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Ion Compositions Determined with Increasing Simplicity

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PART II

TOOLS FOR UNKNOWN IDENTIFICATION USING ACCURATE MASS

CHAPTER 3

ION COMPOSITIONS DETERMINED WITH INCREASING SIMPLICITY

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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 and limit the number of possible compound identities. Double focusing mass spectrometers provided both exact mass and RIA measurements in the early 1990s, while today, less expensive, more robust, and easier to operate time-of-flight mass spectrometers (TOFMSs) 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. This chapter briefly reviews the evolution in the types of mass spectrometers, data acquisitions, and automated determination of ion compositions used in our laboratory to more easily and rapidly identify compounds.

3.1 INTRODUCTION

For 15 years our laboratory has identified compounds in environmental extracts with little or no knowledge of the sampling site history [1–12]. Elements found in molecules have included C, H, N, O, Cl, Br, F, I, S, P, Si, As, and Se. Data acquired with a double focusing, high-resolution mass spectrometer using a successive approximation approach provided exact masses and relative isotopic abundances (RIAs) of ions of sufficient accuracy to establish the elemental composition of the molecular ion or highest-mass product ion. In-house software automated set up for analyte-specific data acquisition and for data processing, including comparison of measured and calculated exact masses for often numerous possible compositions.

Liquid Chromatography Time-of-Flight Mass Spectrometry: Principles, Tools, and Applications for Accurate Mass Analysis, Edited by Imma Ferrer and E. Michael Thurman

Over the past few years, the instrumentation, data acquisition, and automated data evaluation necessary to determine ion compositions have evolved toward greater simplicity. Consequently, others are now identifying contaminants found in water [13] and food [14, 15] with relative ease. This chapter describes our parallel progressions in instrumentation, data acquisition methods, and data interpretation. Double focusing mass spectrometers, an accurate-mass triple quadrupole mass spectrometer, and an orthogonal-acceleration, time-of-flight mass spectrometer have been used to acquire accurate-mass data using selected ion recording, multiple reaction monitoring, and full scanning, respectively. Data processing has evolved from using a mass-resolution-dependent Profile Generation Model to determine ion compositions to using a simpler Ion Correlation Program to determine precursor ion, product ion, and corresponding neutral loss compositions for mass spectra produced by single or multiple analytes.

3.2 COMPOUND IDENTIFICATION

The NIST [16] or Wiley [17] libraries often provide mass spectra similar or related 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 a proton or captured an electron 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 databases [18, 19] such as the SciFinder® compilation of known isomers [20] further reduces the number of plausible isomers. A tentative identification is confirmed when a purchased or synthesized standard with a verified structure provides the same mass spectrum and retention time by gas chromatography or liquid chromatography as the analyte.

3.3 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 lowest-mass isotopes are unique for different sets of atoms. The RIAs of the +1 and +2 isotopes of the elements [21] most commonly found in organic molecules, C, H, N, O, Br, Cl, F, I, P, S, and Si result in the abundance of the M + 1 mass peak providing an estimate of the number of C and Si atoms in an ion, while the abundance of the M + 2 mass peak provides estimates of

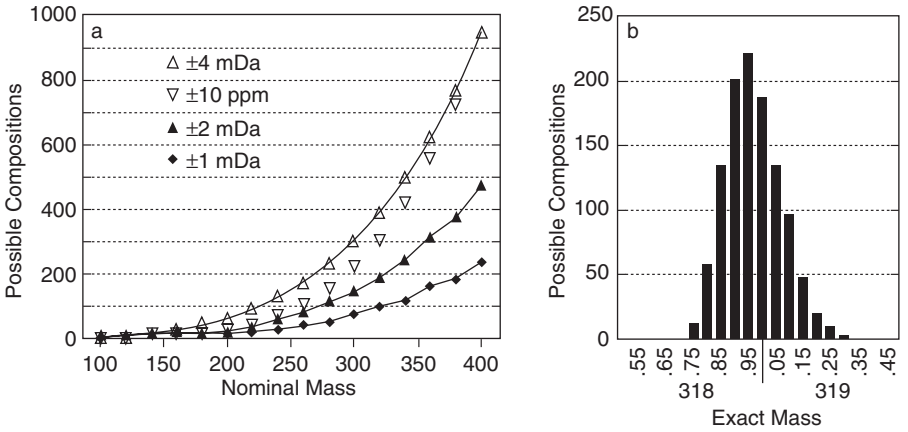

Figure 3.1. (a) the number of possible compositions as a function of mass for four different error limits. The elements C, H, N, O, P, S, and Cl were considered for masses between 100.0000 and 400.0000 Da at 20-Da intervals. At m/z 400, 4 mDa equals 10 ppm and the number of possible compositions is the 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 ±2 mDa [19].

the number of S, Si, Cl, and Br atoms present. Note that the generic (M) can be a molecule that has lost an electron or gained a proton (positive ion) or lost a proton or captured an electron (negative ion).

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 3.1 illustrates that the number of possible compositions increases exponentially with increasing mass (Figure 3.1a), increases nearly linearly with increasing mass error (Figure 3.1a), and is highly dependent on the mass defect measured for an ion (Figure 3.1b). 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 approximately halves the number. The number of possible compositions based on consideration of RIAs alone also increases as an ion's mass or the RIA error limit increases.

