

## Determining Ion Compositions with Increasing Simplicity

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### Abstract

Exact masses of ions and relative isotopic abundances (RIAs) of the +1 and +2 isotopic mass peaks, measured with sufficient accuracy, provide the elemental compositions of ions from a compound, which correspond to a limited number of possible isomers. Double focusing mass spectrometers provided exact mass and RIA measurements in the early 90's, while today, less expensive, more robust, and easier to operate time-of-flight mass spectrometers (TOFMSs) also provide data sufficiently accurate to determine ion compositions. Using exact masses and RIAs measured with a TOFMS, an Ion Correlation Program matches precursor ion and product ion:neutral loss pairs to increase the mass range for which the unique and correct composition of ions can be determined and to provide deconvolution of composite mass spectra.

For 15 years, the authors have identified compounds in environmental extracts with little or no knowledge of the sampling site history (Grange et al. 1998-2003, 2007, Snyder et al. 2001, Brumley et al. 2000). Elements found in molecules have included, C, H, N, O, F, Cl, Br, I, S, P, Si, As, and Se. Both the instrumentation and automated data evaluation have evolved toward greater simplicity. Consequently, others are now identifying contaminants found in water (Bobeldijk et al. 2002) and food (Thurman et al. 2005, Ibáñez et al. 2005).

**Compound Identification.** Often, the NIST (NIST/EPA/NIH) or Wiley (Wiley Registry) libraries will provide similar or related mass spectra to that of an analyte, which can lead to a tentative identification, even when only nominal (or integer) masses are measured. If not, determining the elemental composition of a molecule that has lost an electron or gained a proton to become a positively-charged ion, or lost an H atom to become a negatively-charged ion, limits a compound's identity to a finite number of isomers. Product ions formed from such a precursor ion and the corresponding neutral losses (product ion:neutral loss pairs) provide insight into the structural features of the compound, thereby limiting the number of possible isomers. Consultation of data bases (Grange, 2005, 2006) such as the SciFinder<sup>®</sup> compilation of known isomers (Chemical Abstract Services) further reduces the number of plausible isomers. A tentative identification is confirmed when a purchased or synthesized standard provides a mass spectrum and a retention time by gas chromatography or liquid chromatography similar to those of the analyte.

**Exact Masses and Relative Isotopic Abundances.** The exact mass is only one of two measurable and independent physical properties of an ion that provide differentiation among possible ion compositions. In addition to the exact mass of the monoisotopic ion ( $M$ ), which contains only atoms of the lowest-mass isotopes, the Relative Isotopic Abundances (RIAs) of the

M+1 and M+2 mass peaks that arise from the presence within an ion of atom(s) of isotopes heavier by 1 or 2 Da than the most abundant isotopes are unique for different sets of atoms. The RIAs of the +1 and +2 isotopes of the elements (NIST Physics) most commonly found in organic molecules, C, H, N, O, Br, Cl, F, I, P, and S, result in the abundance of the M+1 mass peak providing an estimate of the number of C atoms in an ion, while the abundance of the M+2 mass peak provides estimates of the number of S, Cl, and Br atoms present.

The mass error limits and RIA error limits determine the relative utility of exact masses and RIAs for determining the unique and correct ion composition. Figure 1 illustrates that the number of possible compositions increases exponentially with increasing mass, increases nearly linearly with increasing mass error, and is highly dependent on the mass defect measured for an ion. Even for a small mass error limit, numerous ion compositions can be possible for high-mass ions. Considering additional elements as possibly present also increases the number of possible compositions, while considering only odd or even electron ions halves the number. The number of possible compositions based on consideration of RIAs alone also increases as an ion's mass and the RIA error limit increase.

Figure 2 illustrates for one set of mass error and RIA error limits (2 mmu and 20%) a major reduction in the number of possible compositions that occurred when both exact masses and RIAs were considered for measured values of 319.1039 Da, 19.00%, (M+1), and 36.78% (M+2) for the  $[M+H]^+$  ion from chlorpromazine, a sedative (Grange et al. 2006). Even assuming that at least 1/3 of the mass of the ion was due C atoms, 47 compositions were possible for the molecular ion. Considering only the RIAs, 402 compositions were possible. But when both the exact masses and RIAs were considered, only 5 compositions remained viable. Clearly, for the purpose of determining ion compositions, it is advantageous to measure both exact masses and

RIAs.

**Instrumentation:** *Double Focusing Mass Spectrometer.* To accurately measure exact masses and RIAs for coeluting compounds in complex environmental extracts, high resolving power was necessary. In the early 90's, double focusing mass spectrometers alone provided resolving powers of 10,000 to 20,000 (10% valley) for routine analyses. In the environmental arena, these instruments were often used for dioxin analyses (Method 8290, Method 1613). To provide low error limits for quantitation  $^{13}\text{C}$ -labeled analogs of dioxins were spiked into extracts. Selected-Ion-Recording (SIR), the monitoring of selected  $m/z$  ratios corresponding to the exact masses of the labeled reference and target analyte compounds, provided 100-fold greater sensitivity, fast scan cycles, and 10-fold higher resolving power (10,000, 10% valley) compared to full scanning. We devised methodology to use double focusing mass spectrometers and SIR to provide compositions of ions from unidentified compounds in complex environmental extracts without reference to lists of target compounds. With 2,800 high production chemicals ( $10^6$  lbs/yr or more)[1990 HPV] and 87,000 compounds used in commerce (Endocrine), most compounds, their synthetic byproducts, and their transformation products do not appear on target lists.

To measure both exact masses and RIAs with high resolving power, four data acquisitions were necessary to provide the plots of full or partial mass peak profiles shown in Figure 3. First, a full scan mass spectrum using the lowest resolving power (1000) to maximize sensitivity and scan speed was obtained for compounds as they eluted from a gas chromatograph (GC) into the electron impact ion source. For each compound, the apparent molecular ion (largest-mass ion observed) was further investigated. Three SIR data acquisitions that covered narrow mass ranges and that delineated full or partial mass peak profiles were then made as analytes eluted from a GC interfaced to either a VG 70-SE mass spectrometer for which 23  $m/z$

ratios were used (Grange et al. 1992-1999) or a Finnigan MAT 950S mass spectrometer for which 31  $m/z$  ratios were used (Grange et al. 2000-2003, Brumley et al. 2000, Snyder et al. 2001, Grange and Sovocool 2007).

In Figure 3a, a narrow mass range about a monoisotopic ion from an analyte found in an extract of water from a monitoring well was monitored with a resolving power of 3,000 and a  $m/z$  increment of 100 ppm between adjacent points. The ion chromatogram for each  $m/z$  ratio was integrated across the chromatographic peak for the monoisotopic ion of interest and plotted in Figure 3a. The two mass peak profiles, each delineated by three points, were obtained for an analyte (left peak) and a perfluorokerosene (PFK) ion (right peak). The ion chromatograms for the  $m/z$  ratios at the maxima of the two peaks are shown as insets. For the PFK peak, two baseline excursions are evident that were induced by changing a lens voltage in the ion source to temporarily divert the ion beam. The excursions enabled area integration across the simulated chromatographic peak between them. The areas were plotted to provide the partial mass peak profiles for PFK lock mass and calibration ions that bracket the analyte ion mass. The exact masses of all full or partial mass peak profiles in Figure 3 were obtained as a weighted average of the top several points delineating the profiles.

