

# Ion Composition Elucidation (ICE) of Ions from Trace Levels of Pharmaceuticals and Disinfection Byproducts in Water Supplies

Andrew H. Grange and Wayne Sovocool

U.S. EPA, Office of Research and Development, NERL, ESD, PO Box 93478, Las Vegas, NV 89193-3478

## 1 INTRODUCTION

Good-quality, low resolution mass spectra provide tentative identifications of compounds through comparison to library mass spectra. When no matches or multiple matches are found, "exact masses" of ions measured by peak matching with a mass resolution of 10,000 can provide unique compositions of ions containing C, H, N, O, P, or S atoms with masses up to 150 Da. Using 20,000 resolution, Ion Composition Elucidation (ICE) increases the mass limit of ions for which compositions can be uniquely determined to 600 Da. ICE utilizes Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) to acquire data and a Profile Generation Model (PGM) for its automated interpretation. ICE is accomplished by measuring the exact masses and relative abundances of mass peak profiles arising from less abundant isotopes of the elements comprising the ions such as  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^{34}\text{S}$  (1). Three examples will illustrate the utility of this recently developed analytical approach for identifying trace levels of compounds in complex sample extracts.

## 2 MPPSIRD

Selected ion recording (or multiple ion detection) is often used to monitor only the maxima of multiple mass peak profiles for analytes and internal standards in order to increase sensitivity by a factor of 100 relative to full scanning. MPPSIRD consists of monitoring multiple m/z ratios across multiple profiles as a chromatographic peak elutes. In these examples, five m/z ratios were monitored across the top portions of lock and calibration profiles and seven m/z ratios were monitored across the analyte profiles. The areas under the chromatographic peaks were plotted to provide partial mass peak profiles. The weighted average of the top several areas delineating a molecular ion (M) or fragment ion (F) profile provided its exact mass. The ratio of the sum of the profile-delineating areas for profiles arising from ions containing higher isotopes (M+1, M+2, F+1, or F+2 profiles) to the sum of areas for the M or F profile provided the relative abundance of the higher-mass profile (2).

## 3 Acetaminophen or Surfynol 104?

Displayed in Figure 1a, 1b, and 1c, respectively, are: the NIST library mass spectrum of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ), a commonly used analgesic; a background subtracted mass spectrum from an extract of 100 L of lake water; and the NIST library mass spectrum for Surfynol 104 ( $\text{C}_{14}\text{H}_{26}\text{O}_2$ ), a wetting agent used in household and industrial cleaners. The lake water extract contained hundreds of compounds, some of which degraded the gas chromatography column interfaced to a Finnigan MAT 900S mass spectrometer. Consequently, background subtracted mass spectra often displayed ions not associated with the compound of interest, and numerous, questionable library matches resulted. The presence of either widely used compound in the lake was plausible and both would normally be purchased to compare mass spectra and retention times to those of the analyte. MPPSIRD was used to acquire data with 10,000 resolution to plot the partial profiles in Figure 2. The three exact mass defects and two relative abundances from Figure 2a were entered into the PGM to provide the first two compositions in Table 1 that were possible based on the exact mass of the m/z 151 ion within the error limits of its determination ( $\pm 6$  ppm for 1 determination with 10,000 resolution). Each "X" indicates disagreement between the experimental and calculated values for a mass defect or relative abundance.

| m/z = 151.11225 ± 6 ppm |           | Elements: C H N O P S Si               |        | Resolution: 10000 |                      |
|-------------------------|-----------|--|--------|-------------------|----------------------|
| #                       | RDB Range | Composition                            | M      | M+1               | M+2                  |
| 1                       | 0.0       | $\text{C}_8\text{H}_9\text{NO}_2$      | .11209 | .11499            | .11686 X             |
| 2                       | 3.5       | $\text{C}_{14}\text{H}_{26}\text{O}_2$ | .11229 | .11570            | .11848               |
| Experimental Values:    |           | .11225                                 | .11581 | .11934            | 11.98                |
| m/z = 190.17201 ± 6 ppm |           | Elements: C H N O P S Si               |        | Resolution: 10000 |                      |
| #                       | RDB Range | Composition                            | M      | M+1               | M+2                  |
| 1                       | 0.5       | $\text{C}_8\text{H}_9\text{NO}_2$      | .17195 | .17493            | 10.62( 8.35-12.93) X |
| 2                       | 4.0       | $\text{C}_{14}\text{H}_{26}\text{O}_2$ | .17215 | .17556            | 15.90(13.35-18.46)   |
| Experimental Values:    |           | .17201                                 | .17553 | 15.67             |                      |

Table 1. Possible Compositions Based on Experimental Exact Mass Defects and Relative Abundances for Fragment Ions with m/z 151, 152, 153 and 190, 191, 192.