Figure 3.2 illustrates for one set of mass error and RIA error limits (±2 mDa 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, an antipsychotic drug [19]. Even assuming that at least one third of the mass of the ion was due to C atoms, 47 compositions were possible for the protonated molecule (82 compositions without the assumption). Considering only the RIAs, 402 compositions were possible. But when both the exact masses and RIAs were

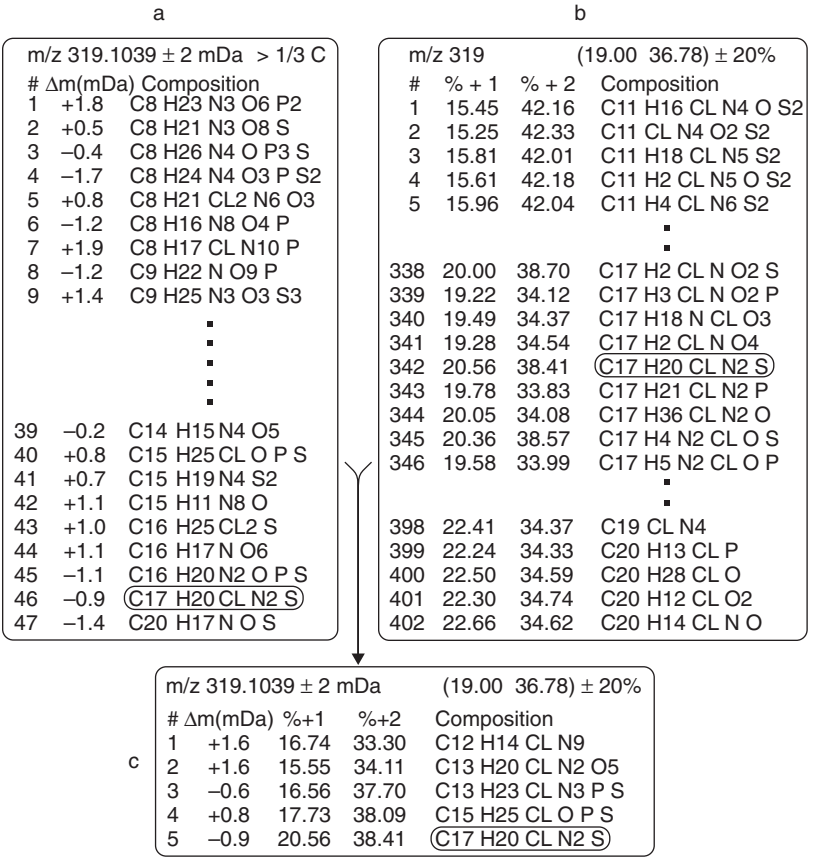

Figure 3.2. (a) partial list of possible compositions for a measured monoisotopic exact mass of 319.1039 Da assuming one third of the ion's mass is due to C atoms and a mass error limit of ±2 mDa with consideration of C, H, N, O, P, S, and Cl atoms. (b) Partial list of possible compositions for a nominal mass of 319 Da and measured RIAs of 19.00% and 36.78% assuming an RIA error limit of ±20% for consideration of the same elements. (c) List of possible compositions found in both partial lists. The correct composition is enclosed by a modified ellipse [19].

considered, only 5 compositions remained viable. Clearly, for the purpose of determining ion compositions, it is advantageous to measure both exact masses and RIAs.

3.4 DOUBLE FOCUSING MASS SPECTROMETER

To accurately measure exact masses and RIAs for coeluting compounds in complex environmental extracts, high mass resolving power was necessary. In the early 1990s, 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 [22, 23]. To provide low error limits for quantitation ^{13}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 [24–26]. With 2200 high production chemicals (10^6 lbs/yr or more) [27] and 87,000 compounds used in commerce [28], 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 3.3. First, a full scan, background-subtracted, 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 ionization ion source. For each compound, the apparent molecular ion (largest-mass, monoisotopic 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 of 25 available *m/z* ratios were used [1–3, 10, 24–26, 29–32] or a Finnigan MAT 950S mass spectrometer for which 31 of 32 available *m/z* ratios were used [4–9, 11, 12].

In the middle part of Figure 3.3a, a narrow mass range about a monoisotopic ion from an analyte found in an extract of ground water was monitored with a resolving power of 3000 and an *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 3.3a. The two mass peak profiles, each delineated by three points, were obtained for an analyte ion (left peak inset) and a perfluorokerosene (PFK) ion (right peak inset). The insets are the ion chromatograms for the *m/z* ratios at the maxima of the two peaks. For the PFK peak, two baseline excursions are evident in the inset 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 (left plot) and calibration (right plot) ions that bracket the analyte ion mass. The exact masses of all full or partial mass peak profiles in Figure 3.3 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 3.3a was used as the center mass in the SIR *m/z* ratio list to acquire the ion chromatograms used to plot the analyte ion profile in Figure 3.3b. 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.

