Determining Ion Compositions Using An Accurate Mass, Triple Quadrupole Mass Spectrometer

Introduction

The Environmental Chemistry Branch identifies compounds found in Superfund sites, monitoring wells, and drinking water sources. When poor-quality analyte mass spectra are obtained, multiple mass spectral library matches are found, or analyte mass spectra are absent from mass spectral libraries, compound identities are deduced from the compositions of ions in their mass spectra.

Mass spectroscopists often use measured exact masses of monoisotopic ions to reduce the number of ion compositions that are possible for a nominal mass. Measured exact masses and relative abundances of the isotopic profiles bear/are by 1 and 2 Da (+1 and +2 profiles) that arise from the presence of atoms of higher isotopes such as ^{13}C, ^{18}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S provide the means for rejecting all but the correct composition for most monoisotopic ions weighing no more than 600 Da.[1] The discriminating power of exact mass and relative abundance measurements depends on their error limits.

For the past decade, our laboratory has used double focusing mass spectrometers with GC sample introduction to accurately measure exact masses and relative abundances to determine the compositions of ions in mass spectra and to thereby tentatively identify compounds before purchasing standards for their confirmation.[2,3] Our analytical methodology, Ion Composition Elucidation (ICE),[4] requires up to three experiments to determine an ion's composition and custom software only executable by older data systems that provide a command line. It is therefore prudent to investigate other types of mass spectrometers that can measure exact masses and relative abundances using standard data system software.

Experimental

A Thermo Finnigan TSQ Quantum Ultra AM™ accurate mass triple quadrupole mass spectrometer was used with electrospray ionization to measure exact masses and relative abundances for several compounds introduced as 10-µL injections of a 1:1 methanol:water solution containing 1% acetic acid and 10 ng/µL of a single analyte. The injection peaks were 24 s wide. The schematic diagram of the quadrupoles provided on the instrument's data system is shown in Figure 1. Several scanning methods were used.

Figure 1. Diagram of the accurate mass triple quadrupole mass spectrometer from its data system.

1. *Selected Ion Monitoring by Q1.* For this instrument, selected ion monitoring is a full scan over a narrow mass window, rather than monitoring of a single m/z ratio atop a mass peak profile. The mass resolution for the first quadrupole was set to 0.1 Da full width at half maximum (FWHM) and mass ranges of 0.3 Da were scanned for the protonated molecular ion and its +1 and +2 profiles. Polyethylene glycol (PEG) ions were used for external mass calibration. Absent mass interferences, accurate mass averages for three consecutive injections were accurate to within 5 mmu for the monoisotopic ion and to within 10 mmu for the +1 and +2 profiles. For +1 and +2 profile relative abundances greater than 1%, single injection values were almost always accurate to within 10% and usually accurate to within 5% of their calculated values. An error limit of ±0.1% about measured relative abundances of less than 1% is used in the Ion Correlation Program described later to permit a proportionally larger error for very low ion abundances.

2. *Product Ion Scanning by Q3.* The monoisotopic protonated molecular ion (MH^+) was selected by Q1 with a peak width of 0.7 Da FWHM. Most of the MH^+ ions were fragmented in Q2 by collisional activation using argon gas at 0.8 mTorr. The product ions were characterized by full scan MS/MS with a Q3 peak width of 0.7 Da. Fragment ions for further investigation were selected from these scans. Three examples of MS/MS spectra are shown in Figure 2.

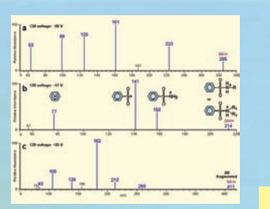

Figure 2. Product Ion spectra for (a) trischloroethylphosphate, (b) n-butyl benzene sulfonamide, and (c) Accent®.

3. *Selected reaction monitoring for fragment ion masses.* Monoisotopic protonated molecular ions were selected by Q1 and fragmented by collision in Q2 to provide monoisotopic fragment ions for mass analysis in Q3. The Q1 peak width was 0.7 Da and the Q3 mass resolution was set to 0.1 Da FWHM. The mass range monitored for each fragment ion was 0.8 Da. External mass calibration against the six ions from tris(chloroethyl)phosphate in Figure 2a was performed. The exact masses of the analyte fragment ions were corrected for the linearly-interpolated mass error between the two adjacent calibrant ions. The tris(chloroethyl)phosphate mass calibrant was superior to PEG for supplying fragment ions with adequate abundance over the working mass range. In Figure 3c-h, six fragment ions were monitored for each injection. The exact mass obtained from each injection peak was the average from the scans across 16 s of the peak maximum. Exact mass averages for three consecutive injections were usually accurate to within 10 mmu, and almost always accurate to within 20 mmu.

