Identifying Compounds Using an Accurate Mass Triple Quadrupole Mass Spectrometer

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INTRODUCTION

Most compounds are not found in mass spectral libraries and must be identified by other means. Often, compound identities can be deduced from the compositions of the ions in their mass spectra and review of the chemical literature. Confirmation is provided by mass spectra and retention time comparisons between analytes and purchased standards.

Two independent physical properties distinguish among ion compositions possible for a given nominal mass: the exact masses of ions and the relative isotopic abundances (RIAs) of ions greater in mass by 1 and 2 Da that arise from the presence of atoms of heavier isotopes of elements, *e.g.*, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, ^{34}S, ^{37}Cl, and ^{81}Br.

Instrumental capabilities that constrain the utility of a mass spectrometer for measuring exact masses and RIAs of ions from compounds that elute into the ion source from a chromatographic column are scan speed, mass accuracy, linear dynamic range, and resolving power (1). For the past decade, our laboratory has used double focusing mass spectrometers in the selected ion recording mode to determine ion compositions (2-4). Our analytical methodology, Ion Composition Elucidation (ICE), requires up to three experiments using custom software written in macro languages available for VG 70SE or Finnigan MAT900 mass spectrometers, but not for newer instruments.

Fortunately, other types of mass spectrometers can now provide mass accuracies and linear dynamic ranges sufficient to determine ion compositions using scan modes provided by the manufacturers. Herein, the utility of an accurate mass triple quadrupole mass spectrometer for identifying compounds is demonstrated.

EXPERIMENTAL

A Thermo Finnigan TSQ Quantum Ultra AM™ accurate mass triple quadrupole mass spectrometer (AM3QMS) was used with atmospheric pressure chemical ionization (APCI) to measure exact masses and RIAs for nine compounds serving as simulated unknowns that were introduced in 10-µL injections of a 1:1 methanol:water solution containing 1% acetic acid and 1 ng/µL of each analyte onto a Zorbax® RX-C18, 5 µm particle, 2.1 mm x 150 mm HPLC column. Gradient elution from 98:2 to 2:98 water:methanol with 0.2% acetic acid separated the nine HPLC peaks. Figure 1 is a diagram of the AM3QMS.

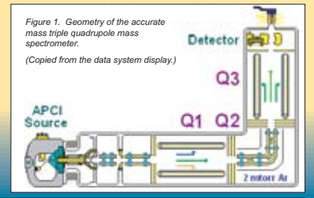

Figure 1. Geometry of the accurate mass triple quadrupole mass spectrometer.

(Copied from the data system display.)

DISCUSSION

Selecting Fragment Ions

For each analyte, mass spectra were acquired with CID voltages of -12, -24, and -36 V and 2 mtorr of Ar within Q2. The mass resolution for both Q1 and Q3 was 0.7 Da (FWHM). Only monoisotopic ions were passed by Q1 into the CID region to provide only monoisotopic fragment ions for analysis by Q3. In Figure 2 these product ion spectra are displayed for the largest-mass analyte.

Measuring Exact Masses

A mixture of six compounds provided [MH]$^+$ calibration ions of m/z 95.0604, 124.0757, 166.1226, 218.0964, 265.0754, and 319.1030 for the nine analytes. For the analyte that provided Figure 2, exact masses were measured for the six "**F**$^+$" labeled fragment ions using internal calibration, while the [MH]$^+$ ion's mass was determined using external calibration, because it was one of the calibration ions. As the analyte eluted into the APCI source, the four highest mass calibration ions and the seven analyte ions were selected sequentially by Q1 with a resolution of 0.7 Da, and 1 Da wide mass windows were scanned by Q2 about each mass using a resolution of 0.1 Da. The CID voltage was -10 V for the [MH]$^+$ calibration ions to minimize fragmentation. The six analyte fragment ions were generated from the [MH]$^+$ ion using CID voltages of -24 or -36 V, depending on which CID voltage provided the greatest ion abundance for each ion in Figure 2. These Selected Reaction Monitoring (SRM) transitions are depicted in Figure 3. Each cycle through the 10 SRM scans required 0.36 s, so that 42 measurements were made and averaged for each mass as the top 0.25-min portion of this compound's chromatographic peak eluted. The average exact masses of the analyte ions were corrected by the linearly interpolated error in the calibration masses that bracketed each one. The average corrected exact masses for triplicate measurements for each of the six fragment ions all agreed to within 7 mmu with the calculated values.