## 4 Phenytoin

Six prominent ions appear in both the poor-quality background subtracted mass spectrum in Figure 3a for a compound in the same lake water extract and the NIST library entry for the sedative phenytoin in Figure 3b. However, the many additional ions in Figure 3a limited confidence in this match. The partial profiles for the molecular ion of phenytoin in Figure 4 were obtained with 25,000 resolution. Even at this resolution, a low-mass interference inflated slightly the %M+1 value, and the M+2 profile was useless. In the top part of Table 2 are listed three compositions that provided calculated mass defects for the M and M+1 partial profiles and a relative abundance for the M+1 partial profile consistent with the measured values. The nine compositions in the lower part of Table 2 were consistent with the measured exact mass of the m/z 180 ion. In Figure 4b, the F+1 and F+2 partial profiles were both dominated by interferences. The neutral loss difference between m/z 252.08992 and m/z 180.08106 is 72.00886 Da and corresponds to  $\text{C}_2\text{H}_2\text{NO}_2$  (72.00855 Da). This neutral loss from composition 36 provided composition 9 for the fragment ion. No other M composition contains the two O atoms required to provide the observed neutral loss. Composition 36 is the composition of the molecular ion from phenytoin. Clearly, purchase of this standard for probable confirmation of the compound's identity would be justified.

| m/z = 252.08992 ± 3 ppm |           | Elements: C H N O P S Si                         |        | Resolution: 25000 |                    |
|-------------------------|-----------|--|--------|-------------------|--------------------|
| #                       | RDB Range | Composition                                      | M      | M+1               | M+2                |
| 30                      | 3.0       | $\text{C}_8\text{H}_9\text{NO}_2$                | .09057 | .09278            | 14.21(11.12-17.60) |
| 35                      | 6.0       | $\text{C}_{11}\text{H}_{15}\text{PPSI}$          | .08994 | .09248            | 17.94(15.00-21.06) |
| 36                      | 11.0      | $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_2$ | .08988 | .09324            | 17.03(14.41-19.70) |
| Experimental Values:    |           | .08992   | .09296 | 17.37             |                    |
| m/z = 180.08106 ± 6 ppm |           | Elements: C H N O P S Si                         |        | Resolution: 10000 |                    |
| #                       | RDB Range | Composition                                      | M      | M+1               | M+2                |
| 1                       | 2.5       | $\text{C}_8\text{H}_9\text{NO}_2$                | .08092 | X                 | X                  |
| 2                       | 0.5       | $\text{C}_{14}\text{H}_{26}\text{O}_2$           | .08044 | X                 | X                  |
| 3                       | 1.5       | $\text{C}_{14}\text{H}_{26}\text{N}_2$           | .08144 | X                 | X                  |
| 4                       | 0.5       | $\text{C}_{14}\text{H}_{26}\text{P}_2$           | .08195 | X                 | X                  |
| 5                       | 0.0       | $\text{C}_{14}\text{H}_{26}\text{PSi}$           | .08020 | X                 | X                  |
| 6                       | 0.0       | $\text{C}_{14}\text{H}_{26}\text{Si}$            | .08179 | X                 | X                  |
| 7                       | 6.0       | $\text{C}_8\text{H}_9\text{NO}_2$                | .08124 | X                 | X                  |
| 8                       | 5.0       | $\text{C}_8\text{H}_9\text{N}_2$                 | .08164 | X                 | X                  |
| 9                       | 9.5       | $\text{C}_{14}\text{H}_{26}\text{N}$             | .08132 |                   |                    |

Table 2. Possible Compositions Based on Experimental Exact Mass Defects and Relative Abundances for Ions with m/z 252, 253, 254 and 180, 181, 182.