The exact mass from the analyte mass peak profile in Figure 3a was used as the center mass in the SIR  $m/z$  ratio list to acquire the ion chromatograms used to plot Figure 3b. The resolving power was 10,000 and the mass increment between points was 10 ppm. The exact mass obtained corresponded to eight possible compositions assuming atoms of C, H, N, O, As, F, P, or S could be present and a mass error limit of 6 ppm.

Experience has shown that the possible composition containing the fewest heteroatoms is most likely to be correct, and it was chosen as the hypothetical composition to create the SIR  $m/z$

ratio list to acquire data for the M, M+1, and M+2 partial profiles plotted in Figure 3c. Summing the chromatographic peak areas used to plot the partial profiles, dividing the summed areas for the M+1 and M+2 partial profiles by the summed area of the M partial profile, and multiplying by 100% provided the %M+1 and %M+2 relative abundances shown on the partial profiles in Figure 3c. This use of SIR to delineate profiles to provide exact masses and RIAs was called Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) (Grange and Brumley 1996b).

**Applying Exact Mass and RIA Criteria.** To compare measured exact masses and RIAs with calculated values for possible compositions, a Profile Generation Model (PGM) was written in QuickBASIC<sup>®</sup> version 4.5 (Microsoft Corp., Bellevue, WA, USA) to calculate the mass of the M profile and to construct the M+1 and M+2 profiles resulting from the ions containing atoms of higher isotopes (Grange and Brumley 1997). The M+1 and M+2 profiles were calculated as sums of the Gaussian distributions for the individual +1 and +2 ions at the resolving power being used. Table 1 prepared by the PGM provided a listing of the possible compositions. The "X"s indicated that the measured and calculated values for a composition did not agree within the mass error limit of 6 ppm and the RIA error limit of about 11%. These error limits were established from measurements for several standards (Grange and Brumley 1997). Using MPPSIRD and the PGM together was referred to as Ion Composition Elucidation (ICE) in several papers (Grange et al. 2001-2007).

Table 1. Calculated Exact Masses and RIAs for 8 Compositions\* and the Measured Values

<u>Composition</u>	182 <u>M</u>	183 <u>M+1</u>	184 <u>M+2</u>	%M+1 <u>(%1 Range)<sup>#</sup></u>	%M+2 <u>(%2 Range)</u>
HNOF <sub>3</sub> P <sub>2</sub> S	.92007	.91896 X	.91623	1.21(1.02-1.39) X	4.63(4.02-5.25) X
HNOAsF <sub>4</sub>	.92047	.91842 X	.92472 X	0.42(0.36-0.48) X	0.20(0.17-0.23) X
H <sub>2</sub> NO <sub>2</sub> F <sub>2</sub> S <sub>3</sub>	.92102	.92029 X	.91707	2.83(2.38-3.29) X	13.72(11.92-15.53) X
N <sub>4</sub> O <sub>2</sub> P <sub>2</sub> S	.92117	.91924 X	.91767 X	2.33(1.99-2.68) X	4.85(4.21-5.50) X
N <sub>4</sub> O <sub>2</sub> AsF	.92157	.91896 X	.92561 X	1.55(1.33-1.76) X	0.41(0.34-0.48) X
C <sub>2</sub> NO <sub>5</sub> S <sub>2</sub>	.92124	.92265	.91794 X	4.34(3.69-4.99)	9.94(8.60-11.28)
C <sub>3</sub> HNOFS <sub>3</sub>	.91988	.93132 X	.91588	6.09(5.19-6.99) X	13.65(11.86-15.44) X
C <sub>3</sub> H <sub>7</sub> AsS <sub>2</sub>	.91996	.92210	.91585	4.97(4.23-5.70)	8.96(7.79-10.13)
Measured: Values	.92056	.92265	.91625	4.72	9.54

\* Some of the compositions are different from those in the original paper (Grange et al. 2002), because the mass of the electron, 0.00055 Da, had not been taken into account.

# The permissible ranges result from consideration of several errors associated with plotting partial profiles (Grange and Brumley 1997).

Initially, two other criteria were examined: the apparent resolution determined from the profile width at 5% of the maximum profile height, which was less than the instrument resolving power when profile broadening occurred and a peak shape parameter, which summed amplitude differences between calculated and measured profiles (Grange and Brumley 1996, 1996b, 1997). To observe such broadened profiles, usually the M+2 profile was acquired as a full profile, which required an additional data acquisition. Broad profiles were most often observed when one or more S atoms were present. It became apparent, however, that these two measures seldom, if ever, eliminated compositions that had passed the exact mass and RIA criteria, and they were soon abandoned as a simplification measure. In Table 1 and numerous similar tables for other analytes, the exact mass criteria of the M+1 and M+2 profiles eliminated many of the same compositions as the RIA criteria, with the RIA criteria eliminating additional compositions to provide a higher level of discrimination.

The sensitivity and wide dynamic range provided by a double focusing mass spectrometer enabled identification of compounds present over about three orders of magnitude. Figure 4 is a partial total ion chromatogram labeled with ion compositions for the apparent molecular ion for most of the chromatographic peaks along with many identifications or tentative identifications (Grange and Sovocool 2007).

While the three exact mass and two RIA criteria were passed for the correct ion composition in Table 1, for low-concentration analytes, passing of only 2-4 criteria was common. Especially for compounds with %M+2 values of less than 2%, even a resolving power of 10,000, was insufficient to eliminate interferences from molecular and product ions formed from coeluting compounds, PFK, or column bleed. The RIAs have been found to be more sensitive to interferences than the exact masses (Grange and Sovocool 2007). When more than one composition passed the same number of criteria, the presence of related compounds resulted in its selection as the preferred one.

**Obsolescence of ICE.** Although three other groups utilized MPPSIRD to determine several compositions of molecular ions (Vetter et al. 1999, Wu et al. 2002, Jörundsdóttir et al. 2006), widespread adoption of ICE was discouraged by the required custom software and time needed to acquire data. Four GC/MS data acquisitions were required to determine the exact masses and RIAs of 15 - 30 ions from individually eluting compounds. To determine ion compositions of apparent molecular ions from 100 chromatographic peaks required several weeks.

ASCII files of macro language instructions for either data system were prepared by a Lotus 123<sup>®</sup> spreadsheet (Lotus Development Corp., Cambridge, MA, USA). The data systems then automatically prepared SIR menus, displayed and integrated chromatographic peaks over

user-specified time-windows, and provided lists of  $m/z$  ratios and chromatographic peak areas from which profiles were plotted and exact masses and RIAs were calculated within a second Lotus 123 spreadsheet. Unfortunately, the data systems of current double focusing mass spectrometers no longer provide a macro language. Hence, performing ICE with newer instruments is not practical.