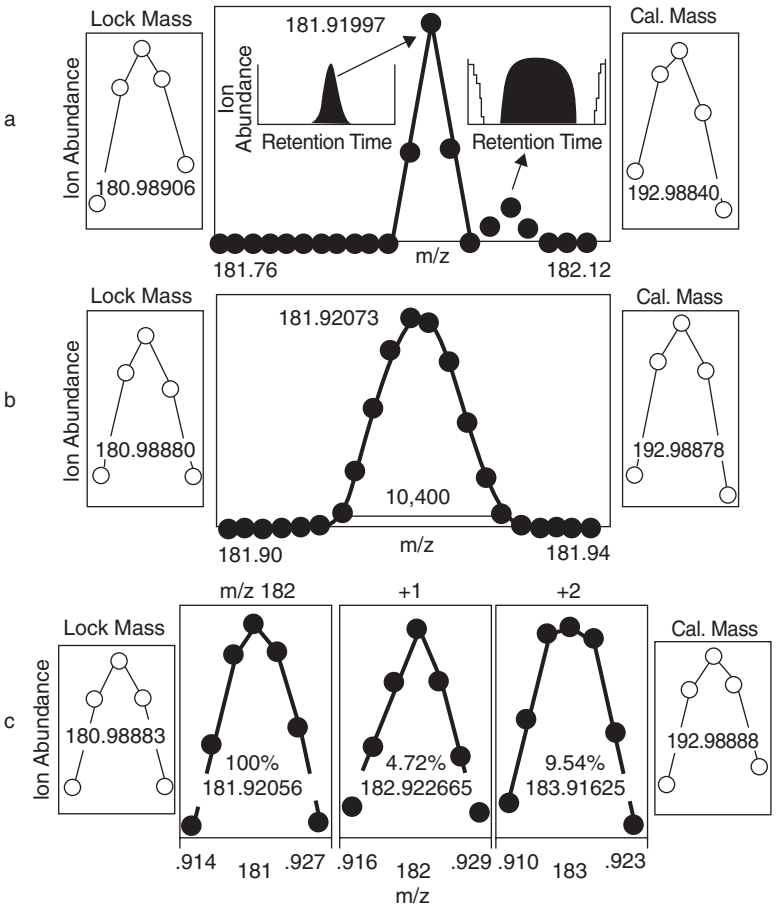

Figure 3.3. Three outputs provided by a Lotus 123® spreadsheet using SIR data: (a) full profile, 100-ppm mass increment, and 3000 resolving power; (b) Full profile, 10-ppm increments, and 10,000 resolving power; and (c) Partial profiles, 10-ppm increment, and 10,000 resolving power.

Experience has shown that the possible composition containing the fewest heteroatoms other than halogen atoms is most likely to be correct when identifying compounds in complex environmental extracts. Hence, 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 3.3c. 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 3.3c. This use of SIR to delineate profiles to provide exact masses and RIAs was called Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) [25].

3.5 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® version 4.5 (Microsoft Corp., Bellevue, WA) 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 [26]. 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 3.1 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 [26]. Using MPPSIRD and the PGM together was referred to as Ion Composition Elucidation (ICE) in several papers [6–10].

Initially, two other criteria based on the width and shape of the full +2 profile 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 was the sum of amplitude differences between measured profiles and profiles calculated for each possible composition [26, 30, 31]. 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 3.1 and numerous similar tables for other analytes, the exact mass and RIA criteria of the M + 1 and M + 2 profiles eliminated many of the same compositions, but additional compositions

TABLE 3.1. Calculated Exact Masses and RIAs for Eight Compositions* and the Measured Values

Commposition	182 M	183 M + 1	184 M + 2	%M + 1 (%1 Range)#	%M + 2 (%2 Range)#
$HNOF_3P_2S$	.92007	.91896 X‡	.91623	1.21 (1.02–1.39) X	4.63 (4.02–5.25) X
$HNOAsF_4$	.92047	.91842 X	.92472 X	0.42 (0.36–0.48) X	0.20 (0.17–1.23) X
$H_2NO_2F_2S_3$	.92102	.92029 X	.91707	2.83 (2.38–3.29) X	13.72 (11.92–15.53) X
$N_4O_2P_2S$	.92117	.91924 X	.91767 X	2.33 (1.99–2.68) X	4.85 (4.21–5.50) X
N_4O_2AsF	.92157	.91896 X	.92561 X	1.55 (1.33–1.76) X	0.41 (0.34–0.48) X
$C_2NO_5S_2$	.92124	.92265	.91794 X	4.34 (3.69–4.99)	9.94 (8.60–11.28)
C_3HNOFS_3	.91988	.93132 X	.91588	6.09 (5.19–6.99) X	13.65 (11.86–15.44) X
$C_3H_7AsS_2$✓	.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 [7], because the mass of the electron, 0.00055 Da, had not been taken into account [33].

The permissible ranges result from consideration of several errors associated with plotting partial profiles [26].

‡ An "X" indicates the measured and calculated values did not agree within mass or RIA error limits.

were eliminated by the RIA criteria, which provided a higher level of discrimination. No chemical reasoning was included in the PGM to eliminate compositions beyond requiring that the rings and double bonds be zero or more for electron ionization or –0.5 or more for ESI or APCI.

The sensitivity and wide dynamic range provided by a double focusing mass spectrometer enabled identification of compounds present over about three orders of magnitude of ion abundance. Figure 3.4 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 [10].

While the three exact mass and two RIA criteria were passed for the correct ion composition in Table 3.1, for low-concentration analytes, passing of only two to four 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 [10]. When more than one composition passed the same number of criteria, the presence of related compounds was used as a criterion to select a single composition.

3.6 OBSOLESCENCE OF ICE

Although two other groups utilized MPPSIRD to determine compositions of several molecular ions [34–36], 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.

Text files of macro language instructions for either data system were prepared by a Lotus 123® spreadsheet (Lotus Development Corp., Cambridge, MA). 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.