## 5 A HIGH-MASS DISINFECTION BYPRODUCT

The low-resolution, electron-impact mass spectrum in Figure 5a with two mass peaks at m/z 415 and 417 visible above the chemical noise suggested a polychlorinated or brominated compound might be present in a chlorine-disinfected, well-water extract provided by an EPA regional lab. Related ions with m/z 430 and 432 became apparent in the background subtracted mass spectrum in Figure 5b for which no library matches were found. In Figure 6 are shown the partial profiles obtained for the m/z 415 and m/z 430 ions and the ions arising from higher-mass isotopes. When Cl or Br atoms are present, the M+2 and F+2 profiles have abundances greater than the M+1 and F+1 profiles and are easily observed. In Table 3 are listed the possible compositions for the m/z 415 and m/z 430 ions based on comparison of calculated and measured mass defects and relative abundances. The exact mass of the neutral loss was measured as 430.15123 - 415.12744 = 15.02379 Da, which corresponded to a methyl group (15.02348 Da). Both possible compositions for m/z 430 could lose  $\text{CH}_3$  to provide the corresponding fragment ion compositions for m/z 415. Here, weighted averages of exact masses of ions, summation of their isotopic abundances, and consideration of the valences of elements were insufficient to reject all but the correct composition. Thus, chemical and commercial reasoning must be invoked to select the most likely composition. Chlorination of the well water containing bromide ions could brominate organic compounds. The structure of quinbolone, an anabolic steroid, is shown in Figure 5 and has three possible allylic bromination sites. Substitution of a Br atom for an H atom would provide the observed composition. A feed lot was located near the well and anabolic steroids are often used to stimulate growth. Purchase of quinbolone, its chlorination in the presence of bromide ions, and examination of the mass spectra of the products would be logical next steps in the identification process for this compound.

| m/z = 415.12744 ± 3 ppm |           | Elements: C H N O P S Cl Br                      |        | Resolution: 20000 |        |
|-------------------------|-----------|--|--------|-------------------|--------|
| #                       | RDB Range | Composition                                      | M      | M+1               | M+2    |
| 109                     | 5.5       | $\text{C}_{11}\text{H}_{15}\text{O}_2\text{FBr}$ | .12841 | .13182            | .12652 |
| 122                     | 9.5       | $\text{C}_{10}\text{H}_{13}\text{O}_2\text{Br}$  | .12727 | .13067            | .12541 |
| Experimental Values:    |           | .12744   | .13066 | .12547            | 26.00  |
| m/z = 430.15123 ± 3 ppm |           | Elements: C H N O P S Cl Br                      |        | Resolution: 20000 |        |
| #                       | RDB Range | Composition                                      | M      | M+1               | M+2    |
| 123                     | 5.0       | $\text{C}_{11}\text{H}_{15}\text{O}_2\text{FBr}$ | .15188 | .15530            | .15001 |
| 136                     | 9.0       | $\text{C}_{10}\text{H}_{13}\text{O}_2\text{Br}$  | .15074 | .15415            | .14891 |
| Experimental Values:    |           | .15123   | .15536 | .14945            | 27.60  |

Table 3. Possible Compositions Based on Experimental Exact Mass Defects and Relative Abundances for Fragment Ions with m/z 415, 416, 417 and 430, 431, 432.

## 6 CONCLUSION

These examples illustrate the great value of determining the composition of ions in mass spectra when seeking to identify trace level compounds in complex water sample extracts that provide poor-quality, low-resolution mass spectra. Library mass spectra containing ions with incorrect compositions are confidently rejected. When interferences overwhelm M+2 or F+2 profiles, exact masses and relative abundances sufficient to establish the composition of the apparent molecular ion are often obtained, especially when multiple ions are investigated and exact masses of neutral losses are considered. When multiple compositions remain viable, chemical reasoning and consideration of an inventory of commercially synthesized compounds can usually select one as most plausible and further experiments can be planned rationally.

ICE is a suite of automated procedures that enable determination of ion compositions using a double focusing mass spectrometer and an ancillary personal computer. ICE is now available from the authors for the Finnigan ICL 10.6 data system upon request.

