THE USE OF ISOTOPE RATIO MEASUREMENTS TO REDUCE THE NUMBER OF CANDIDATE ELEMENTAL COMPOSITIONS FROM ACCURATE MASS DETERMINATION

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ABSTRACT

Accurate mass measurement by mass spectrometry is a common technique to determine elemental composition. Despite technological advances and increases in the mass accuracy the mass accuracy often does not provide unequivocal identification, with the number of candidate formulae increasing exponentially with mass. A novel methodology is described for the simultaneous estimation of chlorine, bromine, sulfur and carbon using a mathematical methodology based on isotope ratios and mass. Restriction of the number of elements in an elemental composition analysis, based on the results of the element prediction, leads to a dramatically reduced number of candidate formulae.

36 pharmaceutical development compounds were analysed using a Q-TOF Premier instrument in ESI positive mode. Compounds composed of carbon, hydrogen, nitrogen, oxygen, fluorine, chlorine, bromine and sulfur in various combinations and ranged in mass from 195 to 710Da.

For each compound the accurate mass and isotope ratios were measured. Mass accuracy was assumed to better than 1.2mDa for the 150-400m/z range and 3ppm for the 400-900m/z range. Errors in measured isotope ratios were assumed to be less than 4%. It was assumed that there would be no phosphorus, no more than eight chlorine and/or bromine, no more than six sulfur, but that there may be any number of carbon, hydrogen, nitrogen, oxygen and fluorine.

The number of chlorine, bromine and sulfur were estimated correctly for all 36 compounds. The number of carbons were typically estimated to a tolerance of one carbon. For 21 of the analyses the number of carbons was estimated correctly, 13 had an error of plus/minus one carbon and 2 had an error of ±2 carbons. During the elemental composition analysis the number of chlorine, bromine and sulfur were restricted to the estimated value, with no tolerance, for each respective element. Carbon was restricted to a range of ±2 carbons around the estimated value. Restriction of the numbers of chlorine, bromine, sulfur and carbon during the elemental composition analysis dramatically reduced the number of formulae, typically by two orders of magnitude.

Elemental composition analysis of the highest mass compound ($C_{32}H_{29}ClF_5N_5O_4S$, 710m/z) gave 2662 formula within 3ppm. Restricting chlorine and bromine to one and zero respectively gave 507 formulae. Further restriction of sulfur to one gave 117 formulae. Further restriction of carbon to a range of 31 to 35 gave 7 formulae. If sulfur was not restricted 41 candidate formulae remained. The described methodology can lead to a significant reduction in the number of candidate formulae by exclusion of those which would give rise to incorrect isotope ratios.

METHODS

Sample Conditions

Sample were received in DMSO at approximately 0.1mg/ml and diluted 100 fold with water:acetonitrile (50:50) + 0.1 % (v/v) formic acid. Diluted samples were infused directly @ 5ul/min.
Data was acquired in continuum mode. Approximately two minutes of data was combined and then 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™. Ionisation Mode: ESI +ve. Sample Cone voltage and capillary voltage tuned to give 0.1 ions per push for the most abundant ion. Reference mass: Leucine Enkephalin 556.2771. Acquisition parameters. 1 spectra per second, Inter-scan delay 20ms . 100-1000m/z.