3.7 ACCURATE MASS TRIPLE QUADRUPOLE MASS SPECTROMETER (AM3QMS)

A Thermo-Finnigan TSQ Quantum Ultra AM® 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

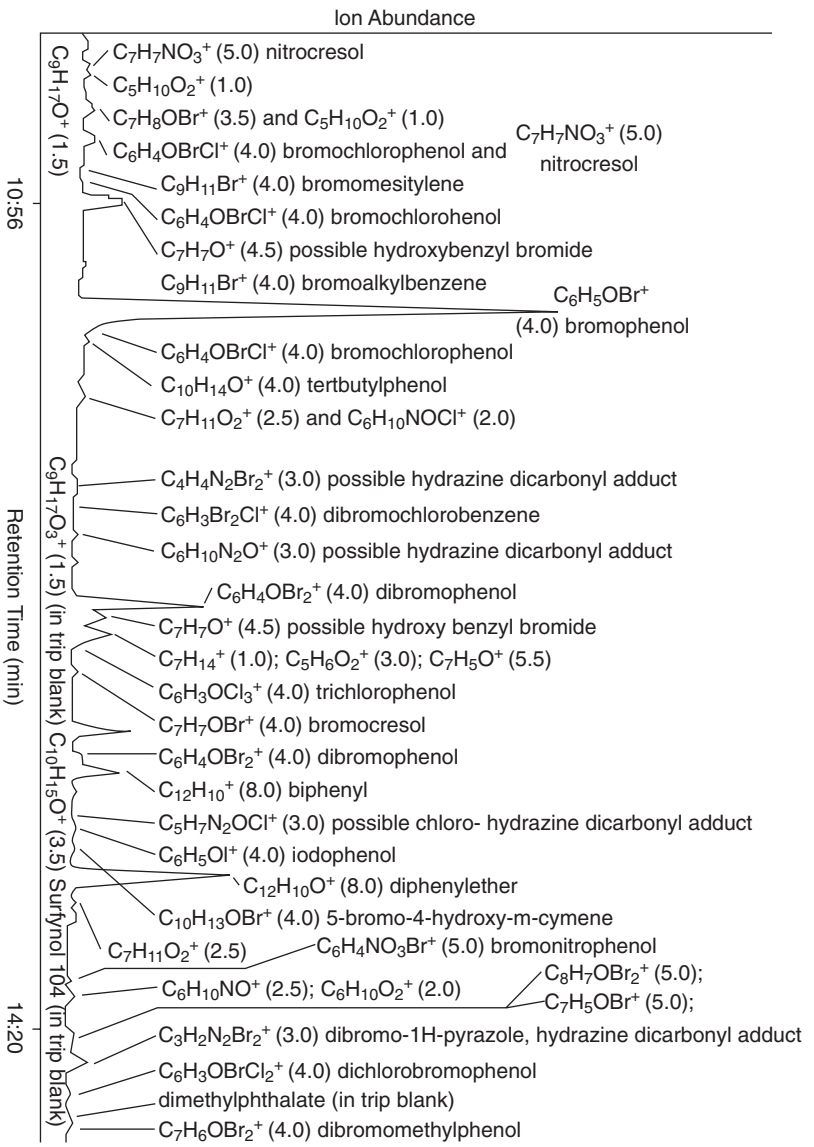

Figure 3.4. A partial total ion chromatogram for a methylene chloride extract from water above a plume of contaminants from a chemical plant [10].

precursor and product ions from nine standards. A solution of the standards was introduced by HPLC and atmospheric pressure chemical ionization (APCI) was used [19]. 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 −36 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) was used to monitor 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 different *m/z* ratios 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 were used when determining their exact mass or RIAs. More detail on these experiments is provided in ref. 18.

3.8 ORTHOGONAL-ACCELERATION, TIME-OF-FLIGHT MASS SPECTROMETER (oa-TOFMS)

Three full scan data acquisitions, one each with different in-source CID voltages, 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 fragmentor (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 nonexistent 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 ref. 19. Note that if GC/oa-TOFMS with electron ionization were used, only one full scan would provide the exact masses and RIAs for the product ions and, in many cases, the molecular ion.

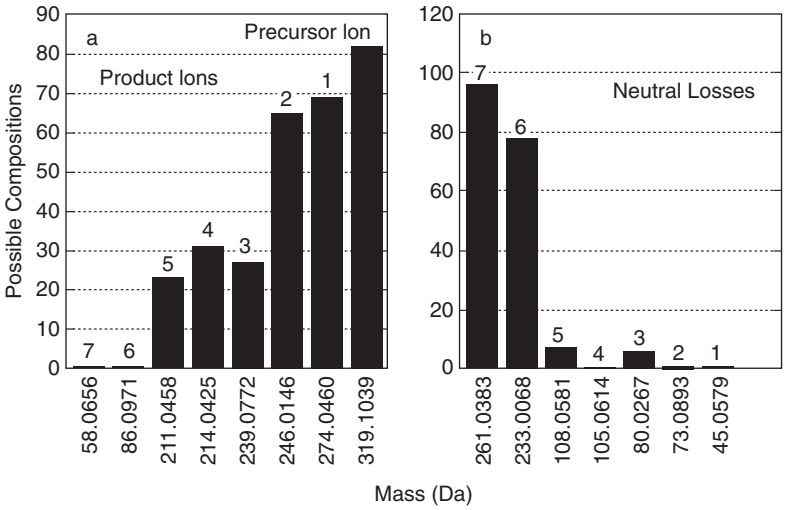

Figure 3.5. (a) A plot of the possible compositions calculated for the measured exact masses of a precursor ion and seven of its product ions and (b) the seven corresponding neutral losses. The mass error limit is ±2 mDa for the ions and ±2.83 mDa ($\sqrt{2} \times 2$ mDa) for the neutral losses, which are determined as the difference in measured exact masses for the precursor and product ions [19]. The numbers atop each vertical bar correlate the product ion:neutral loss pairs.