Fig. 1

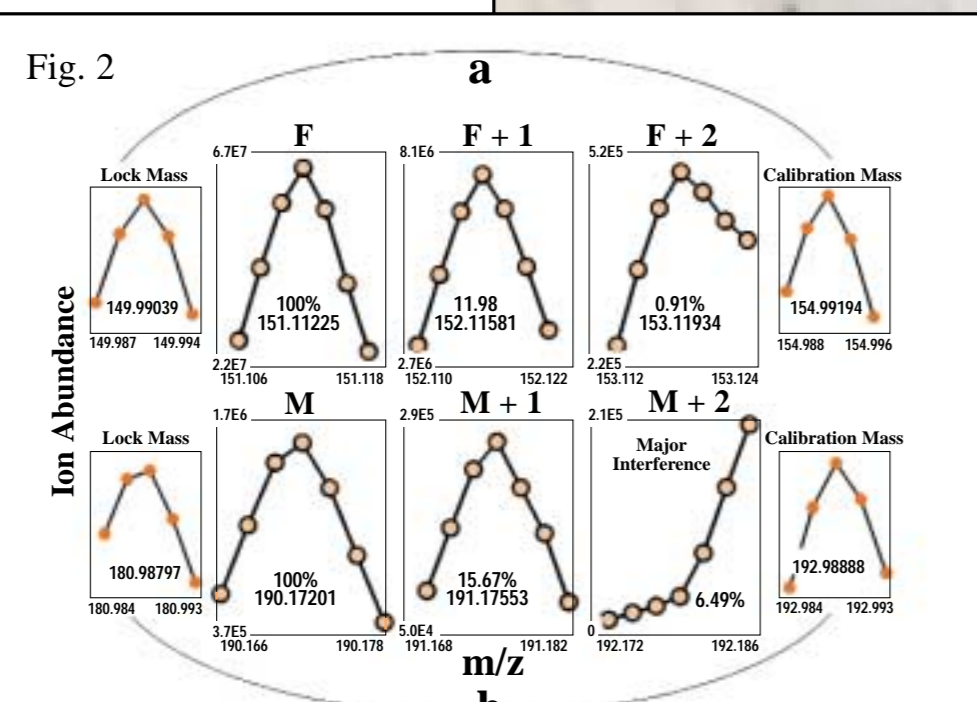
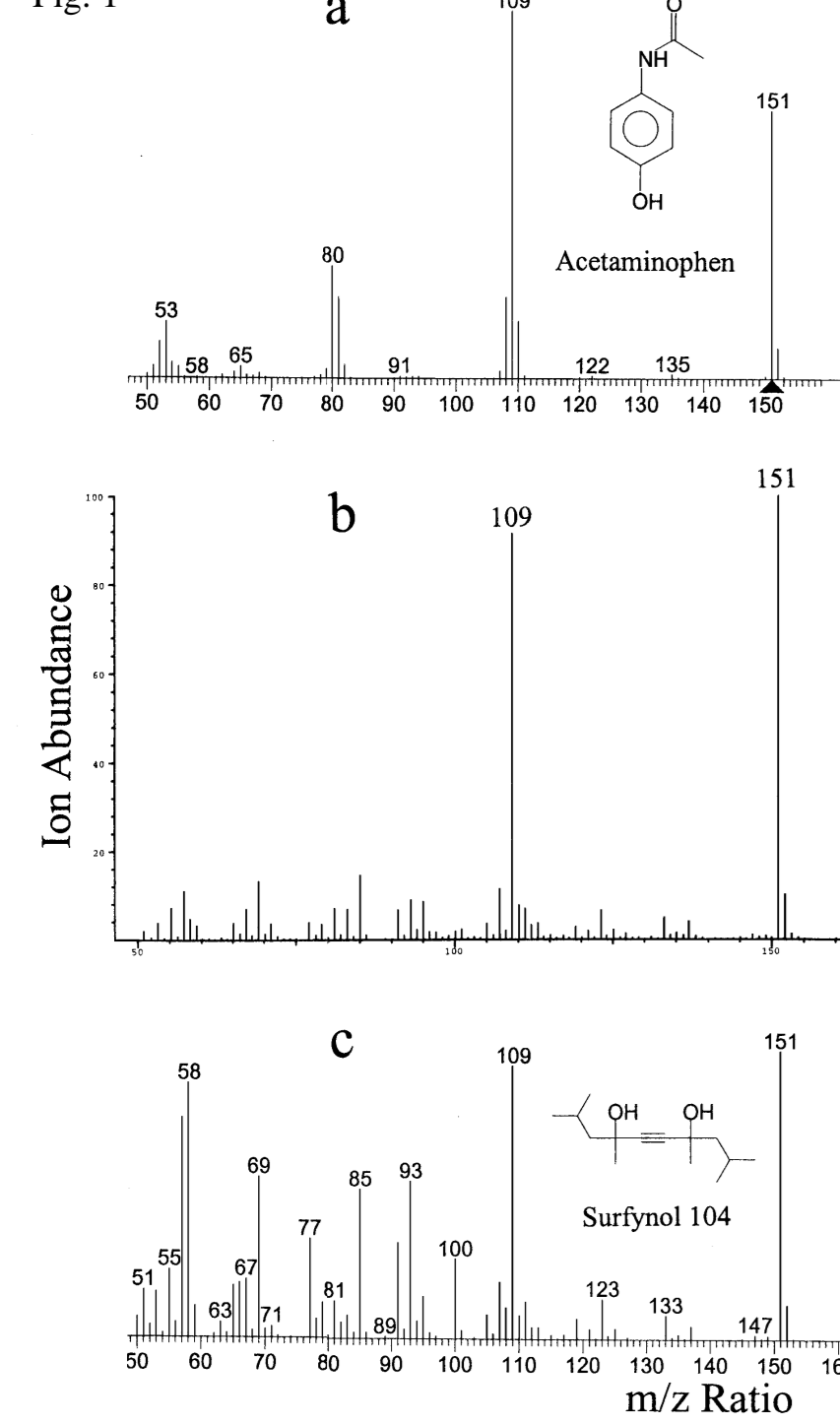