**Instrumentation:** *Accurate Mass Triple Quadrupole Mass Spectrometer (AM3QMS)*. A Thermo-Finnigan TSQ Quantum Ultra AM<sup>®</sup> triple quadrupole mass spectrometer (Thermo-Finnigan, San Jose, CA) provided masses accurate to within 5-10 mDa and RIAs usually accurate to within 10% in the absence of interferences for precursor and product ions from nine standards introduced by HPLC with atmospheric pressure chemical ionization (APCI) [Grange et al. 2006]. Data acquisition was simpler, since no custom software was required, but three types of scans requiring manually prepared  $m/z$  ratio lists and scan parameters were still needed to obtain exact mass and RIA values. First, a single full scan data acquisition was recorded from the third quadrupole (Q3) using a 0.7 mDa mass peak width as the collisionally induced dissociation (CID) voltage in Q2 was switched among -12, -24, or -26 V. Q2 contained 2 mTorr of argon. Mass spectra containing an abundant precursor ion (-12 V) and product ions (-24 V and -36 V) were obtained.

During the single data acquisition to measure exact masses, internal mass calibration was against ions formed from six compounds in a solution that was infused as individual analyte peaks eluted from the HPLC. Selected Reaction Monitoring (SRM) monitored each analyte precursor or product ion and calibration ions that bracketed the analyte ion mass. The mass peak widths for Q1 and Q3 were 0.7 Da and 0.1 Da, respectively. Q3 was scanned over a 1 Da mass window. Between 6 and 12 ions were monitored during elution of each analyte.

For the single data acquisition using SRM that measured RIAs, the mass peak widths were set to 10 Da and 0.5 Da for Q1 and Q3, respectively, and a 1 Da mass range was scanned by Q3. The wide Q1 peak width centered on the M+1 mass peak allowed all M, M+1, and M+2 ions to pass into Q3. Three mass peaks from each of three analytes were monitored during each data acquisition. For both SRM data acquisitions, the CID voltages that produced the greatest abundance for each ion provided their exact mass or RIAs. More detail on these experiments is provided in Grange et al. 2005.

**Instrumentation:** *orthogonal-acceleration, Time-of-Flight Mass Spectrometer (oa-TOFMS)*. Three full scan data acquisitions, one at each in-source CID voltage, were required to measure exact masses and RIAs when using an Agilent G1969A LC/MSD TOF with an Agilent G1948A electrospray ionization source. After initially selecting a mass range, scan rate, and other parameters to optimize instrument performance, all settings remained constant except for the cone (CID) voltage. Manual or automated entries of precursor ion masses or retention time window boundaries into analyte-specific menus were not required to select ions for fragmentation and mass analysis by a non-existent second MS stage. No prior knowledge of target ions and retention times for eight standards was required, because after mass calibration, the full scan data provided the exact masses and RIAs for every precursor ion and their product ions. The exact mass errors were 1-2 mDa and the RIAs were accurate to within 20% in the absence of interferences. Both mass error and RIA error limits were sufficient to greatly limit the number of possible compositions for an ion. Of the three types of mass spectrometer, the oa-TOFMS provided a major advantage in simplicity and greatly reduced the time required to measure exact masses and RIAs. More detail on acquiring such data and using it to identify compounds is provided in Grange et al. 2006. Note that if electron impact ionization were used,

only one full scan would provide the exact masses and RIAs for the product ions and, in many cases, the precursor ion.

**An Ion Correlation Program.** The higher error limits associated with both the AM3QMS and the oa-TOFMS necessitated devising a new data processing strategy to determine unique precursor ( $[M+H]^+$ ) ion compositions. Figure 5 illustrates that in accord with Figure 1a, the numbers of possible compositions for an exact mass within a specified error limit are less for product ions and their corresponding neutral losses than for the precursor ion. When the exact mass and RIAs of a precursor ion correspond to multiple possible compositions, the correct composition can be selected by determining and summing the unique compositions of the two related parts from the precursor ion.

An in-house Ion Correlation Program written in QuickBASIC<sup>®</sup> 4.5 based on a simplified PGM rejects numerous compositions, generally leaving only one for the precursor ion, each fragment ion, and each neutral loss, by comparing their compositions for consistency. All product ion:neutral loss pairs must sum to the composition of the precursor ion. The four-step computational process was described in Grange et al. 2006.

Because the resolving power of the AM3QMS was 3000 (FWHM) and that of the oa-TOFMS was 6000 (FWHM), no peak broadening was observed in mass peak profiles or considered in exact mass and RIA calculations. Calculation of Gaussian distributions was no longer necessary, and to provide additional simplification and to conform with common practice (Suzuki et al. 2005, Ojanperä et al. 2006, Kaufmann 2007), the exact masses of the M+1 and M+2 profiles were no longer considered. The exact masses of the M+1 and M+2 mass peaks are dependent on both the masses of the +1 and +2 isotopes and their isotopic abundances. The M+1 and M+2 mass peak dependence on the RIAs resulted in the redundancy observed in Table 1. In

addition, mass errors in mDa, rather than ppm, were adopted to avoid very large ppm errors for low-mass product ions. For example, a 5 mDa mass error for  $m/z$  77 would be 65 ppm. The observed mass error between 50 and 500 Da will be less variable when expressed in mDa than in ppm for exact masses measured with a TOFMS.

Table 2 compares the discriminating power of the M+1 and M+2 exact masses and RIAs for  $\pm 2$  mDa and  $\pm 20\%$  error limits for 11 compositions containing different sets of heteroatoms. The total number of possible compositions corresponds to a  $\pm 2$  mDa mass window about the calculated monoisotopic ion masses. For all 11 compositions, comparison of the M+1 and M+2 exact masses rejected fewer incorrect compositions than comparison of the RIAs. In four of five cases, unique compositions were not found for precursor ions with masses greater than 200 Da. To obtain unique compositions for these precursor ions, product ion:neutral loss pairs must be considered by the ICP. Only for two compositions did considering the M+1 and M+2 exact masses reject a composition not already eliminated by the M+1 and M+2 RIA criteria. The demonstration that the M+1 and M+2 RIAs provide greater discrimination among compositions than the exact masses would be even more compelling with the larger mass error ( $\pm 10$  mDa) and smaller RIA error ( $\pm 10\%$ ) for the AM3QMS data. Conversely, if a different type of mass spectrometer provided tighter mass error limits, but was incapable of providing RIAs accurate to within 20%, consideration of the M+1 and M+2 exact masses, rather than the RIAs, would be more effective for eliminating incorrect compositions. Because measured RIA values are more subject to interferences than measured exact mass values (Grange et al. 2005), instances might arise where considering the exact masses might be beneficial for the error limits used above. But for good-quality mass spectra, ignoring the exact masses of the M+1 and M+2 mass peaks is a simplification entailing minimal sacrifice.

Table 2. Rejection of Incorrect Compositions by M+1 and M+2 Exact Mass and RIA Criteria for 11 Compositions. Elements considered: C, H, N, O, F, P, S, and Cl when present.