3.9 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 3.5 illustrates that in accord with Figure 3.1a, 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 a product ion:neutral loss pair produced from the precursor ion.

An in-house Ion Correlation Program written in QuickBASIC® 4.5 and based on a simplified PGM rejects numerous compositions, generally leaving only one for the precursor ion, each product 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 ref. 19.

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 for a given resolving power was no longer necessary. To provide additional simplification and to conform with common practice [37–39], the exact

TABLE 3.2. Rejection of Incorrect Compositions by M + 1 and M + 2 Exact Mass and RIA Criteria for 15 Compositions[&]

Nominal Mass	Composition	M + 1 and M + 2 Exact Masses # Rejected (# Possible)	M + 1 and M + 2 RIAs # Rejected (# Possible)
124	$C_6H_{10}N_3^+$	2 (4)	3 (4)
166	$C_{10}H_{16}NO^+$	3 (6)	5 (6)
170	$C_{12}H_{12}N^+$	5 (10)	9 (10)
181	$C_{12}H_9N_2^+$	11 (17)	16 (17)
182	$C_8H_8NS_2^+$	30 (38)	37 (38)
195	$C_8H_{11}N_4O_2^+$	10 (19)	18 (19)
214	$C_{10}H_{16}NO_2S^+$	22 (27)	25 (27)[*]
237	$C_{15}H_{13}N_2O^+$	28 (42)	41 (42)
265	$C_{11}H_{13}N_4O_2S^+$	69 (96)	88 (96)[*]
285	$C_6H_{13}Cl_3O_4P^{+\#}$	9 (20)	13 (20)
319	$C_{17}H_{20}N_2ClS^+\wedge$	12 (49)	42 (49)
330	$C_{19}H_{21}NO_3F^+$	74 (105)	100 (105)
380	$C_{24}H_{22}N_5^+$	103 (158)	152 (158)
419	$C_{25}H_{39}O_5^+$	43 (80)	74 (80)
436	$C_{24}H_{30}N_5O_3^+$	131 (194)	182 (194)

[&] Elements considered: C, H, N, O, F, P, S, and Cl when present. Mass window: ±2 mDa. RIA window: ±20%.
[*] One composition not rejected by RIA criteria was rejected by exact mass criteria.
[#] Three Cl atoms were assumed to be present as would be apparent from the M + 2 and M + 4 peak abundances.
∧ One Cl atom was assumed to be present as would be apparent from the M + 2 and M + 4 peak abundances.

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 similarity of composition rejections observed in Table 3.1 for the M + 1 and M + 2 exact masses and RIAs. 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 3.2 compares the discriminating power for rejecting compositions of the M + 1 and M + 2 exact masses (third column) and RIAs (last column) for ±2 mDa and ±20% error limits for 15 compositions containing different sets of heteroatoms. The total number of possible compositions corresponds to a ±2 mDa mass window about the calculated monoisotopic ion masses. For all 15 compositions, comparison of the M + 1 and M + 2 exact masses rejected fewer incorrect compositions than comparison of the RIAs. In eight of nine 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 (±10 mDa) and smaller RIA error (±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 [18], 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.

3.10 ION CORRELATION AND MASS SPECTRAL DECONVOLUTION

Our current research utilizes a Direct Analysis in Real Time (DART) JEOL AccuTOF® oa-TOFMS. The DART is an open-air, surface-sampling ion source that desorbs and ionizes analytes with a heated stream of metastable helium atoms. Elimination of sample extraction, extract clean up, and chromatography decreases the time per sample analysis more than 100-fold. An autosampler [40] and field sample carrier [41] were designed to permit analysis of 1000 cotton swab wipe samples in a single shift by one analyst. This Autosampler/DART/oa-TOFMS instrument should prove useful for rapidly characterizing and monitoring remediation of Superfund or dispersive sites.

A disadvantage of the DART ion source is that composite mass spectra are often obtained. Figures 3.6a, 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 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 3.6 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. A data acquisition method was written to record the data at the three CID voltages during a single data acquisition [42].

Generally, the low, moderate, and high CID voltages used to acquire the mass spectra in Figure 3.6 provide mass spectra containing: 1) precursor ions, protonated dimer ions for some analytes, protonated A:B adduct ions for some analyte mixtures, and few, if any, product ions; 2) precursor ions and easily formed product ions; and 3) product ions and lower abundances of precursor ions, respectively. The three possible precursor ions in Figure 3.6a at *m/z* 182, 265, and 319 are also evident in Figure 3.6b, which confirms that they are precursor ions, because dimeric ions and their products are not observed at a moderate Orifice 1 voltage. No dimeric ions were observed in the low CID voltage spectrum for the three analytes. The *m/z* 335 ion is an $[M + H + O]^+$ ion related to the *m/z* 319 ion.