Figure 2. Partial mass peak profiles for (a) m/z 151, 152, and 153 and (b) m/z 190, 191, and 192.

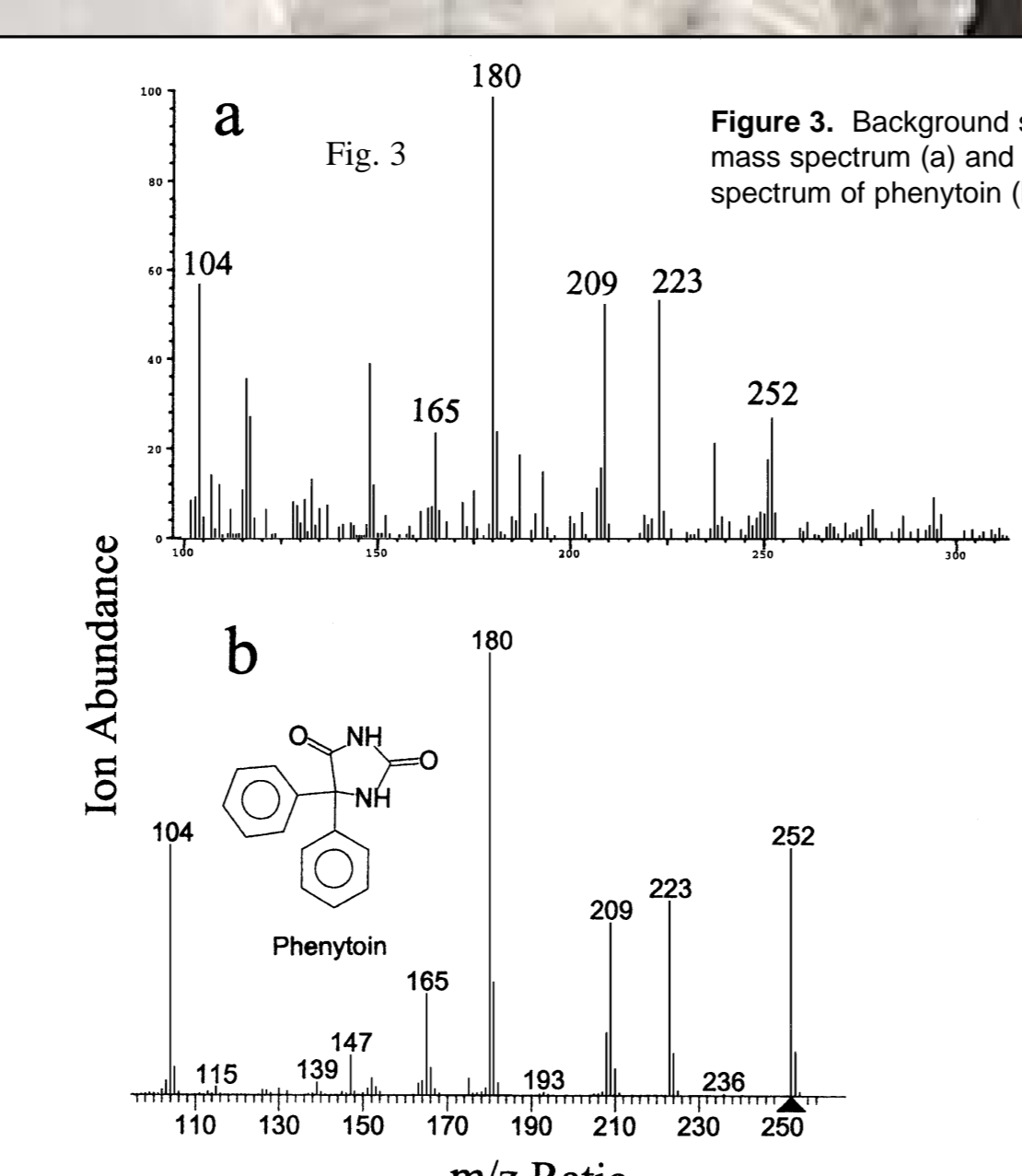


Figure 3. Background subtracted mass spectrum (a) and NIST mass spectrum (b) of phenytoin.

