A NOVEL METHOD OF ISOTOPE PREDICTION APPLIED TO ELEMENTAL COMPOSITION ANALYSIS

Waters

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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.

¹³C 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

R	ESl	JLTS

	F	Raw Data			Resu	lts of Isotopic Pred	iction	Number of eleme within	
Compound	Formula (ion)	Measured Mass	1 st Isotope % abundance	2nd Isotope % abundance	Estimated Chlorines or Bromines	Estimated Sulphurs	Estimated Carbons	Before Filtering	After Filtering
Sulfamethizole	C9H10N4O2S2 (M+H)*	271.0316	14.21	11.09	0	2	11	16	1
Sulfamethazine	C12H14N4O2S (M+H)⁺	279.0917	16.50	6.67	0	1	13	12	1
Sulphadimethoxine	C12H14N4O4S (M+H)+	311.0811	16.84	7.10	0	1	13	39	3
Chloramphenicol	C11H12N2O5Cl2 (M-H) ⁻	321.0046	13.23	65.89	2	0	11	26	2
Bromopride	C14H22BrN3O2 (M+H)*	344.0975	17.76	99.19	1	0	15	22	1
Thioridazine	C21H26N2S2 (M+H)⁺	371.1619	25.10	11.54	0	2	20	20	1
Methotrexate	C20H22N8O5 (M-H) ⁻	453.1633	25.95	4.91	0	0	22	59	3
Verapamil	C27H38N2O4 (M-H)⁺	455.2905	31.44	6.31	0	0	26	19	3
Terfenadine	C32H41NO2 (M+H)⁺	472.3218	36.88	7.58	0	0	31	17	2
Reserpine	C33H40N2O9 (M+H) ⁺	609.2820	36.83	9.41	0	0	31	142	4
Erythromycin	C37H68NO13 (M+H)⁺	734.4687	44.71	11.67	0	0	37	120	6
Actinomycin D	C62H86N12O16 (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.

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Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula		i-FIT	C	н	N	0	
371.1607	100.00	371.1616	-0.9	-2.4 -0.5	9.5	C21 H27 N2		36.0	21	27	2		<u> </u>
		371.1609 371.1614	-0.2 -0.7	-0.5	0.5 6.5	C13 H31 N4 C13 H23 N8		477.6 509.8	13 13	31 23	4 8	2 3	
		371.1600	0.7	1.9	1.5	C12 H27 N4		668.1	12	27	4	7	
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(RH_0405)	05_021 14	31 (2.675) AM	12 (Ar,136										.836
100	05_021 14	31 (2.675) AM	12 (Ar,135									3	.836
(RH_0405)	05_021 14	31 (2.675) AM	12 (Ar,136		1649		373.1	602				3	.836
(RH_0405)	05_021 14	31 (2.675) AM			1649	372.50		602		374.	1623		- m/