RESULTS

Sample No.	Formula	m/z	Estimated Cl or Br	Estimated S	Estimated C	Error in C estimate
1	$C_{10}H_{11}N_2Cl$	195.0689	1	0	10	0
2	$C_{35}H_{48}FN_5O_3$	606.3819	0	0	34	-1
3	$C_{17}H_{23}N_3O_2$	302.1869	0	0	18	1
4	$C_{14}H_{12}N_2$	209.1079	0	0	14	0
5	$C_{14}H_{12}N_2O$	225.1028	0	0	14	0
6	$C_{36}H_{29}ClF_2N_6O_3S$	699.1757	1	1	35	-1
7	$C_{32}H_{42}FN_5O_3$	564.335	0	0	33	1
8	$C_{34}H_{38}F_2N_6O_2$	601.3103	0	0	34	0
9	$C_{34}H_{35}N_4O_3SF$	601.2649	0	1	33	-1
10	$C_{24}H_{29}N_4O_3S$	453.1960	0	1	24	0
11	$C_{31}H_{32}ClN_5O_4S$	606.1942	1	1	31	0
12	$C_{32}H_{27}Cl_2NO_5$	576.1345	2	0	30	-2
13	$C_{29}H_{22}Cl_2N_2O_4$	533.1035	2	0	27	-2
14	$C_{31}H_{27}F_2N_5O_2S$	572.1932	0	1	31	0
15	$C_{37}H_{44}F_2N_6O_2$	643.3572	0	0	37	0
16	$C_{31}H_{32}ClN_5O_3S_2$	622.1713	1	2	30	-1
17	$C_{34}H_{26}F_3N_7O$	606.2229	0	0	34	0
18	$C_{23}H_{26}F_3NO_4S$	470.1613	0	1	23	0
19	$C_{29}H_{22}Cl_2N_2O_4$	533.1035	1	0	28	-1
20	$C_{36}H_{31}F_2N_7O$	616.2636	0	0	35	-1
21	$C_{35}H_{29}F_2N_7O$	602.248	0	0	34	-1
22	$C_{32}H_{31}F_2N_6O_4S$	633.2096	0	1	32	0
23	$C_{32}H_{30}F_5N_5O_4S$	676.2017	0	1	32	0
24	$C_{34}H_{27}ClF_3N_7O_2$	658.1945	1	0	34	0
25	$C_{32}H_{31}FN_6O_4S$	615.219	0	1	32	0
26	$C_{23}H_{28}N_4$	361.2392	0	0	23	0
27	$C_{28}H_{36}ClN_5O_5S_2$	622.1925	1	2	28	0
28	$C_{32}H_{29}ClF_5N_5O_4S$	710.1638	1	1	33	1
29	$C_{31}H_{30}ClF_2N_5O_4S$	642.1753	1	1	31	0
30	$C_{21}H_{24}N_4$	333.2079	0	0	22	1
31	$C_{35}H_{28}F_3N_7O$	620.2386	0	0	35	0
32	$C_{32}H_{31}FN_6O_5S_2$	663.186	0	2	32	0
33	$C_{33}H_{30}F_4N_6O_4S$	683.2064	0	1	33	0
34	$C_{32}H_{35}N_5O_4S$	586.2488	0	1	32	0
35	$C_{27}H_{22}NO_4SBr$	536.0531	1	1	26	-1
36	$C_{28}H_{21}Cl_2NO_5$	522.0875	2	0	27	-1

Table 1: Summary of the elements predicted. Chlorine, bromine and sulfur were correctly estimated in all of the 36 analyses. Carbon was correctly estimated in 21 of the 36 analyses, 13 analyses were within ± 1 carbon and 2 analyses were within ± 2 carbons.