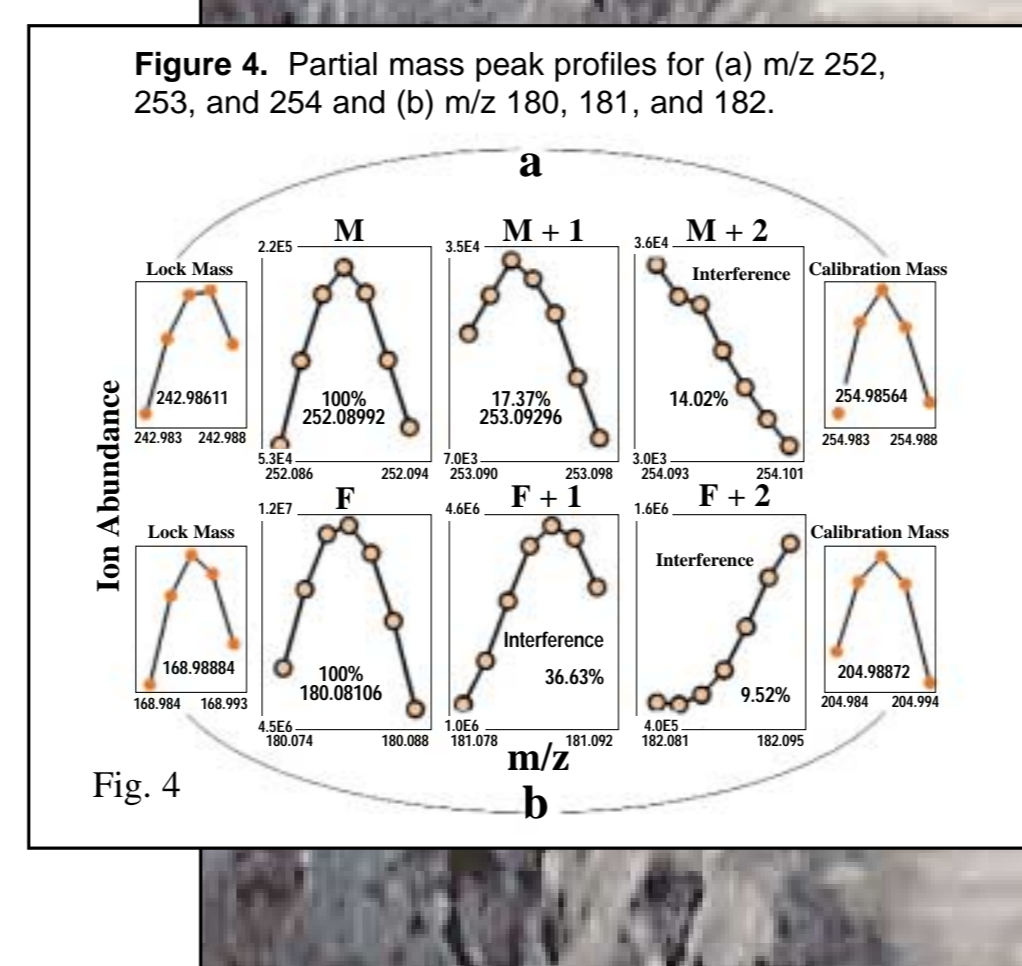


Figure 4. Partial mass peak profiles for (a) m/z 252, 253, and 254 and (b) m/z 180, 181, and 182.

