of elements the i-FIT can give a definitive answer as seen in figure 1 where the correct elemental formula has an i-FIT value more than an order of magnitude lower than the next proposed elemental composition. In this example the data filtering algorithms confirm this to be the case by removing all other possibilities leaving only the correct elemental composition. Using the i-FIT will not always give a definitive result at higher mass and with a greater range of elements due to small statistical errors in the measurement by the instrument. In this case the interpretation is aided significantly by utilising the isotope prediction algorithms to decrease the number of proposed formulae. In some cases suggested formulae with a better i-FIT than the correct formula can be removed and the correct formula moved higher in the list of putative formulae.

CONCLUSIONS

- Filtering of elemental compositions using estimates of the numbers of Sulphur, Chlorine, Bromine and Carbon to restrict the number of proposed elemental compositions is a powerful methodology to assist in the identification of unknowns.
- An absolute estimate of number of Sulphurs, Chlorines or Bromines in a variety of molecules up to 1500m/z has been demonstrated.
- An estimate of number of carbons to typically plus/minus carbon in a variety of molecules up to 1500m/z has been demonstrated.

RESULTS

Raw Data					Results of Isotopic Prediction			Number of elemental compositions within 5ppmm	
Compound	Formula (ion)	Measured Mass	1st Isotope % abundance	2nd Isotope % abundance	Estimated Chlorines or Bromines	Estimated Sulphurs	Estimated Carbons	Before Filtering	After Filtering
Sulfamethizole	$C_9H_{10}N_4O_2S_2$ $(M+H)^+$	271.0316	14.21	11.09	0	2	11	16	1
Sulfamethazine	$C_{12}H_{14}N_4O_2S$ $(M+H)^+$	279.0917	16.50	6.67	0	1	13	12	1
Sulphadimethoxine	$C_{12}H_{14}N_4O_4S$ $(M+H)^+$	311.0811	16.84	7.10	0	1	13	39	3
Chloramphenicol	$C_{11}H_{12}N_2O_5Cl_2$ $(M-H)^-$	321.0046	13.23	65.89	2	0	11	26	2
Bromopride	$C_{14}H_{22}BrN_3O_2$ $(M+H)^+$	344.0975	17.76	99.19	1	0	15	22	1
Thioridazine	$C_{21}H_{26}N_2S_2$ $(M+H)^+$	371.1619	25.10	11.54	0	2	20	20	1
Methotrexate	$C_{20}H_{22}N_8O_5$ $(M-H)^-$	453.1633	25.95	4.91	0	0	22	59	3
Verapamil	$C_{27}H_{38}N_2O_4$ $(M-H)^+$	455.2905	31.44	6.31	0	0	26	19	3
Terfenadine	$C_{32}H_{41}NO_2$ $(M+H)^+$	472.3218	36.88	7.58	0	0	31	17	2
Reserpine	$C_{33}H_{40}N_2O_9$ $(M+H)^+$	609.2820	36.83	9.41	0	0	31	142	4
Erythromycin	$C_{37}H_{68}NO_{13}$ $(M+H)^+$	734.4687	44.71	11.67	0	0	37	120	6
Actinomycin D	$C_{62}H_{86}N_{12}O_{16}$ $(M+H)^+$	1255.6364	73.45	29.12	0	0	62	3933	10

Table 1: Summary of the pharmaceutical compound raw data, estimated values for Bromine, Chlorine, Sulphur and Carbon and the number of elemental compositions before and after isotopic predictive filtering. The following values were used in the elemental composition calculation: C = 0-500, H = 0-1000, N = 0-500, O = 0-500, S = 0-6, Cl = 0-8. In the case of Bromopride Br = 0-8 & Cl=0.

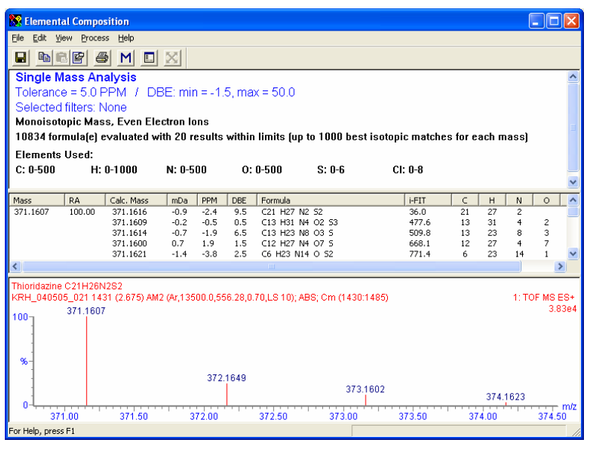

Figure 1: Elemental composition report for Thioridazine. There are 20 possible elemental compositions within 5ppm.