Nominal Mass	Composition	Exact Mass $\pm$ 2 mDa # Rejected (# Possible)	RIA $\pm$ 20% # Rejected (# Possible)
124	C <sub>6</sub> H <sub>10</sub> N <sub>3</sub> <sup>+</sup>	2 (3)	3 (3)
166	C <sub>10</sub> H <sub>16</sub> NO <sup>+</sup>	3 (5)	5 (5)
170	C <sub>12</sub> H <sub>12</sub> N <sup>+</sup>	5 (9)	9 (9)
181	C <sub>12</sub> H <sub>9</sub> N <sub>2</sub> <sup>+</sup>	11 (16)	16 (16)
182	C <sub>8</sub> H <sub>8</sub> NS <sub>2</sub> <sup>+</sup>	30 (37)	37 (37)
195	C <sub>8</sub> H <sub>11</sub> N <sub>4</sub> O <sub>2</sub> <sup>+</sup>	10 (18)	18 (18)
214	C <sub>10</sub> H <sub>16</sub> NO <sub>2</sub> S <sup>+</sup>	22 (26)	25 (26)*
237	C <sub>15</sub> H <sub>13</sub> N <sub>2</sub> O <sup>+</sup>	28 (41)	41 (41)
265	C <sub>11</sub> H <sub>13</sub> N <sub>4</sub> O <sub>2</sub> S <sup>+</sup>	69 (95)	92 (95)*
285	C <sub>6</sub> H <sub>13</sub> Cl <sub>3</sub> O <sub>4</sub> P <sup>+#</sup>	9 (19)	13 (19)
319	C <sub>17</sub> H <sub>20</sub> N <sub>2</sub> ClS <sup>+^</sup>	12 (48)	42 (48)

\* 1 composition not rejected by RIA criteria was rejected by exact mass criteria.

# 3 Cl atoms were assumed to be present as would be apparent from the M+2 and M+4 peak abundances

^ 1 Cl atom was assumed to be present as would be apparent from the M+2 and M+4 peak abundances

**Ion Correlation and Mass Spectrum Deconvolution.** Our current research utilizes a Direct Analysis in Real Time (DART) JEOL AccuTOF<sup>®</sup> oa-TOFMS. The DART is a open-air, surface-sampling ion source that desorbs and ionizes analytes with a heated, metastable stream of helium. Elimination of sample extraction, extract clean up, and chromatography decreases the time per sample analysis more than 100-fold. An autosampler (Grange 2008a) and field sample carrier (Grange 2008b) were designed to permit analysis of 1000 cotton swab wipe samples in a single shift by one analyst. The disadvantage of the DART ion source is that composite mass spectra are often obtained. Figures 6a, b, and c are mass spectra acquired for a mixture of three compounds containing different sets of heteroatoms. Three cotton swabs were dipped into a methanol solution of the compounds, air dried, and transported through the 300°C helium stream in front of the acceptance cone (Orifice 1) into the mass spectrometer. The three mass spectra in Figure 6 were acquired at three Orifice 1 (CID) voltages: -15, -40, and -70 V. Swabs dipped into calibrant solutions customized for each Orifice 1 voltage were exposed to the stream immediately

after each analyte swab to provide external mass calibration. The three possible precursor ions in Figure 6a at m/z 182, 265, and 319 are also evident in Figure 6b, which confirms that they are precursor ions, because dimeric ions and their products are no longer observed at a moderate Orifice 1 voltage. In Figure 6c, product ions from all three precursor ions are evident. The ICP is also an ion non-correlation program. Ions that could only be produced from one of the three precursor ions will not be correlated with the other two. Table 5 lists the ions automatically gleaned from ASCII files of m/z ratios and mass peak areas provided by the data system for the three mass spectra by the ICP.

Table 5. Exact Masses and RIAs for Ions Gleaned from the Mass Spectra in Figure 6.

<u>Precursor and Product Ions</u>			<u>Precursor Ions</u>			
319.10143	923.1353	939.8882	319.10143			
274.04434	0	0	265.07498			
265.07498	13.09923	5.277359	182.00881			
246.01500	14.86971	36.5697				
239.07747	0	0				
233.00690	0	0				
204.04545	0	0				
199.10196	0	0				
182.00881	909.893	908.6573	<u>Oxides, Adducts, &amp; Dimers</u>			
166.98477	10.10156	9.237447	335.09830	0	0	O
156.01086	0	0				
135.01606	0	0				
124.07559	0	0				
110.07156	6.828284	0				
109.05297	0	0				
108.04546	0	0				
92.04958	0	0				
88.07481	0	0				
86.09627	6.086304	0				

Because RIAs are more susceptible to interferences, they are only calculated and listed above two monoisotopic mass peak area thresholds determined empirically from mass spectra obtained for 15 compounds. For the higher threshold, 900 is added to the %RIA so that during

ion correlation, a 15% RIA error limit will be used; above the lower threshold, a 20% RIA is used initially. Criteria testing with RIAs from only the isotopic mass peaks of the precursor ion and abundant product ions is effective, because once the composition of the precursor ion is limited by RIA criteria, all product ion:neutral loss pairs fragmented from the precursor ion are likewise limited to portions of the precursor ion.

The ICP first considers the data for the highest mass ion,  $m/z$  319, and then the data for each lower-mass ion sequentially. If no correlation is found, the second ion is also assumed to be a precursor ion to be correlated with other ions later. When a correlation is found, both the  $m/z$  319 ion data and that for the correlated ion are retained and the next lower-mass ion is considered. The previous comparisons are made before the lower-mass ion is considered to ensure all previously applied constraints on the possible precursor ion compositions are retained. The lowest-mass ion is compared only to the possible precursor ions, since multiple fragmentation pathways are possible, and one cannot be sure the lowest-mass ion fragmented from one of the previously correlated ions. This sequence is repeated until all lower-mass ions have been considered or skipped, because they are precursor ions. Table 6 illustrates this process for the  $m/z$  319 ion.

Table 6. Testing Sequence for Correlating Ions Using the ICP.

<u>Nominal Mass</u>	<u>Compositions Found</u>
319	4
319 & 274	2:2:1*
319, 274 & 246	1:1,1:1,1
319, 274, 246 & 239	1:1,1,1:1,1,1
319, 274, 246, 239 & 233	1:1,1,1,1:1,1,1,1
319, 274, 246, 239, 233 & 204	1:1,1,1,1,1:1,1,1,1,1
319, 274, 246, 239, 233, 204 & <del>199</del>	No compositions
319, 274, 246, 239, 233, 204 & <del>167</del>	No compositions
319, 274, 246, 239, 233, 204 & <del>156</del>	No compositions
319, 274, 246, 239, 233, 204 & <del>135</del>	No compositions
319, 274, 246, 239, 233, 204 & <del>124</del>	No compositions
319, 274, 246, 239, 233, 204 & <del>110</del>	No compositions
319, 274, 246, 239, 233, 204 & 109	1:1,1,1,1,1,1:1,1,1,1,1,1
319, 274, 246, 239, 233, 204, 109 & 108	1:1,1,1,1,1,1,1:1,1,1,1,1,1,1

\*Colons separate the number of compositions found for the precursor ion from those found for the product ions and those found for the product ions from those found for the neutral losses.