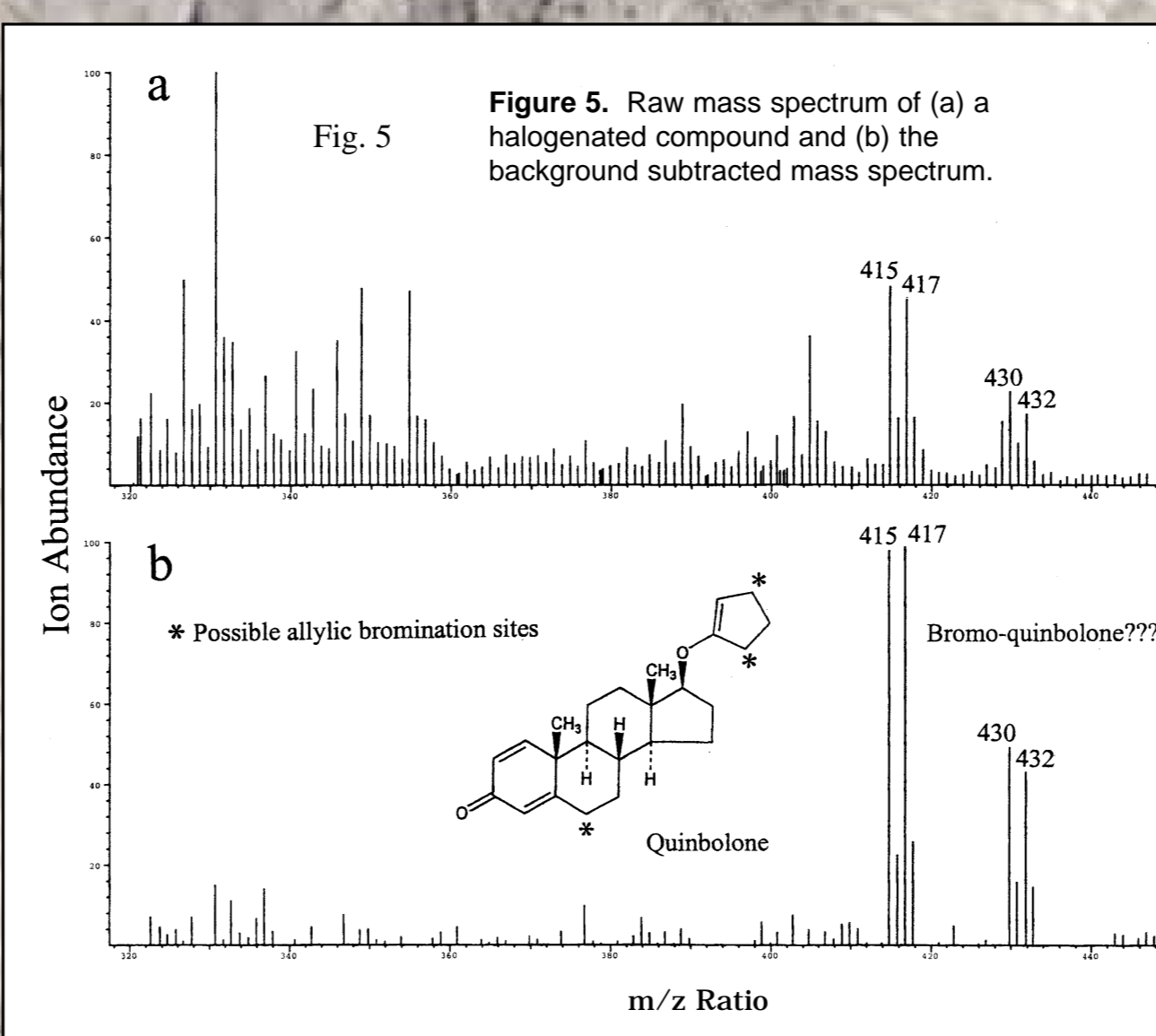


Figure 5. Raw mass spectrum of (a) a halogenated compound and (b) the background subtracted mass spectrum.

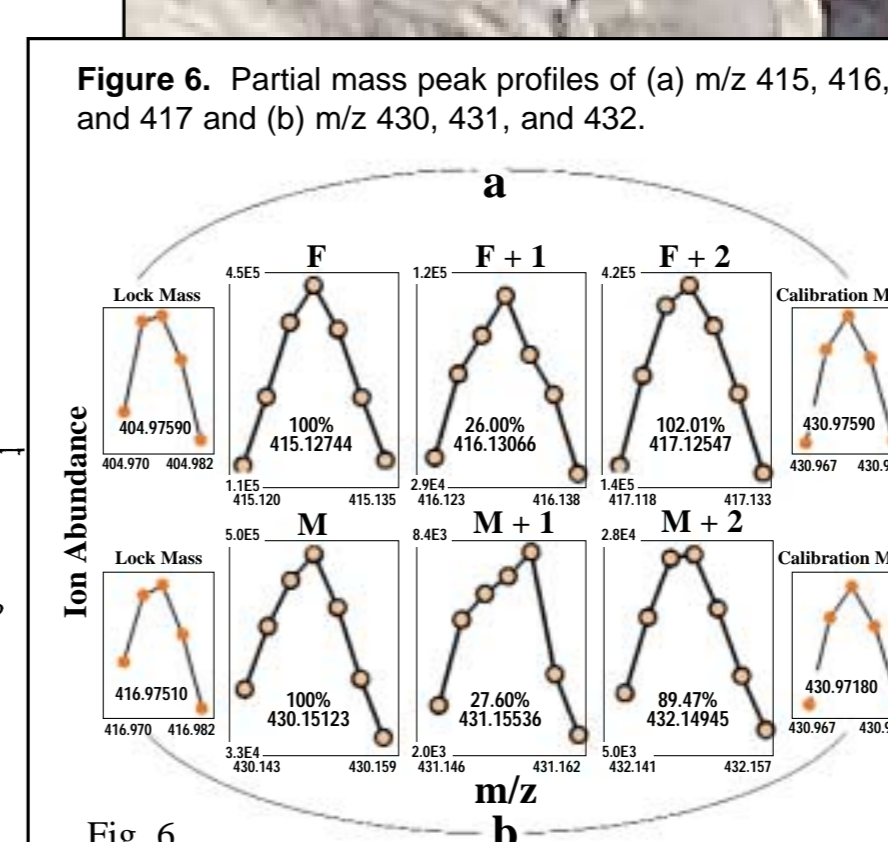


Figure 6. Partial mass peak profiles of (a) m/z 415, 416, and 417 and (b) m/z 430, 431, and 432.

## 7 REFERENCES

- Grange, A.H. & Brumley, W.C. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 170-182.
- Grange, A.H., Donnelly, J.R., Sovocool, G.W., & Brumley, W.C. *Anal. Chem.* **1996**, *68*, 553-560.

Notice: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), prepared this poster presentation. It does not necessarily reflect the views of the EPA or ORD.