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

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Mass	RA	Calc. Mass		PPM	DBE	Formula			i-FIT	С	н	N	0	S	CI		
371.1607	100.00	371.1616	-0.9	-2.4	9.5	C21 H27	N2 S2		36.0	21	27	2		2			
	100.00																
		1202															
Thioridazine	e C21H26N	1282 31 (2.675) AM	12 (Ar,1350	0.0,55	6.28,0.	.70,LS 10)	; ABS; (30:1485)					1:	TOF M	SES
Thioridazine KRH_0405(e C21H26N	31 (2.675) AM	12 (Ar,1350	10.0,55	i6.28,0.	.70,L8 10)	ABS;	Dm (143	30:1486))					1:		
Thioridazine KRH_0405(∋ C21H26N 05_021 143	31 (2.675) AM	12 (Ar,1360	0.0,55	6.28,0.	70,L8 10)	(ABS)	Dm (143	30:1485))					1:		
Thioridazine	∋ C21H26N 05_021 143	31 (2.675) AM	12 (Ar,1350	0.0,55	6.28,0.	.70,LS 10)	(ABS))		30:1485;)					1:		
Thioridazine KRH_0405(∋ C21H26N 05_021 143	31 (2.675) AM	12 (Ar,1350	0.0,55	6.28,0.	.70,LS 10)	(ABS;)	Dm (143	30:1486))					1:		
Thioridazine KRH_04050	∋ C21H26N 05_021 143	31 (2.675) AM	12 (Ar,1350	·		.70,LS 10)	(ABS))	Dm (143	30:1485;)					1:		
Thioridazine KRH_04050	∋ C21H26N 05_021 143	31 (2.675) AM	12 (Ar,1350	·	i6.28,0. 1649	70,LS 10)	(ABS;)		30:1485))						:	
Thioridazine KRH_04050	∋ C21H26N 05_021 143	31 (2.675) AM		372.		70,LS 10)	(ABS) (37	3.1602					374	1:	3	3.83e
Thioridazine KRH_0405(100 %	∋ C21H26N 05_021 143	31 (2.675) AM		·		70,LS 10) 372.50	(ABS)	37	3.1602		3.50		37	374		3	S ES 3.83e T m 4.50

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

HPLC System: Waters Alliance 2695 or Waters ACQUITY UPLC. Column: Waters Symmetry $C_8 2.1 \text{ mm} \cdot 50 \text{ mm} 3.5 \text{ mm}$. Flow Rate: 300 ml/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

lonisation 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.

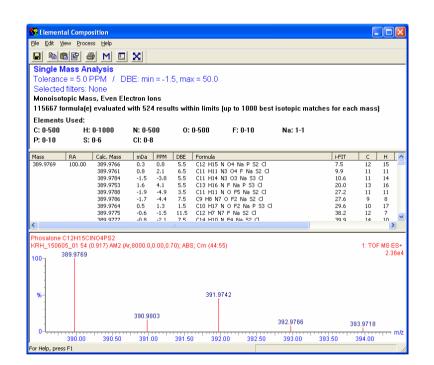
The second sample set of pesticides was a difficult analysis because of the presence of Phosphorus and Fluorine in some of the compounds. Phosphorus and Fluorine do not have isotopes and their presence in a molecule will not significantly alter the observed isotope distribution. The addition of Phosphorus 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.

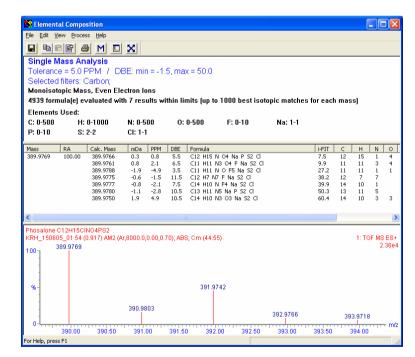
The third set of samples has two examples at higher mass and shows that the absolute number of Sulphurs can be estimated successfully at elevated mass. The data for the highest mass peptide at 1531.8m/z shows a 2.3 order decrease in the number of proposed formula after application of Carbon and Sulphur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

		Raw Data			Resul	ts of Isotopic Pred	iction	Number of eleme within	ntal compositions 5pmm
	Formula	Measured	1 st Isotope %	2nd Isotope %	Estimated	Estimated	Estimated		
Compound	(ion)	Mass	abundance	abundance	Chlorines	Sulphurs	Carbons	Before Filtering	After Filtering
	C12H16CINOS								
Thiobencarb	(M+H)+	258.0719	14.78	37.62	1	1	12	32	1
	C9H21O2PS3								
Terbufos	(M+Na)+	311.0330	13.76	15.68	0	3	10	98	2
	C9H11Cl2FN2O2S2								
Dichlofluanid	(M+Na)+	354.9521	13.29	75.59	2	2	10	400	2
	C14H7ClF3NO8								
Acifluorfen	(M-H) ⁻	359.9887	15.98	35.68	1	0	13	474	7
/ centrol in the second s	C11H15Cl2O2PS2	007.7007	10.70	00.00			10		,
Prothiofos	(M+Na)+	366.9520	14.56	75.31	2	2	11	463	3
	C12H15CINO4PS2								
Phosalone	(M+Na)+	389.9768	16.4	44.1	1	2	12	524	7
	C23H22ClF3O2								
Bifenthrin	(M+Na)+	445.1142	26.35	37.58	1	0	22	404	9
	C22H21CIN3O5								
Propaquizafop	(M+Na)+	466.1140	27.27	38.03	1	0	23	524	8
· · · ·	C16H20O6P2S3								
Temephos	(M+H)+	466.9981	21.00	17.72	0	3	16	2099	18
	C16H8Cl2F6N2O3								
Hexaflumuron	(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.