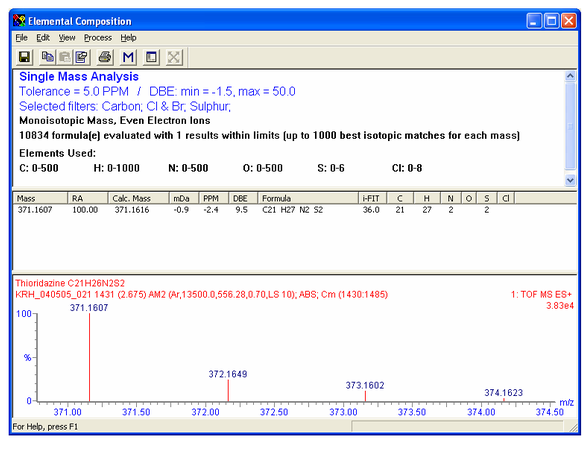

Figure 2: Elemental composition report for Thioridazine after isotope predictive filtering. There is 1 possible elemental composition within 5ppm.

Raw Data					Results of Isotopic Prediction			Number of elemental compositions within 5ppmm	
Compound	Formula (ion)	Measured Mass	1st Isotope % abundance	2nd Isotope % abundance	Estimated Chlorines	Estimated Sulphurs	Estimated Carbons	Before Filtering	After Filtering
Thiobencarb	$C_{12}H_{16}ClNOS$ $(M+H)^+$	258.0719	14.78	37.62	1	1	12	32	1
Terbufos	$C_9H_{21}O_2PS_3$ $(M+Na)^+$	311.0330	13.76	15.68	0	3	10	98	2
Dichlofluanid	$C_9H_{11}Cl_2FN_2O_2S_2$ $(M+Na)^+$	354.9521	13.29	75.59	2	2	10	400	2
Acifluorfen	$C_{14}H_7ClF_3NO_8$ $(M-H)^-$	359.9887	15.98	35.68	1	0	13	474	7
Prothiofos	$C_{11}H_{15}Cl_2O_2PS_2$ $(M+Na)^+$	366.9520	14.56	75.31	2	2	11	463	3
Phosalone	$C_{12}H_{15}ClNO_4PS_2$ $(M+Na)^+$	389.9768	16.4	44.1	1	2	12	524	7
Bifenthrin	$C_{23}H_{22}ClF_3O_2$ $(M+Na)^+$	445.1142	26.35	37.58	1	0	22	404	9
Propaquizafop	$C_{22}H_{21}ClN_3O_5$ $(M+Na)^+$	466.1140	27.27	38.03	1	0	23	524	8
Temephos	$C_{16}H_{20}O_6P_2S_3$ $(M+H)^+$	466.9981	21.00	17.72	0	3	16	2099	18
Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$ $(M+Na)^+$	482.9721	19.09	66.12	2	0	16	2096	34

Table 2: Summary of the pesticide compound raw data, estimated values for Chlorine, Sulphur and Carbon and the number of elemental compositions before and after isotopic predictive filtering. The following values were used in the elemental composition calculation: C = 0-500, H = 0-1000, N = 0-500, O = 0-500, F = 0-10, P = 0-10, S = 0-6, Cl = 0-8. When the main species was $(M+Na)^+$ Na was set to 1.

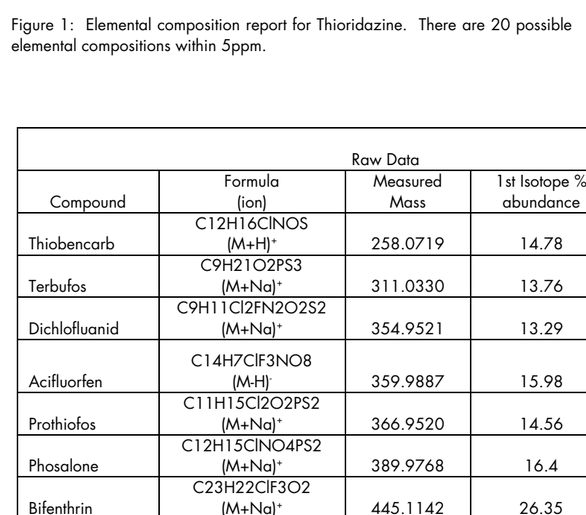

Figure 3: Elemental composition report for Phosalone. There are 524 possible elemental compositions within 5ppm.

