Identifying Compounds Using Source CID on an Orthogonal Acceleration Time-of-Flight Mass Spectrometer

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Introduction

Exact mass libraries of ESI and APCI mass spectra are not commercially available. In-house libraries are dependent on CID parameters and are instrument specific. The ability to identify compounds without reliance on mass spectral libraries is therefore more crucial for liquid sample introduction techniques. Determining the elemental compositions of a precursor ion, product ions, and neutral losses provides structural details of an analyte and yields candidate compounds after a limited review of the chemical literature.

Historically, exact masses of mono-isotopic ions have been measured to limit the number of possible compositions for ions in a mass spectrum. The relative isotopic abundances (RIAs) of the ions 1 and 2 Da greater in mass that arise from heavier isotopes such as ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{33}S, ^{34}S, ^{37}Cl, and ^{81}Br provide an independent and underutilized means for distinguishing among multiple compositions that remain after considering an ion's exact mass.

Double focusing mass spectrometers (1,2), an accurate mass triple quadrupole mass spectrometer (3), and, as this poster demonstrates, an orthogonal acceleration time-of-flight mass spectrometer (oa-TOFMS) measure exact masses and RIAs with sufficient accuracy to provide the compositions of precursor ions, product ions, and neutral losses.

Experimental

An Agilent G1969A LC/MSD TOF orthogonal acceleration time-of-flight mass spectrometer (Figure 1) was coupled to an Agilent 1100 Series HPLC using an Agilent G1948A ESI source for electrospray ionization to measure exact masses (< 3 ppm mass accuracy) and RIAs for nine compounds serving as simulated unknowns that were introduced in 10-µL injections of a 1:1 water/MeOH solution containing 1% acetic acid and 1 ng/µL of each analyte.

LC Conditions
Columns: ZORBAX SB - C18 Narrow Bore Rapid Resolution, 2.1 x 50 mm, 3.5 µm particle size (Agilent PN: 871700-902); **Mobile phases:** A = 0.2% acetic acid/water, B = 0.2% acetic acid/MeOH; **Flow rate:** 200 µL/min; **Linear Gradient:** 2 min @ 2% B, 98% B at 17.00 min, post run 2 min.

MSD TOF Conditions
Source (ESI): Drying gas 10 L/min @ 325 °C, Nebulizer 30 psi, **Capillary voltage:** 4000 V; **Fragmentor (4 settings):** 100, 150, 200, and 250 V, **Scan mode:** m/z 100-950, 10,000 transients/scan, 0.88 scans/sec; **Reference Masses:** purine, m/z 121.050873, and HP-0921, m/z 922.009798. All other conditions were based on the system autotune.
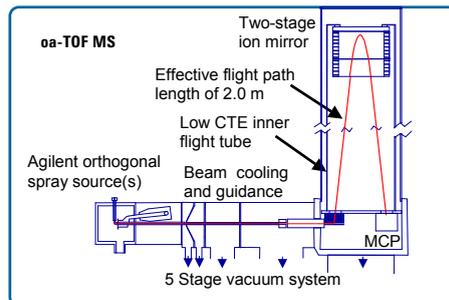
Experimental

oa-TOF MS
- Two-stage ion mirror
- Effective flight path length of 2.0 m
- Low CTE inner flight tube
- Agilent orthogonal spray source(s)
- Beam cooling and guidance
- MCP
- 5 Stage vacuum system

Figure 1. Schematic of the oa-TOFMS.

The identity of compounds was known to the second author, who provided them as a mixture to the first author to analyze by LC/MS. The data from the analysis was then used by the second author to determine the chemical formulae for the precursors, products, and neutrals, using his Ion Correlation Program (ICP) (3).

In this poster, the data from an averaged product ion spectrum in Figure 2 for one of the compounds that provided a precursor ion (MH+) at 319.1039 is examined in detail.   Although the chromatographic analysis was run at four different fragmentor voltages, the highest setting of 250 V provided the largest ion abundances for the product ions (P+) while maintaining adequate ion abundance for the precursor ion.

The measured exact masses are labeled in Figure 2. The RIAs were calculated using the peak area counts for the A, A+1, and A+2 isotopes as shown in Figure 3 for MH+ and one of the product ions.