The ICP automatically uses multiple sets of mass and RIA error limits to ensure low abundance product ions are not discarded when the error in their measurement slightly exceeds the mass error limit passed by the precursor ion and more abundant product ions. The number of possible compositions listed is based on a  $\pm 2$  mDa mass error range when measured exact masses are accurate to within 2 mDa. More possible compositions may be listed when a  $\pm 4$  mDa mass error range and  $\pm 30\%$  RIA error range are required for a product ion to be recognized as such. More detail on how error limits are adjusted during ion correlation is available in Grange and Sovocool 2008. In Table 6, no more ions were checked after seven product ions were found due to memory limitations of QuickBASIC<sup>®</sup>. When the mass error limits for product ions and neutral losses were increased to  $\pm 4$  mDa and  $\pm 6$  mDa, respectively, m/z 199 and 135 were also found to correlate.

The sequence in Table 6 was then repeated for the other two precursor ions starting with m/z 265 or m/z 182. As listed in Table 7, multiple possible compositions were found for the m/z 319 and 265 ions when the elements C, H, N, O, F, P, S, (and 1 Cl for m/z 319) were considered. When the largest-mass product ion (m/z 274) and its corresponding neutral loss were considered, two of four possible compositions for the precursor ion (m/z 319) were eliminated, while two compositions were possible for the m/z 274 ion. M/z 265 was a precursor ion and was not considered as a possible product ion of m/z 319. The m/z 246 ion was found to correlate with the m/z 319 and 274 ions and only the correct composition remained for all three ions. The single possible composition for the m/z 246 ion rejected the incorrect precursor ion composition, which in turn eliminated the possible m/z 274 ion composition that was not a subunit of the remaining precursor ion composition.

Table 7. Reduction of Possible Precursor Ion Compositions when Product Ions Are Considered.\*

319.10143 (274.04434) (246.01500)	<b>C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>ClS<sup>+</sup></b> , C <sub>17</sub> H <sub>17</sub> N <sub>2</sub> OCIF <sup>+</sup> , C <sub>18</sub> H <sub>21</sub> OCIP <sup>+</sup> , C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> Cl <sup>+</sup> <b>C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>ClS<sup>+</sup></b> , C <sub>17</sub> H <sub>17</sub> N <sub>2</sub> OCIF <sup>+</sup> <b>C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>ClS<sup>+</sup></b>
274.04434 (246.01500)	C <sub>15</sub> H <sub>13</sub> NCIS <sup>+</sup> or C <sub>15</sub> H <sub>10</sub> NOCIF <sup>+</sup> + C <sub>2</sub> H <sub>7</sub> N C <sub>15</sub> H <sub>13</sub> NCIS <sup>+</sup> + C <sub>2</sub> H <sub>7</sub> N
246.01500	C <sub>13</sub> H <sub>9</sub> NCIS <sup>+</sup> + C <sub>4</sub> H <sub>11</sub> N
<hr/>	
265.07498 (204.04546) (156.01086)	C <sub>8</sub> H <sub>14</sub> N <sub>4</sub> O <sub>3</sub> FS <sup>+</sup> , C <sub>10</sub> H <sub>17</sub> O <sub>6</sub> S <sup>+</sup> , C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> F <sub>2</sub> PS <sup>+</sup> , C <sub>11</sub> H <sub>20</sub> FP <sub>2</sub> S <sup>+</sup> , <b>C<sub>11</sub>H<sub>13</sub>N<sub>4</sub>O<sub>2</sub>S<sup>+</sup></b> C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> F <sub>2</sub> PS <sup>+</sup> , <b>C<sub>11</sub>H<sub>13</sub>N<sub>4</sub>O<sub>2</sub>S<sup>+</sup></b> <b>C<sub>11</sub>H<sub>13</sub>N<sub>4</sub>O<sub>2</sub>S<sup>+</sup></b>
204.04546 (156.01086)	C <sub>8</sub> H <sub>12</sub> O <sub>4</sub> S <sup>+</sup> or C <sub>9</sub> H <sub>8</sub> N <sub>4</sub> S <sup>+</sup> + C <sub>2</sub> H <sub>5</sub> O <sub>2</sub> C <sub>9</sub> H <sub>8</sub> N <sub>4</sub> S <sup>+</sup> + C <sub>2</sub> H <sub>5</sub> O <sub>2</sub>
156.01086	C <sub>4</sub> H <sub>4</sub> N <sub>4</sub> OS <sup>+</sup> + C <sub>7</sub> H <sub>9</sub> O or C <sub>6</sub> H <sub>6</sub> NO <sub>2</sub> S <sup>+</sup> + C <sub>5</sub> H <sub>7</sub> N <sub>3</sub>

\* The correct precursor ion composition is in bold type.

The  $m/z$  204 ion was the first ion lower in mass than the  $m/z$  265 precursor ion to correlate and had the two possible product ion compositions and one corresponding neutral loss composition listed in Table 7. The next lower-mass ion to correlate with the  $m/z$  265 and 204 ions was the  $m/z$  156 ion. Although it could have two possible compositions with two corresponding neutral losses, both pairs of subunits could only be fragmented from one of the two remaining possible precursor ion compositions. Hence, only the correct precursor ion composition remained. These two examples from the composite mass spectra in Figure 6 illustrate the power for determining the unique composition of precursor ions by correlating their product ion:neutral loss pairs with them when multiple analytes provide ions.

Table 8 lists the correlations that were found. Several low-mass ion compositions were subunits of multiple precursor ions and were correlated with more than one precursor ion. Hence, deconvolution of composite mass spectra by applying exact mass and RIA criteria is not as complete as is deconvolution based on small differences in elution times or chromatographic peak shapes of ions when chromatography is used. In Table 8, the  $m/z$  135 ion is listed under all three precursor ions, because its composition is a subunit of each one, and which precursor ion(s) produced it is indiscernible. The  $m/z$  108, 109, and 204 ions correlated with both the  $m/z$  319 and 265 precursor ions, but the  $m/z$  108 and 204 ions can be ascribed to the  $m/z$  265 precursor ion, since no single Cl atom isotopic pattern is evident for these ions in Figure 6c. The  $m/z$  92 is a subunit of all three precursor ions but is only listed under the  $m/z$  265 and 182 precursor ions. For the  $m/z$  319 ion, seven correlated ions were found using both error limits before the lower-mass  $m/z$  92 ion could be tested. The high-abundance  $m/z$  86 ion in Figure 6b is due to  $C_3H_{12}N^+$ , a subunit of both the  $m/z$  265 and 319 precursor ions, but in both cases, seven higher masses had already been correlated.