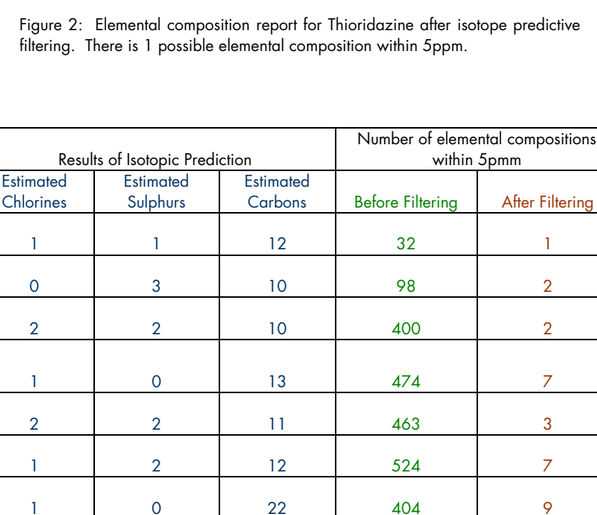

Figure 4: Elemental composition report for Phosalone after isotope predictive filtering. There are 7 possible elemental compositions within 5ppm.

Raw Data					Results of Isotopic Prediction		Number of elemental compositions within 5ppmm	
Compound	Formula (ion)	Measured Mass	1st Isotope % abundance	2nd Isotope % abundance	Estimated Sulphurs	Estimated Carbons	Before Filtering	After Filtering
Val-Tyr-Val	$C_{19}H_{29}N_3O_5$ $(M-H)^-$	378.2020	23.1	3.99	0	19	18	2
Leucine Enkephalin	$C_{28}H_{37}N_5O_7$ $(M-H)^+$	556.2775	33.92	7.56	0	28	78	4
Methionine Enkephalin-Arg-Phe	$C_{42}H_{56}N_{10}O_9S$ $(M-H)^+$	877.4008	50.93	19.8	1	42	222	4
PPPPPPPPPPPPPR	$C_{76}H_{112}N_{18}O_{16}$ $(M-H)^-$	1531.8429	91.23	44.95	0	76	2419	13

Table 3: Summary of the peptide compound raw data, estimated values for Sulphur and Carbon and the number of elemental compositions before and after isotopic predictive filtering. The following values were used in the elemental composition calculation: C = 0-500, H = 0-1000, N = 0-500, O = 0-500, S = 0-10.

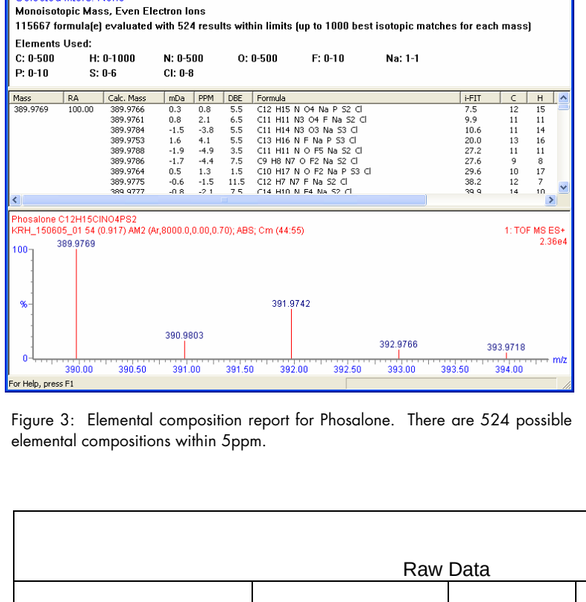

Figure 5: Elemental composition report for Methionine Enkephalin-Arg-Phe. There are 222 possible elemental compositions within 5ppm.

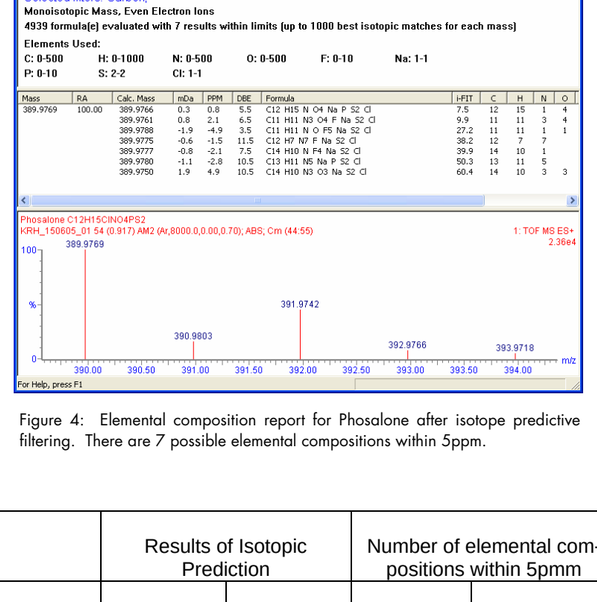

Figure 6: Elemental composition report for Methionine Enkephalin-Arg-Phe after isotope predictive filtering. There are 4 possible elemental compositions within 5ppm.

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