The ICP uses both exact masses and RIAs to discriminate among compositions that are possible based on either property alone. This point is illustrated in Figure 4. Shown in the "Exact Mass of MH+" box, 47 compositions were calculated assuming at least 1/3 of the m/z 319 ion's mass was from C atoms, a mass error limit of 2 mmu (6.3 ppm for m/z 319), and the possible presence of C, H, N, O, P, S, and Cl atoms. On the RIA side, 297 compositions were calculated assuming an RIA error limit of 20% for a nominal mass of 319 Da. Combining these results still leaves four possibilities.

Additional discrimination among precursor ion compositions is provided by consideration of the product ions and their corresponding neutral losses, each of which has fewer possible compositions than the precursor ion due to their lower masses.
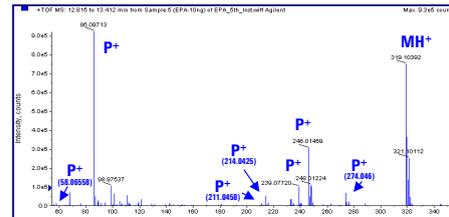
Results and Discussion

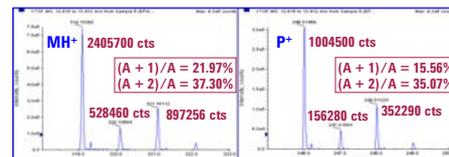**Figure 2.** Averaged CID spectrum of MH+ 319.1039 at fragmentor voltage = 250 V.

Figure 3. Magnified and normalized portions of the CID spectrum in Figure 2. The RIAs were calculated using isotope peak areas.
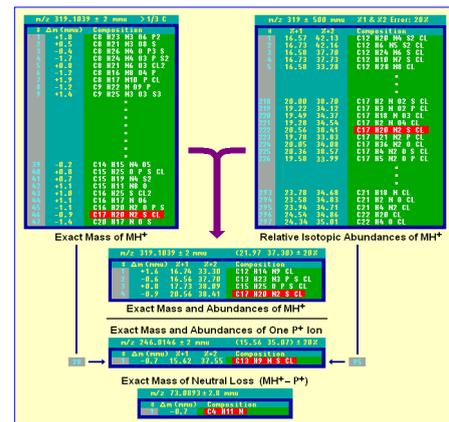

Figure 4. Tables listing possible compositions based on consideration of precursor ion exact masses, RIAs, and both exact masses and RIAs. The two bottom tables show the unique composition found by the ICP for a product ion and its corresponding neutral loss. Their summed compositions provide that of the precursor ion.

Entering the exact masses of all eight ions (one MH+ and seven P+s) into ICP with error limits of 2 mmu and 20% (Figure 5) provided only one composition, $C_{17}H_{20}N_2SCl$, for MH+ and the unique compositions for the product ions and neutral losses listed in Figure 6. Notice that inclusion of RIAs for only two ions restricted the possible compositions for the closely related ions as well.
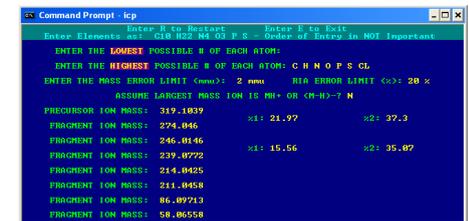

Figure 5. Input of exact masses and RIAs into the ICP. A 2 mmu mass error for the m/z 58 ion would correspond to 34.4 ppm. For m/z 319, 34.4 ppm would be 11 mmu. By using an mmu-based error limit, even low mass ions can be considered without unnecessarily relaxing the mass error limit for higher mass ions.
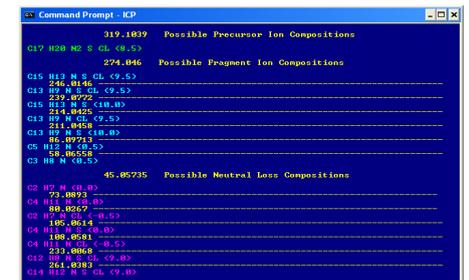

Figure 6. ICP output of unique ion and neutral compositions.