Measuring Relative Isotopic Abundances

To measure RIAs all of the monoisotopic, +1, and +2 ions must enter Q2 for fragmentation. As illustrated in Figure 4, the Q1 resolution was set to 10 Da with m/z 320 (the +1 profile mass) as the center mass. Each monoisotopic, +1, and +2 profile was scanned across 1 Da in the SRM mode with the Q3 resolution set to 0.5 Da to ensure none of the profile was missed. The ratios of the areas under the chromatographic peaks (*e.g.*, areas for m/z 320/319 and m/z 321/319) multiplied by 100% provided the RIAs, %1 RIA and %2 RIA. Three ions for each of three analytes (nine in all) were investigated for each injection. To obtain accurate RIAs, no other monoisotopic fragment ions must be observed within ±2 Da of the fragment ion under scrutiny. Figure 5 displays portions of the monoisotopic product ion spectra in Figure 2, magnified and normalized about each analyte ion for which exact masses were measured and for m/z 58 and 86. RIAs were measured for the nine ions featured in Figure 5. The RIAs obtained for m/z 86, 246, 274, and 319 were accurate to within 5% of the calculated values for each of triplicate measurements for these analyte ions. The monoisotopic ions in Figure 5 circled in red distorted the measured values for three fragment ions. A larger %1 RIA error was obtained for the m/z 233 ion present with low abundance in Figure 2. The largest % error in %1 RIA for m/z 58 was 6.9%, while the measured %2 RIA of 0.06% agreed well with the calculated value of 0.05%.

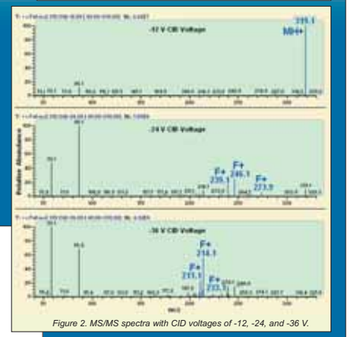

Figure 2. MS/MS spectra with CID voltages of -12, -24, and -36 V.

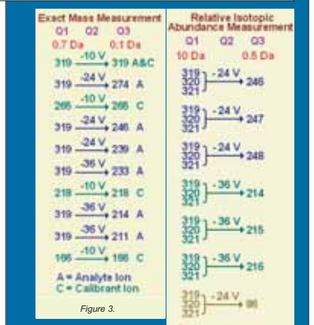

Figure 3.

Figure 4.

Figure 5. Normalized segments of the mass spectra in Figure 2 used to reveal interferences for RIA measurements.

DISCUSSION *(Continued)*

Determining Ion Compositions

Measuring two complementary physical properties, exact masses and RIAs, greatly limited the possible compositions for the m/z 319 ion as illustrated by Figure 6. Assuming at least 1/3 of the mass was from C atoms and a mass error limit of 7 mmu, there were 172 possible compositions based on consideration of C, H, N, O, P, S, and Cl atoms. Even for a mass error of only 0.32 mmu (1 ppm), seven compositions would still remain. To better illustrate complementarity, an error limit of 10% in the +1 and +2 RIAs was assumed (twice the error observed for abundant ions free of interferences and greater in mass than 100 Da), which yielded 154 possible ion compositions. When both properties were considered simultaneously, only the seven possible compositions present in both lists remained viable. Six compositions must still be eliminated.

The AM3QMS provides larger mass error limits (5 mmu for abundant ions and 10 mmu for ions with low abundance) than a double focusing mass spectrometer (6 ppm or 1.9 mmu at m/z 319), and many more compositions are possible. However, the AM3QMS was able to measure either exact masses or RIAs of the precursor ion and multiple fragment ions from an analyte for a single injection. This capability provides additional complementary information that allows the composition of the precursor ion to be determined, despite the larger mass error limit. This point is illustrated by the lower portion of Figure 6. A unique composition was found for the most abundant fragment ion and for its corresponding neutral loss. The sum of the atoms of each element in these compositions provided the unique precursor ion composition.

Mass and RIA Errors

Exact masses for the abundant monoisotopic ions are seldom distorted by mass interferences. As Figure 5 illustrated, interferences with RIA measurements are more common. The ions produced from a precursor ion are closely related. Knowing the unique composition for one ion greatly limits the possible compositions for the others. To exploit this observation an Ion Correlation Program was developed.

Ion Correlation Program (ICP)

An in-house Ion Correlation Program written in QuickBASIC® 4.5 permits simultaneous consideration of the precursor ion, multiple fragment ions, and neutral losses. The ICP performs several steps:

1. All possible compositions consistent with the elemental limits, exact masses, mass error limit, RIAs, and RIA mass error entered by the user that have at least -0.5 rings and double bonds (RDB) are calculated and stored for all ions. (A saturated [MH]$^+$ ion has -0.5 RDB). The precursor ion compositions are calculated first to establish upper elemental limits for calculating the possible fragment ion compositions.
2. All possible neutral loss compositions based on the mass differences between fragment ions and the precursor ion having no less than -2.0 RDB are calculated and saved. RIAs of the neutral losses are not considered. (The neutral loss of H_6O_3 corresponding to three water molecules is calculated to have -2.0 RDB).
3. Precursor ion compositions that do not contain the sum of the atoms of each element in a fragment ion composition and a corresponding neutral loss composition for each fragment ion exact mass are rejected.
4. Fragment ion compositions that do not provide a remaining possible precursor ion when summed with a corresponding neutral loss composition are rejected, as are neutral loss compositions that do not provide a remaining possible precursor ion when summed with a corresponding fragment ion composition.