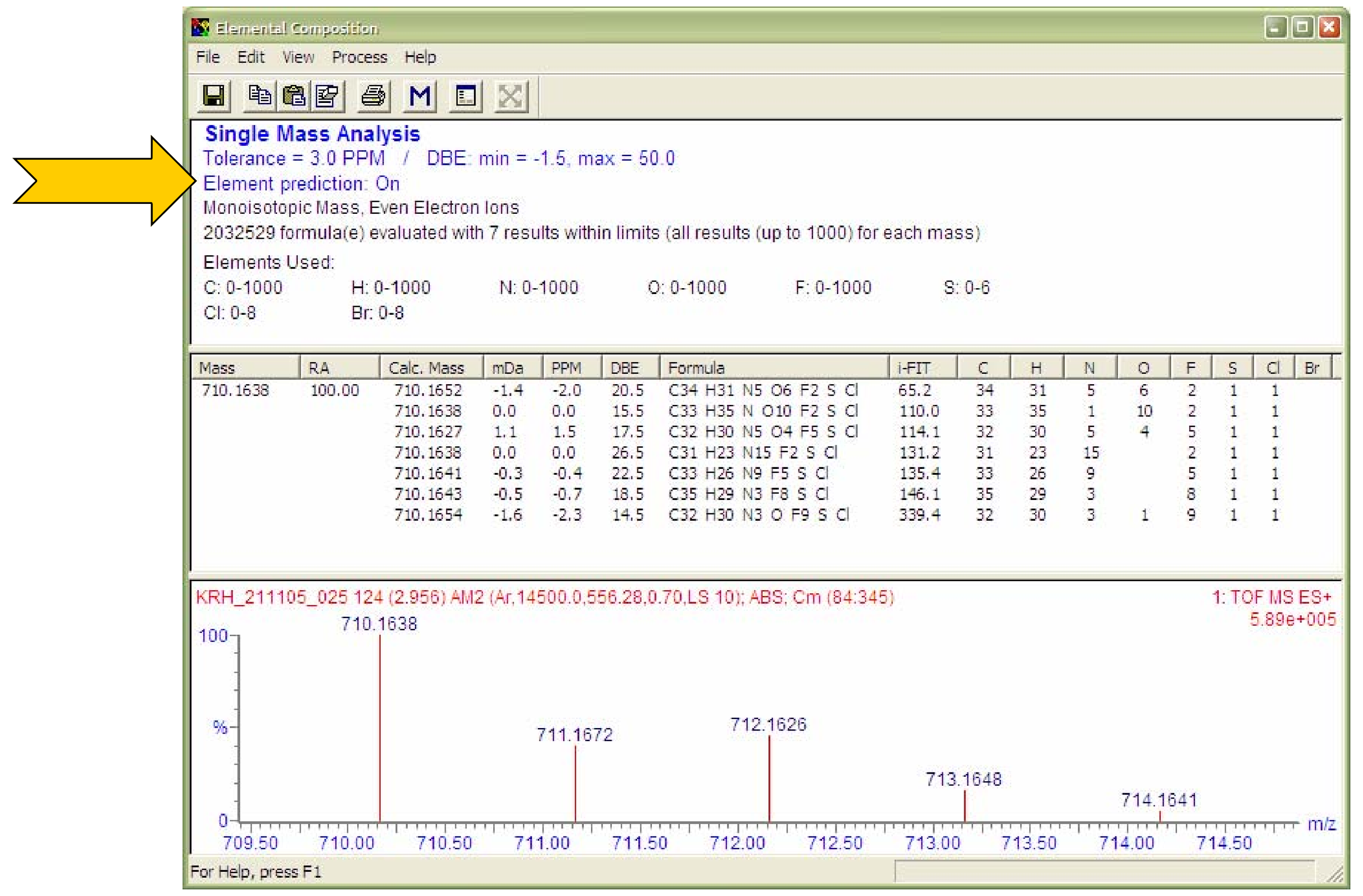

Figure 1: Elemental composition report for sample 28 ($C_{32}H_{29}N_5O_4F_5SCl$) after application of element prediction filters. There are 7 possible formulae with the 3ppm tolerance window.