To determine if the new approach just described provided an advantage over conventional means for determining precursor ion compositions, the composition generator included in the Analyst QS for Agilent TOF (Applied Biosystems | MDS SCIEX) data processing software was used to determine possible precursor ion compositions.

The instrument's mass error limit, 3 ppm, was entered and C, H, N, O, P, S, and Cl were the elements considered. The RIAs determined in Figure 3 justified the assumptions that one Cl and at least 15 C atoms were present. The two precursor ion compositions listed in Figure 7 were found.
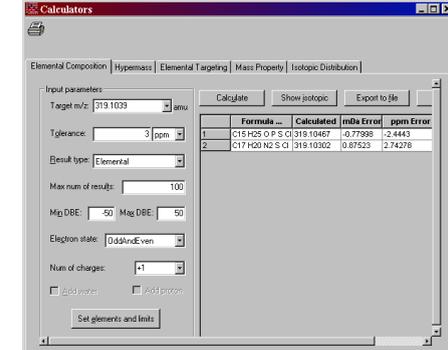
Results and Discussion

Figure 7. Possible compositions calculated for MH+ 319.1039 ± 3 ppm.

Having limited the composition of the precursor ion to one or two possibilities, the Merck database of compound structures (13th Edition) was searched using ChemFinder Net, v8.0.5 (CambridgeSoft). No matches were found for $C_{15}H_{24}OPSCl$, but one was found for $C_{17}H_{20}N_2SCl$ as seen in Figure 8.
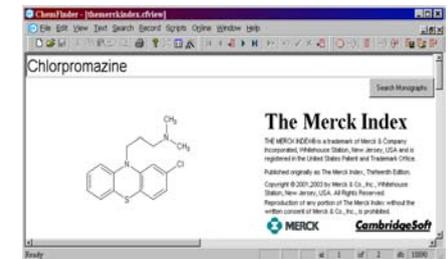

Figure 8. Compound structure match in the Merck database for $C_{17}H_{19}N_2SCl$.

To provide confidence for the analyte's tentative identification, the compositions of the product ions found by the ICP were compared with those predicted from the protonated structure of chlorpromazine by MS Fragmentor, v 8.0.4 (Advanced Chemistry Development, Inc.). The predicted structures of four product ions found by the ICP are depicted in Figure 9.

Eight of the nine unknowns were successfully identified using these methods. The ninth compound's ion abundance was inadequate due to co-elution of another analyte that was preferentially ionized.
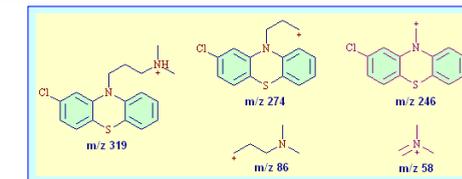

Figure 9. Product ions generated in ACD by MS Fragmentor.

Conclusions

The mass accuracy of the Agilent LC/MSD TOF (< 3 ppm) was adequate for identifying chlorpromazine from the measured exact mass of its precursor ion and from the measured RIAs of its +1 Da and +2 Da isotopic peaks. Greater confidence in the tentative identification was provided by measuring exact masses of several product ions and the RIAs for an abundant product ion. These values were entered into an Ion Correlation Program (ICP) to provide unique compositions for the precursor ion, seven product ions, and their seven corresponding neutral losses. Four of the product ion compositions were consistent with those predicted by fragmentation software. For larger mass compounds, the more complete use of information provided by product ion mass spectra demonstrated herein might be essential for determining unique precursor ion compositions before conducting literature searches to hypothesize tentative identifications.

References

1. Grange A.H., Donnelly J.R., Sovocool G.W., Brumley W.C. "Determination of Elemental Compositions from Mass Peak Profiles of the Molecular (M), M+1 and M+2 Ions" Anal. Chem., 1996, 68, 553-560.

2. http://www.epa.gov/nerlesd1/chemistry/ice/default.htm "Ion Composition Elucidation (ICE) and Environmental Forensics: Identification of Pollutants of Unknown Origin" U.S. EPA, Las Vegas, LV.

3. Grange A.H., Winnik W., Sovocool, G.W. "Identifying Compounds Using an Accurate Mass Triple Quadrupole Mass Spectrometer" 53rd ASMS Meeting, San Antonio, TX, June 5-9, Poster TP03-043.

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