In Figure 3.6c, 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

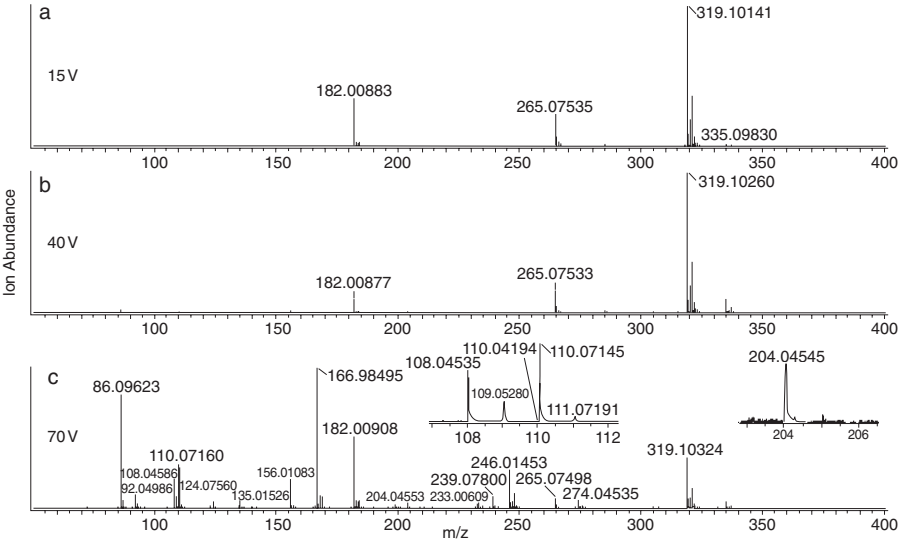

Figure 3.6. Mass spectra acquired with a DART/oa-TOFMS for three compounds on cotton swabs at Orifice 1 (CID) voltages of (a) 15 V, (b) 40 V, and (c) 70 V. No Cl isotopic pattern was observed in the magnified portions of the spectrum for the *m/z* 108 or 204 ions in (c).

of the three precursor ions will not be correlated with the other two. Table 3.3 lists the ions automatically gleaned by an Ion Extraction Program [42] from text files of *m/z* ratios and mass peak areas provided by the data system for the three mass spectra in Figure 3.6. The three lists contain monoisotopic precursor and product ions; precursor ions; and oxide, adduct, and dimer ions, if present.

Because RIAs are more susceptible to interferences, the RIAs in Table 3.3 were only calculated and listed when one of two monoisotopic mass peak area thresholds were exceeded. The thresholds were determined empirically from mass spectra obtained for 15 compounds. For the higher threshold, 900 was added to the %RIA to serve as a software flag. During ion correlation, 900 was subtracted from the %RIA and the software applied a 15% RIA error limit; above the lower threshold, the %RIA was unaltered and a 20% RIA was used. 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.

In ref. 19, the ICP first considered the data for the highest mass ion, *m/z* 319, and then the data for each lower-mass ion sequentially. If no correlation was found, the second ion was also assumed to be a precursor ion to be correlated with other ions later. When a correlation was found, both the *m/z* 319 ion data and that for the correlated ion were retained and the next lower-mass ion was considered. The previous comparisons were made before the lower-mass ion was considered to ensure

TABLE 3.3. Exact Masses and RIAs for Ions Gleaned from Text Files of the Mass Spectra in Figure 3.6

Precursor and Product Ions		
Exact Mass	%1 RIA	%2 RIA
319.10141	923.42	940.41
265.07535	13.28	6.82
182.00884	910.63	910.00
166.98495	11.41	10.19
86.09623	6.40	0.00
246.01453	13.40	36.06
110.07160	0.00	0.00
156.01084	0.00	0.00
108.04586	0.00	0.00
109.05344	0.00	0.00
135.01526	0.00	0.00
239.07801	0.00	0.00
283.96495	0.00	0.00
297.21216	0.00	0.00
92.04986	0.00	0.00
274.04535	0.00	0.00
124.07560	0.00	0.00
204.04553	0.00	0.00
111.04825	0.00	0.00
233.00609	0.00	0.00
218.07256	0.00	0.00
93.05705	0.00	0.00

Precursor Ions
Exact Mass
319.10141
265.07535
182.00884

Oxides, Adducts, and Dimers			
Exact Mass	%1 RIA	%2 RIA	Label
335.09830	0.00	0.00	O

all previously applied constraints on the possible precursor ion compositions were retained. This sequence was repeated until all lower-mass ions were considered or skipped, because they were precursor ions. Table 3.4 illustrates this process for the *m/z* 319 ion.

The current 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

TABLE 3.4. Testing Sequence for Correlating Ions Using the ICP

Nominal Mass	Compositions Found
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 &~~199~~	No compositions
319, 274, 246, 239, 233, 204 &~~167~~	No compositions
319, 274, 246, 239, 233, 204 &~~156~~	No compositions
319, 274, 246, 239, 233, 204 &~~135~~	No compositions
319, 274, 246, 239, 233, 204 &~~124~~	No compositions
319, 274, 246, 239, 233, 204 &~~110~~	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.

more abundant product ions. The number of possible compositions listed is based on a ±2 mDa mass error range when measured exact masses are accurate to within 2 mDa. More possible compositions may be listed when a ±4 mDa mass error range and ±40% RIA error range are required for a product ion to be recognized as such. More detail on how error limits are automatically adjusted during ion correlation is available in ref. 42. In Table 3.4, no more ions were tested for correlation after seven product ions were found due to memory limitations of QuickBASIC®. The sequence in Table 3.4 was then repeated for the other two precursor ions starting with *m/z* 265 or *m/z* 182.

Considering up to seven product ions was satisfactory for individual analytes, but analyte mixtures can provide many more than seven ions. In these cases, this approach sometimes ignored prominent low-mass ions. A software simplification improved the situation. Starting with the most abundant product ion in the product ion mass spectrum and ending with the least abundant ion, each product ion was tested for correlation with each precursor ion. As illustrated in Table 3.5 for a single analyte, metoprolol, the number of possible precursor ion compositions could decrease as more individual product ions were tested for correlation.