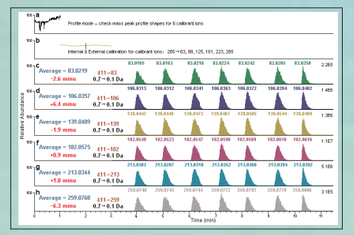

Figure 3. (a) total ion chromatogram (TIC) for six calibrant ions using the profile mode, (b) TIC for one of the calibrant ions, (c-h) selected-reaction-monitoring ion chromatograms for six fragment ions from Accent®.

4. *Selected reaction monitoring for fragment ion relative abundances.* The Q1 peak width was 10 Da FWHM to ensure all MH^+ ions including those that contain atoms of higher isotopes entered Q2 to be fragmented. The mass of the M+1 profile was the center mass. The Q3 peak width was 0.5 Da and each fragment ion was scanned over a 1 Da mass range. In Figure 4, nine profiles were monitored for each injection. The relative abundances for the fragment ions determined from the ratios of flow-injection RIC peak areas were usually accurate to within 5% of a calculated value, and almost always accurate to within 10% for single injections.

Figure 4. Total ion chromatograms for nine fragment ions from Accent®. The profile mode for the first injection provided the mass peak profiles in green, blue, and yellow. The centroid mode was used for the remaining seven injections.

Quality assurance

The profile mode was used at the start of each data acquisition to check the mass peak profile shapes, to verify that the entire profiles were included in the scanning range, and to check that no portions of adjacent profiles were scanned. Mass peak profiles for nine fragment ions are shown in Figure 4. The centroid mode was used to determine exact masses and relative abundances.

Results and Discussion

Table 1. Possible compositions for a protonated molecular ion based on exact masses of the monoisotopic, +1 and +2 profiles or the relative abundances of the +1 and +2 profiles. Elements considered: C H N O P S

#	Composition	Mass Defects 214	+1	+2	#	Composition	Relative Abundances %1	%2
1	$H_{15}N_{10}O_4$	.08865	.08631	.09217	1	C_6N_6OS	11.48	5.24
2	$C_{\cdots}{}_{14}N_8P_2$	.08474	.08370	.09235	2	$C_6H_{12}N_4O_2S$	11.37	5.62
3	$_{14}C_{\cdots}N_{14}$	.08999	.08825	.08631	3	$C_6H_{14}N_2O_4S$	11.73	5.46
4	$_{14}C_2H_{15}N^7O_5$	.08999	.09032	.09383	4	$C_6H_{16}N_2OS$	12.09	5.30
5	$C_2H_{15}N_8P_2S$	.08780	.08774	.08374	5	$C_6N_8O_2S$	11.89	5.48
6	$C_2H_{15}N_8O_2P_2$	.08608	.08716	.08930	6	$C_6H_{16}N_8S$	12.45	5.14
7	$C_2H_{15}N_8O_2S$	.08481	.08570	.08179	7	$C_6H_8N_2OS$	12.24	5.32
8	$C_2H_{16}N_2S_2$	.09086	.09138	.08880	8	$C_6H_8N_8S$	12.60	5.16
9	$C_2H_{16}N_{10}O$	.09133	.09133	.09303	9	$C_6H_{13}OPS$	12.07	5.30
10	$C_2H_{17}N_8OPS$	.08914	.09059	.08562	10	$C_6H_{14}O_2S$	12.13	5.70
11	$C_2H_{17}N_8O_2P_2$	.08743	.08990	.09186	11	$C_6H_{14}NPS$	12.43	5.14
12	$C_2H_{17}N_8O_2S$	.08615	.08832	.08359	12	$C_6H_{14}NOPS$	12.23	5.31
13	$C_2H_{18}N_4OS_2$	.09220	.09390	.08846	13	$C_6H_{18}NO_2S$	12.49	5.55
14	$C_2H_{18}N_4O_2PS$	.09049	.09303	.08751	14	$C_6N_4O_2S$	12.29	5.72
15	$C_2H_{19}N_7S$	.08749	.08899	.08396	15	$C_6H_7N_5PS$	12.59	5.16
16	$C_2H_{19}O_4P_2$	.08877	.09224	.09390	16	$C_6H_{19}N_5OS$	12.85	5.39
17	$C_2H_{19}O_6S$	.08749	.09061	.08540	17	$C_6H_7N_5O_2S$	12.65	5.57
18	$C_2H_{20}NO_3S_2$	.09355	.09614	.09013	18	$C_6H_9O_4N_3S$	13.21	5.24
19	$C_2H_{21}N_6OP$	.08577	.08800	.09007	19	$C_6H_{21}N_3OS$	13.01	5.41
20	$C_2H_{21}N_6OS$	.08883	.09115	.08594	20	$C_6H_{21}PS$	13.20	5.23
21	$C_2H_{21}N_6O_2P$	.08711	.09011	.09234	21	C_6H_7OPS	12.99	5.41
22	$C_{\cdots}{}_6N_6O_2S$	.09017	.09315	.08789	22	$C_6H_{15}O_2S$	13.26	5.64
23	$C_{\cdots}{}_6H_{19}S_2$	.08499	.08796	.08172	23	$C_6H_7O_2S$	13.06	5.81
24	$C_{\cdots}{}_6H_{19}PS$	.09451	.09769	.09192				
25	$C_{\cdots}{}_6H_{21}N_6O$	.08546	.08818	.09062				
26	$C_{\cdots}{}_6H_{21}NO_2$	.08680	.09004	.09265				
Experimental Values:		.08966	.08803	.08474			12.33	5.56
Error Limits:		±5 mmu	±10 mmu				±8%	