Table 8. Ion Correlation Test Results for Ions from Three Analytes

Ion Exact Mass	Composition	Mass (Error)	Ion Exact Mass	Composition	Mass (Error)	Ion Exact Mass	Composition	Mass (Error)
<b>319.10143*</b>	<b>C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>ClS<sup>+</sup></b>	<b>(-1.6)</b>	<b>265.07498</b>	<b>C<sub>11</sub>H<sub>13</sub>N<sub>4</sub>O<sub>2</sub>S<sup>+</sup></b>	<b>(-0.4)</b>	<b>182.00881</b>	<b>C<sub>8</sub>H<sub>8</sub>NS<sub>2</sub><sup>+</sup></b>	<b>(-0.5)</b>
<b>274.04434</b>	<b>C<sub>15</sub>H<sub>13</sub>NCIS<sup>+</sup></b>	<b>(-0.8)</b>	204.04545	C <sub>9</sub> H <sub>8</sub> N <sub>4</sub> S <sup>+</sup>	(-0.0)	<b>166.98477</b>	<b>C<sub>7</sub>H<sub>5</sub>NS<sub>2</sub><sup>+</sup></b>	<b>(-1.0)</b>
	C <sub>2</sub> H <sub>7</sub> N <sup>#</sup>	(-0.2)		C <sub>2</sub> H <sub>5</sub> O <sub>2</sub>	(+1.1)		CH <sub>3</sub>	(1.1)
<b>246.01500</b>	<b>C<sub>13</sub>H<sub>9</sub>NCIS<sup>+</sup></b>	<b>(+1.1)</b>	<b>156.01086</b>	<b>C<sub>4</sub>H<sub>4</sub>N<sub>4</sub>OS<sup>+</sup></b>	<b>(+0.8)</b>	135.01606	C <sub>7</sub> H <sub>5</sub> NS <sup>+</sup>	(2.3)
	C <sub>4</sub> H <sub>11</sub> N	(-2.2)		C <sub>7</sub> H <sub>9</sub> O	(-0.7)		CH <sub>3</sub> S	(-2.2)
<b>239.07747</b>	<b>C<sub>15</sub>H<sub>13</sub>NS<sup>+</sup></b>	<b>(+1.1)</b>		C <sub>6</sub> H <sub>6</sub> NO <sub>2</sub> S <sup>+</sup>	(-0.5)	92.04958	C <sub>6</sub> H <sub>6</sub> N <sup>+</sup>	(0.1)
	C <sub>2</sub> H <sub>7</sub> NCl	(-2.2)		C <sub>5</sub> H <sub>7</sub> N <sub>3</sub>	(+0.7)		C <sub>2</sub> H <sub>2</sub> S <sub>2</sub>	(-0.0)
<b>233.00690</b>	<b>C<sub>12</sub>H<sub>8</sub>NCIS<sup>+</sup></b>	<b>(+0.9)</b>	135.01606	C <sub>6</sub> H <sub>3</sub> N <sub>2</sub> O <sub>2</sub> <sup>+</sup>	(-2.8)			
	C <sub>5</sub> H <sub>12</sub> N	(-0.2)		C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> S	(+3.0)			
204.04545	C <sub>11</sub> H <sub>9</sub> N <sub>2</sub> Cl <sup>+</sup>	(+0.6)		C <sub>7</sub> H <sub>5</sub> NS <sup>+</sup>	(+2.3)			
	C <sub>6</sub> H <sub>11</sub> S	(-1.6)		C <sub>4</sub> H <sub>8</sub> N <sub>3</sub> O <sub>2</sub>	(-2.2)			
<b>199.10196</b>	<b>C<sub>10</sub>H<sub>16</sub>N<sub>2</sub>Cl<sup>+</sup></b>	<b>(+2.3)</b>	<b>124.07559</b>	<b>C<sub>5</sub>H<sub>8</sub>N<sub>4</sub><sup>+</sup></b>	<b>(+1.2)</b>			
	C <sub>7</sub> H <sub>4</sub> S	(-3.4)		C <sub>6</sub> H <sub>5</sub> O <sub>2</sub> S	(-1.1)			
135.01606	C <sub>7</sub> H <sub>5</sub> NS <sup>+</sup>	(+2.3)		C <sub>7</sub> H <sub>10</sub> NO <sup>+</sup>	(-0.1)			
	C <sub>10</sub> H <sub>15</sub> NCl	(-3.4)		C <sub>4</sub> H <sub>3</sub> N <sub>3</sub> OS	(+0.3)			
109.05297	C <sub>3</sub> H <sub>10</sub> N <sub>2</sub> Cl <sup>+</sup>	(+0.3)	<b>110.07156</b>	<b>C<sub>5</sub>H<sub>8</sub>N<sub>3</sub><sup>+</sup></b>	<b>(+0.3)</b>			
	C <sub>15</sub> H <sub>10</sub> S	(-1.3)		C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> S	(-0.1)			
108.04546	C <sub>3</sub> H <sub>9</sub> N <sub>2</sub> Cl <sup>+</sup>	(+0.6)		C <sub>7</sub> H <sub>10</sub> O <sup>+</sup>	(-1.1)			
	C <sub>14</sub> H <sub>11</sub> S	(-1.6)		C <sub>4</sub> H <sub>3</sub> N <sub>4</sub> OS	(+1.2)			
			109.05297	CH <sub>9</sub> N <sub>4</sub> S <sup>+</sup>	(-1.3)			
				C <sub>10</sub> H <sub>4</sub> O <sub>2</sub>	(+1.4)			
				C <sub>6</sub> H <sub>7</sub> NO <sup>+</sup>	(+0.8)			
				C <sub>5</sub> H <sub>6</sub> N <sub>3</sub> OS	(-0.6)			
			108.04546	CH <sub>8</sub> N <sub>4</sub> S <sup>+</sup>	(-0.0)			
				C <sub>10</sub> H <sub>5</sub> O <sub>2</sub>	(+1.1)			
				C <sub>6</sub> H <sub>6</sub> NO <sup>+</sup>	(+1.1)			
				C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> OS	(-0.9)			
			92.04958	C <sub>6</sub> H <sub>6</sub> N <sup>+</sup>	(+0.1)			
				C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> S	(+0.1)			

\*Ions in bold type correlated with only one precursor ion.

# Indented compositions are neutral losses corresponding to the preceding product ion.

When a surface sampling ionization technique is used, chromatography is not employed and uncertainly remains for the source of such low-mass ions. However, the unique composition of all three precursor ions was obtained in this example due to unequivocal assignment of multiple product ions each to the m/z 319 and 265 precursor ions as illustrated in Table 8. The exact mass and RIA criteria provided only one composition for the lowest-mass precursor ion (m/z 182) even before product ion:neutral loss pairs were correlated.

To tentatively identify the three compounds, the measured exact masses of the protonated molecules and the RIAs could be compared to calculated values within an exact mass and RIA library. Mass spectra acquired for standards are not required to compile the library; exact masses and RIAs are simply calculated from the composition of the protonated molecule. For example, several compounds with a molecular weight of 264 g/mole were found in Pflieger et al. 1992. Those compositions with exact masses for the protonated molecules closest to the exact mass of the m/z 265 precursor ion in Figure 6 are listed in Table 9. The structures of the compounds in Table 9 contained at least one primary, secondary, or tertiary amine and would be expected to provide a protonated molecule with ESI, APCI, or DART ionization.

Table 9. An exact mass and RIA library for m/z 265.