This approach compensates for the exponential increase in the number of possible compositions as an ion's mass increases. The precursor ion is the sum of its fragment ion and corresponding neutral loss parts, each of which has many fewer possible compositions. For the inputs into the ICP illustrated in Figure 7, the precursor ion was assumed to contain one Cl atom. The mass error limit assumed was 7 mmu and the assumed RIA error limit was 10%. Also entered were the average (N=3) measured exact masses of the seven ions and the average (N=3) RIAs for the m/z 319 and 246 ions. These inputs provided the unique compositions for all of the ions and neutral losses shown in Figure 8.

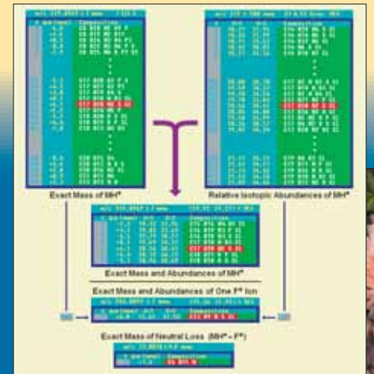

Figure 6. Exact Mass and Relative Isotopic Abundance Tables illustrating the complementarity of these properties for determining ion compositions. Also shown are unique fragment ion and corresponding neutral loss compositions that sum to the correct precursor ion composition.

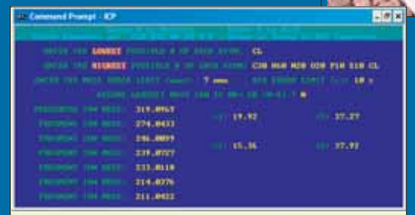

Figure 7. Input into the ICP of seven exact masses, two pairs of RIAs, a mass error limit of 7 mmu, and an RIA error limit of 10%. The presence of one Cl atom was assumed.

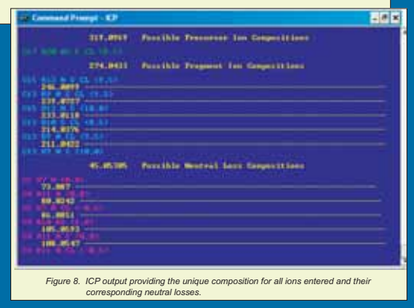

Figure 8. ICP output providing the unique composition for all ions entered and their corresponding neutral losses.

Searching the Chemical Literature for Isomers

Commercial mass spectral libraries of APCI mass spectra are not available. SciFinder® was used to examine the known structures for $C_{17}H_{19}N_2SCl$. There is a strong correlation among compounds used commercially, compounds available in chemical catalogs, the number of literature references for a compound, and compounds found in the environment. The structure of chlorpromazine, a sedative, tallied 13,843 references, far more than any others. This structure could produce the four fragment ions in Figure 9, predicted by Thermo Finnigan Mass Frontier® 3.0 software, which have the compositions found by the ICP. Had this analyte been an unknown, chlorpromazine would have been purchased to compare its fragment ion mass spectra at different CID voltages and its retention time to those of the analyte, which would confirm its identity. This strategy would be less effective for byproducts and degradation products not found in the literature.

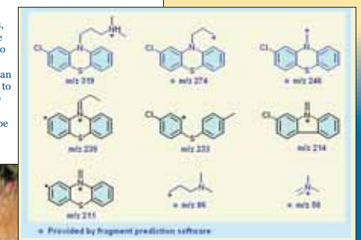

Figure 9. Four fragment ions predicted by Thermo Finnigan's Mass Frontier® 3.0 software and four others rationalized by a mass spectra interpretation expert.

CONCLUSIONS

Accurate Mass Triple Quadrupole Mass Spectrometer (AM3QMS)

- An AM3QMS provided sufficient mass accuracy and relative isotopic abundance (RIA) accuracy for precursor and fragment ions to determine ion compositions of environmental pollutants that provided robust ESI or APCI signals.
- All 21 exact mass measurements of the monoisotopic ions were accurate to within 11 mmu, while RIAs were often distorted by ion abundance contributions from ions within 2 Da of their nominal masses.
- The AM3QMS made these measurements with far fewer and simpler data acquisitions than using a double focusing mass spectrometer. No custom software was required.
- The APCI and ESI sources permit liquid sample introduction, while the double focusing mass spectrometer is still used when analytes provide gas chromatographic peaks.

Complementarity of Exact Masses and RIAs

- Considering both exact masses and RIAs to limit the number of possible ion compositions is a powerful approach for determining ion compositions.

Ion Correlation Program (ICP)

- Correlating the precursor ion with its fragment ions and corresponding neutral losses is an effective method for rejecting incorrect compositions for prominent ions and neutral losses produced by an analyte.
- The ICP provides a convenient means to calculate the possible compositions for the ions in a product ion spectrum and their corresponding neutral losses based on both exact masses and RIAs and to discard compositions that are inconsistent with each other.
- Using the ICP relaxes the error limits for mass and RIA measurements necessary for determining precursor ion compositions.
- The ICP provides unique compositions for ions for which accurate RIA measurements cannot be made by providing a unique composition for another ion produced from the analyte for which no RIA interferences were observed.

REFERENCES

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ACKNOWLEDGMENT

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NOTICE