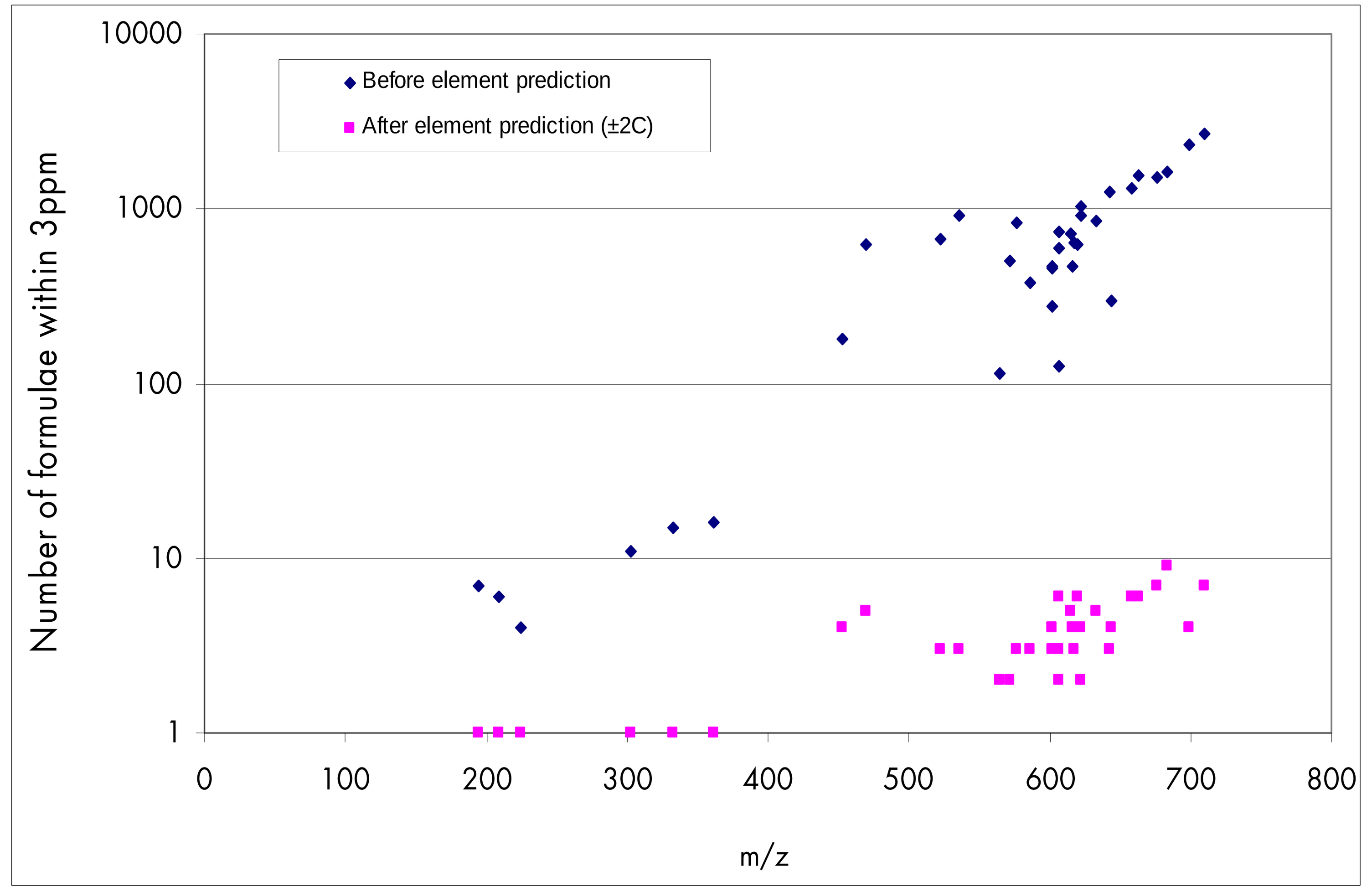

Figure 2: The effect of elemental composition filters on the total number of elemental compositions . A single formula was returned for compounds below 400m/z. The element prediction filters were particularly effective at high m/z where there were typically several thousand candidate formulae before filtering and less than 10 candidate formulae after filtering.

Mass tolerance (ppm)	No interpretation	Manual Interpretation (Cl=1)	Filters applied Br=0, Cl=1, S=1	
			C±4	C±2
<5	4441	843	26	14
<4	3554	678	24	14
<3	2662	507	14	7
<2	1761	337	11	7
<1	891	163	4	2

Table 2: Comparison of effect of mass tolerance and 'Element prediction filters' on number of candidate formulae for $C_{32}H_{29}N_5O_4F_5SCl$ (710.1627m/z). The first column shows the total number of candidate formulae at each of the given mass tolerances when none of the elements are restricted. The second column shows the effect of manual interpretation of the mass spectrum in figure 1 (chlorine and bromine restricted to 1 and 0 respectively) on the number of formulae. No further manual interpretation is possible. The third and fourth columns show the effect of absolute restriction of Br to 0, Cl to 1 and S to 1 with C restricted to a range around the estimated number of C of C±4 and C±2 respectively.

The element prediction filters are more effective in reducing the number of candidate formulae than an increase in the mass accuracy of the measurement.

DISCUSSION

The element prediction algorithms applied to the spectra generated for the 36 test compounds have unequivocally identified the presence (or absence) of sulphur, chlorine or bromine in every case (Table 1). In some examples simultaneous estimation of chlorine and sulfur or bromine and sulfur has been shown.

The element prediction algorithms have also been very successful in estimation of the number of carbons. The number of carbons in 21 compounds were estimated exactly, 13 compounds were estimated to ±1 carbon and 2 compounds to ±2 carbons. The application of a tolerance of ±2 carbons around the estimated number of carbons to create a range of carbons was very successful in reducing the number of formulae.

Figure 1 is a representative example of the elemental composition browser window after application of the element prediction filters.

Figure 2 is a summary of the effect of the element prediction filters on the data generated for the 36 compounds. Application of the filters gives a decrease in the number of proposed formulae by up to two orders of magnitude.

CONCLUSIONS

- Filtering of elemental compositions using estimates of the numbers of sulfur, 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 sulfur, chlorine or bromine and an estimate of number of carbons to typically plus/minus one carbon has been demonstrated.
- In all cases the correct formula was never removed.

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