Four possible compositions were found for the *m/z* 268 precursor ion when the elements C, H, N, O, F, P, and S were considered. The most abundant product ion, *m/z* 116, was correlated with the *m/z* 268 ion and provided the two possible product ion compositions and four corresponding neutral loss compositions listed in Table 3.5. One possible precursor ion was eliminated. The next most abundant product ion, *m/z* 191, also correlated with the *m/z* 268 ion, providing two possible product ion compositions and two corresponding neutral losses. Only two possible precursor ions remained viable. Correlation between the *m/z* 268 ion and the seventh most abundant product ion (*m/z* 226) yielded only one precursor ion, one product

TABLE 3.5 Reduction of the Number of Possible Precursor Ion Compositions for Metoprolol When Product Ions Are Considered*

Precursor Ion	
268.18933	$C_{11}H_{22}N_7O^+$, $C_{13}H_{28}F_2NS^+$, $C_{13}H_{25}F_3NO^+$, $\mathbf{C_{15}H_{26}NO_3^+}$
Correlations	
268.18933	$C_{11}H_{22}N_7O^+$, $C_{13}H_{25}F_3NO^+$, $\mathbf{C_{15}H_{26}NO_3^+}$
116.10705	$(C_4H_{12}N_4^+ + C_7H_{10}N_3O)$ or $(C_6H_{14}NO^+ + C_5H_8N_6$, $C_7H_{11}F_3$, or $C_9H_{12}O_2)$
268.18933	$C_{11}H_{22}N_7O^+$, $\mathbf{C_{15}H_{26}NO_3^+}$
191.10638	$(C_{10}H_{13}N_3O^+ + CH_9N_4)$ or $(C_{12}H_{15}O_2^+ + C_3H_{11}NO)$
268.18933	$\mathbf{C_{15}H_{26}NO_3^+}$
226.14404	$C_{12}H_{20}NO_3 + C_3H_6$
Re-correlations	
268.18933	$\mathbf{C_{15}H_{26}NO_3^+}$
116.10705	$C_6H_{14}NO^+ + C_9H_{12}O_2$
268.18933	$\mathbf{C_{15}H_{26}NO_3^+}$
191.10638	$C_{12}H_{15}O_2^+ + C_{13}H_{11}NO$

*The correct precursor ion composition is in bold type.

ion, and one corresponding neutral loss. This cycle was repeated for all product ions starting with upper elemental limits provided by the single precursor ion composition, and only one possible composition was found for each product ion and neutral loss. After this re-correlation, the constraints on the precursor ion provided by all correlated ions provided constraints for all product ions. When only one precursor ion composition is found for the first correlation, the second cycle is unnecessary and is not performed.

For the three-compound mixture that provided the mass spectra in Figure 3.6, Table 3.6 lists the correlations that were found among the three precursor ions and the product ions produced from them. Twelve product ions were correlated with both the *m/z* 319 and 265 precursor ions. Several low-mass ion compositions were subunits of multiple precursor ions and thus 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 for ions when chromatography is used. In Table 3.6, the *m/z* 92, 93, and 135 ions are listed under all three precursor ions, because their compositions are subunits of each one, and which precursor ion(s) produced the ions is indiscernible. The *m/z* 86, 108, 109, 204, and 218 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 the isotopic pattern for a single Cl atom is not evident for these ions in Figure 3.6c. The *m/z* 218 ion is not evident in the spectrum and was an artifact that appeared when the raw data was centroided.