<u>Compound</u>	<u>Composition</u>	<u>Exact Mass</u>	<u>%1 RIA</u>	<u>%2 RIA</u>	<u><math>\Delta M(\text{mDa})^*</math></u>
Sulfadizole	$\text{C}_7\text{H}_{13}\text{N}_4\text{O}_3\text{S}_2^+$	265.04236	11.03	10.02	
Furazidine	$\text{C}_{10}\text{H}_9\text{N}_4\text{O}_5^+$	265.05675	12.78	1.76	14.4
Sulfamerazine	$\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2\text{S}^+$	265.07537	14.61	5.83	18.6
Sulfaperin	$\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2\text{S}^+$	265.07537	14.61	5.83	0.0
Temodox	$\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_5^+$	265.08190	14.30	1.95	6.5
Sulfirame	$\text{C}_{10}\text{H}_{21}\text{N}_2\text{S}_3^+$	265.08614	14.36	14.25	4.2
Analyte	$\text{C}_{11}\text{H}_{13}\text{N}_4\text{O}_2\text{S}^+$	265.07498	13.10	5.28	

\* The mass difference between the exact masses of the previous composition and the one in this row.

The measured exact masses for precursor ions from 2-(methylthio)-benzothiazole, sulfamerazine, and chlorpromazine, 182.00881, 265.07498, and 319.10143, differed by -0.5, -0.4, and -1.6 mDa, respectively, from their calculated exact masses. In each case, the correct composition would be further investigated in data bases of compounds. For example, in the SciFinder<sup>®</sup> (Chemical Abstract Services) data base, 14,474, 2,022, and 403 references were found for chlorpromazine, sulfamerazine, and 2-(methylthio)-benzothiazole, respectively. For 3-methyl-2(3H)-benzothiazolethione, 159 references were found and for Sulfaperin (isosulfamerazine) 138 references. To determine the correct isomers producing m/z 182 and 265

and to confirm the tentative identification of chlorpromazine, all five standards would be purchased and HPLC/TOFMS would be used to compare retention times and mass spectra acquired with different CID voltages.

**Conclusion.** The measurement of exact masses and RIAs to determine compositions of ions observed in mass spectra has become much simpler over the past decade. Initially, our group plotted mass peak profiles from SIR data acquired with double focusing mass spectrometers. Full scan and three SIR data acquisitions were required, with automatically-prepared, analyte-specific SIR menus, which required prior input from the operator. Today, with ESI, APCI, or DART ionization, three full scan data acquisitions at different CID voltages with a single-MS-stage oa-TOFMS provide exact masses and RIAs of sufficient accuracy to determine ion compositions of precursor ions, product ions, and neutral losses without the need to limit data collection to ions from a list of target analytes. Concurrently, data interpretation has been streamlined and made conceptually simpler. Initially, exact masses and RIAs were calculated based on summations of Gaussian distributions calculated for each +1 and +2 ion that contributed to the M+1 and M+2 profiles, which could be broadened at 10,000 (10% valley) resolving power. Now, simple weighed averages based on the exact masses of +1 and +2 ions and their RIAs are summed, since a resolving power of 6000 (FWHM) does not cause significant profile broadening. To compensate for the wider mass and RIA error limits of an oa-TOFMS relative to a double focusing instrument, an Ion Correlation Program was developed to restrict the number of possible compositions of precursor ions by correlating the compositions of the product ion:neutral loss pairs with the precursor ions. In addition, the simplicity of an oa-TOFMS provides a lower purchase cost, greater reliability, and a smaller footprint. Less operator expertise is required to acquire data and the time needed to determine ion compositions has been

greatly reduced.

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## Captions

Figure 1. (a) the number of possible compositions as a function of mass for four different error limits. The elements C, H, N, O, P, S, and Cl were considered for masses between 100.0000 and 400.0000 Da at 20 Da intervals. At  $m/z$  400, 4 mDa equals 10 ppm and the number of possible compositions is the same for the two error limits. For masses below 400 Da, 4 mDa is greater than 10 ppm and fewer compositions are possible for the 10 ppm error limit. The opposite is true from masses greater than 400 Da. (b) the number of possible compositions as a function of the mass defect calculated at 0.05 Da intervals between 318.5500 and 319.4500 Da for the same elements and a mass error limit of  $\pm 2$  mDa (Grange et al. 2006).

Figure 2. (a) partial list of possible compositions for a measured monoisotopic exact mass of 319.1039 Da assuming 1/3 of the ion's mass is due to C atoms and a mass error limit of  $\pm 2$  mDa. (b) partial list of possible compositions for a nominal mass of 319 Da and measured RIAs of 19.00% and 36.78% assuming an RIA error limit of  $\pm 20\%$ . (c) list of possible compositions found in both partial lists. The correct composition is enclosed by a rectangle (Grange et al. 2006).

Figure 3. Three outputs provided by a Lotus 123<sup>®</sup> spreadsheet using SIR data: (a) full profile, 100 ppm mass increment, and 3,000 resolving power; (b) full profile, 10 ppm increments, and 10,000 resolving power; and (c) partial profiles, 10 ppm increment, and 10,000 resolving power.

Figure 4. A partial total ion chromatogram for a methylene chloride extract from water above a plume of contaminants from a chemical plant (Grange and Sovocool 2007).

Figure 5. (a) A plot of the possible compositions calculated for the measured exact masses of a precursor ion and seven of its product ions and (b) the seven corresponding neutral

losses. The mass error limit is  $\pm 2$  mDa for the ions and  $\pm 2.83$  mDa ( $\sqrt{2} \times 2$  mDa) for the neutral losses, which are determined as the difference in measured exact masses for the precursor and product ions (Grange et al. 2006).

Figure 6. Mass spectra acquired with a DART/oa-TOFMS for three compounds on cotton swabs at Orifice 1 (CID) voltages of -15, -40, and -70 V. No Cl isotopic pattern was observed in the magnified portions of the spectrum for the m/z 108 or 204 ions in (c).



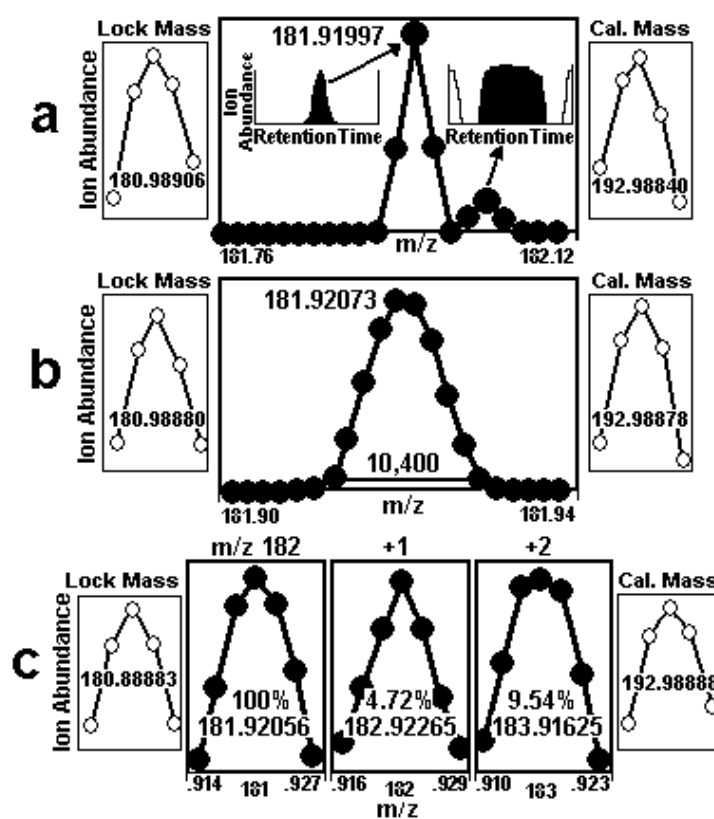


Figure 3.