Discriminating power of exact masses and relative abundances

Exact masses (summed atomic masses) and relative abundances (summed isotopic abundances) provide orthogonal discrimination among possible compositions. Table 1 contains two lists of possible compositions calculated for the protonated molecular ion from one of the compounds studied. The first list results from the measured exact masses of the monoisotopic, +1, and +2 profiles, while the second list derives from the measured relative abundances of the +1 and +2 profiles. Both lists contain more than 20 possible compositions, but only the two in bold print appear in both lists.

Table 2 lists the numbers of possible compositions for the three fragment ions in Figure 2b from the same compound. Only the monoisotopic masses were measured and an error limit of 20 mmu was assumed.

Table 2. Possible compositions for fragment ions from m/z 214.

Measured Mass	Measured %1	Measured %2	Possible compositions based on: Exact Mass	Relative Abundances	Both
77.0314	6.57	Interference	15	1	1
140.9991	7.45	5.13	72	3	2
158.0296	8.07	5.33	89	3	1

Again, only one or two compositions remained possible when exact masses and relative abundances were both considered.

Ion Correlation Program

The number of possible compositions for the protonated molecular ion and its fragment ions can be reduced by rejecting:

(1) protonated molecular ions that cannot produce at least one possible fragment ion or neutral loss for each fragment ion or neutral loss exact mass,

(2) fragment ions and neutral losses that cannot be produced from the remaining possible protonated molecular ions,

(3) neutral losses for which there is no corresponding fragment ion, and

(4) fragment ions for which there is no corresponding neutral loss.

The ion correlation program written in QuickBASIC 4.5® determines the possible compositions for the protonated molecular ion, each fragment ion, and each neutral loss and then applies criteria 1 through 4. In Figure 5 are displayed the inputs and outputs for this compound. The numbers in parentheses are ranges of rings and double bonds.

The unique compositions of these fragment ions and neutral losses reveal structural details of the molecule as shown in Figure 2b. The composition of the m/z 77 fragment ion corresponds to a benzene ring. The composition of the m/z 141 ion indicates addition of an SO_2 group to the ring, and the m/z 158 ion's composition suggests NH_2 is attached to the SO_2 group. The neutral loss corresponding to this ion, C_4H_6, suggests one or two alkyl groups are attached to the N atom.