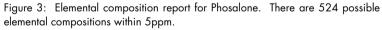


Figure 4: Elemental composition report for Phosalone after isotope predictive filtering. There are 7 possible elemental compositions within 5ppm.

	Raw	Data	-			f Isotopic		elemental com- within 5pmm
Compound	Formula (ion)	Measured Mass	1st Isotope % abundance	2nd Isotope % abundance	Estimated Sulphurs	Estimated Carbons	Before Filtering	After Filtering
Val-Tyr-Val	C19H29N3O5 (M-H)	378.2020	23.1	3.99	0	19	18	2
Leucine Enkephalin	C28H37N5O7 (M-H)⁺	556.2775	33.92	7.56	0	28	78	4
Methionine Enkephalin- Arg-Phe	C42H56N10O9S (M-H)⁺	877.4008	50.93	19.8	1	42	222	4
PPPPPPPPPPPPR	C76H112N18O16 (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.



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.

0333410	rmula(e)	evaluated w		ons result	s withi	in limits	(up to 1000	best isotopic	matches f	or eac	h mas	s)
Elements												
C: 0-500	H:	0-1000	N: 0-5	00	0:	0-500	S: 0-10	l -				
Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula		i-FII	· [c	Н	N	0
877.4008	100.00	877.4031	-2.3	-2.6	19.5	C42 H57	N10 09 5	12.5	5 42	57	10	9
		877.4044	-3.6	-4.1		C45 H65		34.4		65		15
		877.4044 877.4017	-3.6 -0.9	-4.1 -1.0	24.5 14.5		N14 05 5	66.1 77.3		53 61	14 6	5 13
		877.4017 877.4017	-0.9	-1.0	25.5		N6 013 5	//.: 99.0		49	ь 20	13
		877.4004	0.4	0.5			N16 07 5	181			16	7
		877.4051	-4.3	-4.9	20.5	C36 H53	N20 03 52	212	.9 36	53	20	3
		877.4004	0.4	0.5	9.5	C40 H65	5 N2 O17 5	272	.2 40	65	2	17
<												
		n-Arg-Phe C4					0.4.000					
KKH_0/0/	00_04 83 ((1.419) AM2 (A	a, rouuu.	u,u.uu,u	. 7 U), Al	55, UN (6	0.123)					FOF M
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				8	378.403	17						
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Figure 5: Elemental composition report for Methionine Enkephalin-Arg-Phe. There are 222 possible elemental compositions within 5ppm.

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Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula			i-FIT	С	н	N	0	S	Т
877.4008	100.00	877.4031 877.4044 877.4044 877.4044	-2.3 -3.6 -3.6	-2.6 -4.1 -4.1	19.5 13.5 24.5	C45 H65 C43 H53	N14 O5 5		12.5 34.4 66.1	42 45 43	57 65 53	10 14	9 15 5	1 1 1	
		077.4017	-0.9	-1.0	14.5	C41 H61	N6 O13 S		77.3	41	61	6	13	1	
		n-Arg-Phe C4 1.419) AM2 (/	2H56N10	0095					77.3	41	61	6	13	1 TOF	
		n-Arg-Phe C4	2H56N10	0095					77.3	41	61	6	13		MS 1

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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