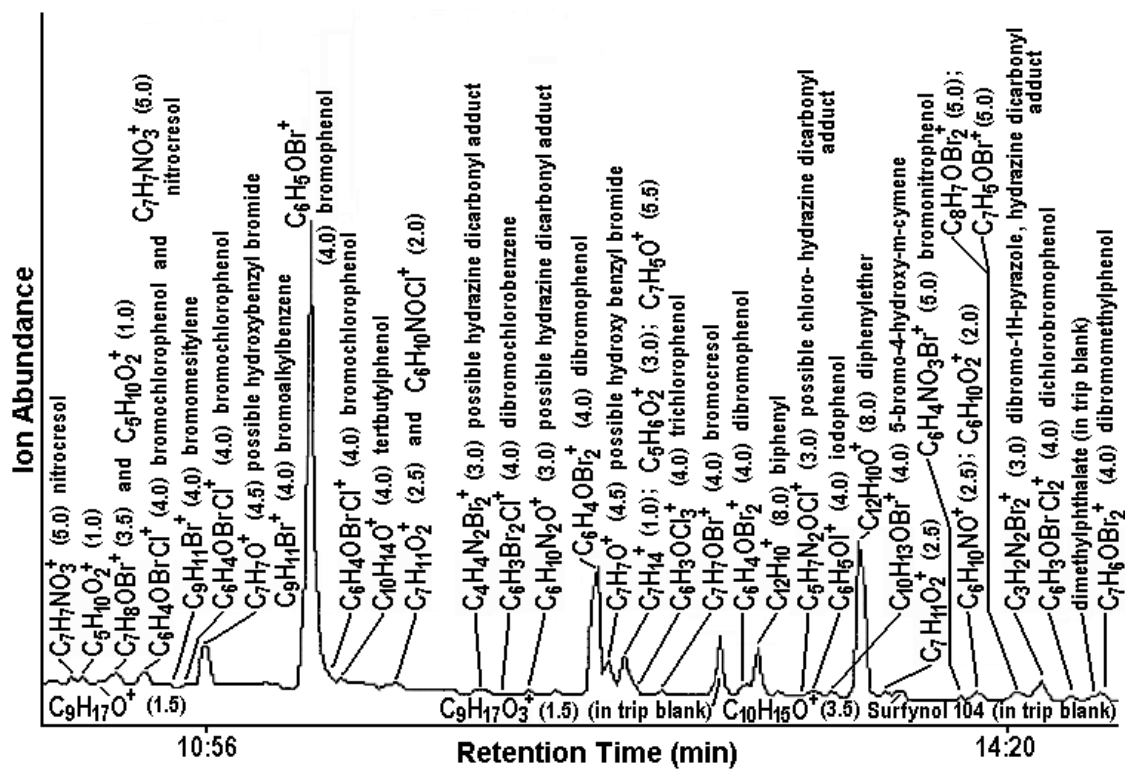


Figure 4.

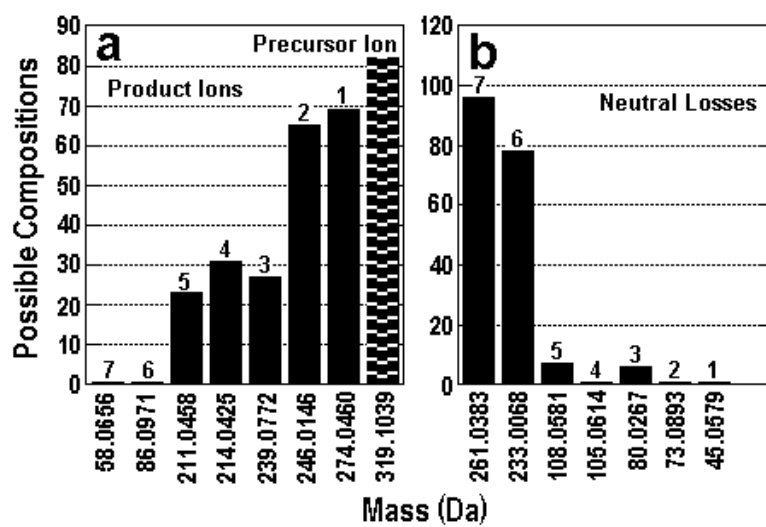


Figure 5.

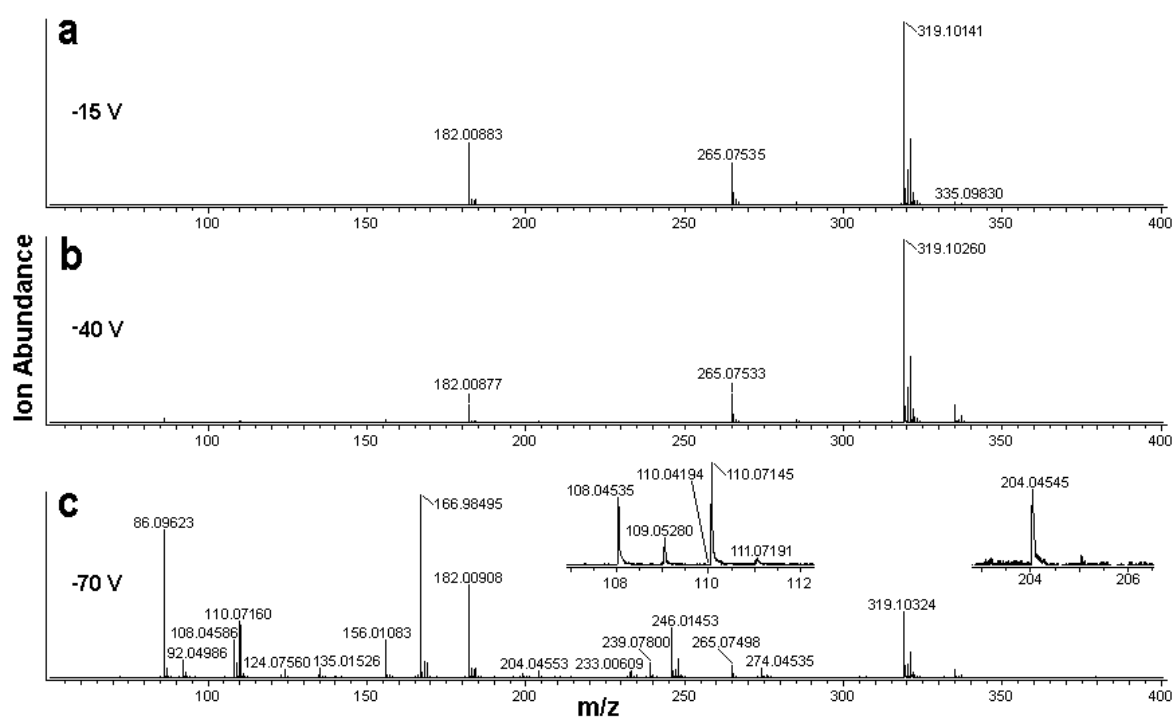


Figure 6.