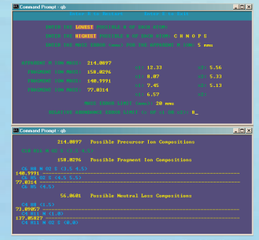

Figure 5. The input and output screens for the Ion Correlation Program.

SciFinder® in lieu of a mass spectral library

No commercial library of electrospray ionization mass spectra is available. To compensate, SciFinder®, an on-line service from the American Chemical Society, was used to provide the known structures for a molecular formula and the number of literature references available for each structure.

Example I

Shown in Figure 6a are the three structures consistent with those determined from the compositions of the fragment ions and neutral losses. More references exist for the first structure than for the other two. This compound, n-butyl benzene sulfonamide, is the only one available in the Aldrich chemical catalog with the correct molecular formula that contains an SO_2 group. It was purchased for earlier work and was used as a simulated unknown in this study.

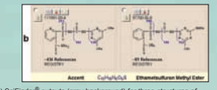

Figure 6. (a) SciFinder® outputs (gray background) for three structures of $C_{10}H_{15}NO_2S$ similar to the structures for the protonated molecular ion in Figure 2b and (b) the only two structures for $C_{15}H_{18}N_6O_6S$, and $C_{17}H_{21}N_4O_7S$ with more than 4 references. *SciFinder output is used with the permission of CAS, a division of the American Chemical Society.*

Example II

The product ion spectrum in Figure 2c contains six fragment ions from the protonated molecular ion at m/z 411. The exact masses and relative abundances measured for the MH^+ and fragment ions including those from Figures 3 and 4 were entered into the ion correlation program. Multiple compositions remained for most ions and neutral losses, including two for the protonated molecular ion: $C_{15}H_{19}N_4O_6S^+$ (9.5 12.5) and $C_{17}H_{23}N_2O_7S^+$ (9.0 13.0).

SciFinder® provided 44 structures for $C_{15}H_{18}N_4O_6S$, and 55 for $C_{17}H_{22}N_2O_7S$. Only for the two structures in Figure 6b were more than 4 references listed. A large number of references implies possible commercial importance of a compound and potential presence in environmental extracts. For the first structure, the blue lines indicate bond breakages that would produce fragment ions with compositions among the list of possibilities for the m/z 213, 182, and 139 ions. Breaking these bonds in the second structure provides ions with different masses. If this compound were an unknown, only Accent® would be purchased in hope of confirming its tentative identification by comparative LC/MS.

Conclusions

These preliminary results, obtained during the first month of research using the Thermo Finnigan TSQ Quantum Ultra AM™ accurate mass triple quadrupole mass spectrometer, allow for the following conclusions:

- Relative abundances of the +1 and +2 isotopic profiles for both the protonated molecular ion measured with Q1 and fragment ions measured with Q3 are almost always accurate to within 10% for a single determination.
- Relative abundances of this accuracy provide a powerful means orthogonal to exact mass measurements for distinguishing among possible molecular and fragment ion compositions.
- Average exact masses from triplicate determinations measured by Q1 for monoisotopic MH^+ ions were almost always accurate to within 5 mmu. (This result is consistent with the instrument's specifications.)
- Average exact masses from triplicate determinations measured by Selected Reaction Monitoring were almost always accurate to within 20 mmu. (This error limit can probably be reduced with more experience.)
- The ability to simultaneously obtain exact masses for six monoisotopic ions or three pairs of relative abundances for fragment ions reduces the number of experiments needed to determine the compositions of the predominant ions in an ion product spectrum relative to using a double focusing mass spectrometer.
- An Ion Correlation Program reduces the possible compositions for the MH^+ ion, fragment ions, and the corresponding neutral losses.
- Remaining molecular compositions can then be searched in the SciFinder® data base, which substitutes for mass spectral libraries by providing one or more structures that have been frequently described in the chemical literature.
- Most of these compounds should be purchasable for confirmation of tentative identifications. Degradation products and byproducts may not be available.
- We anticipate that this *accurate mass* and *accurate relative abundance* triple quadrupole mass spectrometer can be a valuable tool for identifying compounds in environmental extracts that do not provide gas chromatographic peaks or molecular ions in GC/MS spectra.

References

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4. http://www.epa.gov/nerlesd1/chemistry/ice/default.htm

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