TABLE 3.6. Ion Correlation Test Results from Three Analytes

Ion Exact Mass	Composition	Mass Error (mDa)	Ion Exact Mass	Composition	Mass Error (mDa)	Ion Exact Mass	Composition	Mass Error (mDa)
319.10141	**$C_{17}H_{20}N_2ClS^+$**	**(–1.6)***	**265.07535**	**$C_{11}H_{13}N_4O_2S^+$**	**(–0.0)**	**182.00883**	**$C_8H_8NS_2^+$**	**(–0.4)**
86.09623	$C_5H_{12}N^+$	(–0.2)	86.09623	$C_5H_{12}N^+$	(–0.2)	**166.98495**	**$C_7H_5NS_2^+$**	**(–0.8)**
	$C_{12}H_8NClS^{\#}$	(–0.5)		$C_6HN_3O_2S$	(+0.2)		CH_3	(+0.4)
246.01453	**$C_{13}H_9NClS^+$**	**(+0.7)**	**110.07160**	**$C_5H_8N_3^+$**	**(+0.3)**	135.01526	$C_7H_5NS^+$	(+1.5)
	$C_4H_{11}N$	(–2.3)		$C_6H_5NO_2S$	(–0.3)		CH_3S	(–2.0)
108.04586	$C_3H_9N_2Cl^+$	(+1.0)		**$C_7H_{10}O^+$**	(–1.0)	92.04986	$C_6H_6N^+$	(+0.4)
	$C_{14}H_{11}S$	(–2.6)		$C_4H_3N_4OS$	(+1.0)		$C_2H_2S_2$	(–0.8)
109.05344	$C_3H_{10}N_2Cl^+$	(+0.7)	**156.01083**	**$C_3H_4N_4OS^+$**	(+0.8)	93.05705	$C_6H_7N^+$	(–0.3)
	$C_{14}H_{10}S$	(–2.4)		C_7H_9O	(–0.8)		C_2HS_2	(–0.2)
135.01526	$C_7H_5NS^+$	(+1.5)		**$C_6H_6NO_2S^+$**	(–0.5)			
	$C_{10}H_{15}NCl$	(–3.2)		$C_5H_7N_3$	(+0.5)			
239.07800	**$C_{15}H_{13}NS^+$**	**(+1.7)**	108.04586	$CH_8N_4S^+$	(–0.6)			
	C_2H_7NCl	(–3.3)		$C_{10}H_5O_2$	(+0.5)			
92.04986	$C_6H_6N^+$	(+0.4)		$C_3H_{10}NOS^+$	(–1.9)			
	$C_{11}H_{14}NSCl$	(–2.0)		$C_8H_3N_3O$	(+1.9)			
274.04535	**$C_{15}H_{13}NSCl^+$**	**(+0.2)**		$C_6H_6NO^+$	(+1.5)			
	C_2H_7N	(–1.8)		$C_5H_7N_3OS$	(–1.5)			
204.04553	$C_{11}H_9N_2Cl^+$	(+0.7)	109.05344	$CH_9N_4S^+$	(–0.8)			
	$C_6H_{11}S$	(–2.3)		$C_{10}H_4O_2$	(+0.8)			
233.00609	**$C_{12}H_8NSCl^+$**	**(+0.0)**		$C_6H_7NO^+$	(+1.2)			
	$C_5H_{12}N$	(–0.7)		$C_5H_6N_3OS$	(–1.2)			
218.07256	$C_{13}H_{13}NCl^+$	(–0.5)	135.01526	$C_7H_5NS^+$	(+1.5)			
	C_4H_7NS	(–1.1)		$C_4H_8N_3O_2$	(–1.6)			
93.05705	$C_6H_7N^+$	(–0.3)	92.04986	$C_6H_6N^+$	(+0.4)			
	$C_{11}H_{13}NSCl$	(–1.4)		$C_5H_7N_3O_2S$	(–0.4)			
			124.07560	**$C_5H_8N_4^+$**	**(+1.3)**			
				$C_6H_5O_2S$	(–1.3)			
				$C_7H_{10}NO^+$	**(–0.1)**			
				$C_4H_3N_3OS$	(+0.1)			
			204.04553	$C_9H_8N_4S^+$	(–0.9)			
				$C_2H_5O_2$	(+0.9)			
			111.04825	**$C_3H_{11}O_2S^+$**	**(+0.8)**			
				$C_8H_2N_4$	(–0.8)			
			218.07256	$C_{11}H_{12}N_3S^+$	(–2.1)			
				HNO_2	(+2.1)			
			93.05705	$C_6H_7N^+$	(–0.3)			
				$C_5H_6N_3O_2S$	(+0.2)			

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

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

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 compositions of the protonated molecules. For example, several compounds with a molecular weight of 264 g/mole were found in ref. 43. Those compositions with exact masses for the protonated molecules closest to the exact mass of the *m/z* 265 precursor ion in Figure 3.6 are listed in Table 3.7. The structures of the compounds in Table 3.7 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 3.7. An Exact Mass and RIA Library for m/z 265*

Compound	Composition	Exact Mass	%1 RIA	%2 RIA	ΔM(mDa)‡
Sulfadizole	$C_7H_{13}N_4O_3S_2^+$	265.04236	10.91	10.20	
Furazidine	$C_{10}H_9N_4O_5^+$	265.05675	12.59	1.76	14.4
Sulfamerazine	$C_{11}H_{13}N_4O_2S^+$	265.07537	14.40	5.90	18.6
Sulfaperin	$C_{11}H_{13}N_4O_2S^+$	265.07537	14.40	5.90	0.0
Temodox	$C_{12}H_{13}N_2O_5^+$	265.08190	14.06	1.94	6.5
Sulfirame	$C_{10}H_{21}N_2S_3^+$	265.08614	14.20	14.50	4.2
Analyte	$C_{11}H_{13}N_4O_2S^+$	265.07498	13.10	5.28	

*Exact masses and RIAs calculated using the atomic masses and isotopic abundances from ref. 21.
‡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, 182.00883, 265.07535, and 319.10141, differed by −0.4, −0.0, 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® [20] data base, 14,474; 2,022; and 403 references were found for chlorpromazine, sulfamerazine, and 2-(methylthio)-benzothiazole, respectively. For an isomer of 2-(methylthio)-benzothiazole, 3-methyl-2(3H)-benzothiazolethione, 159 references were found and for an isomer of sulfamerazine, Sulfaperin (isosulfamerazine), 138 references. To determine the correct isomers producing the *m/z* 182 and 265 ions 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.

3.11 CONCLUSIONS

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, full scan data acquisitions acquired at different CID voltages using a single-MS-stage oa-TOFMS provide exact masses and RIAs of sufficient accuracy to determine elemental compositions of precursor ions, product ions, and neutral losses without the need to target data collection to a pre-specified list of analyte ions. Concurrently, data interpretation has been streamlined and made conceptually simpler. Initially, exact masses and RIAs for partial mass peak profiles 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 or partially resolved at 10,000 (10% valley) resolving power. Now, only the measured and calculated RIAs are compared

and the individual contributions by +1 and +2 ions to the RIAs are totaled based on full profiles, 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. Final drafts of the authors' work referenced herein are available at http://www.epa.gov/nerlesd1/chemistry/ice/default.htm.

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