Deconvoluting Overlapping Isotopic Patterns Using Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)

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1 ABSTRACT

Isotopic abundance patterns arising from Cl or Br atoms in an ion aid in determining the ion's elemental composition. For mixtures of polychlorinated or polybrominated compounds, however, isotopic patterns can overlap to yield composite patterns that do not clearly correlate with a single combination of Cl and Br atoms. For a synthetic product mixture, a VG70-250SE double focusing mass spectrometer provided high mass resolution (20,000) to resolve overlapping isotopic patterns. Because each KVE scan (variable electrostatic sector voltage and constant magnet current) required 6.4 sec, unreliable patterns were obtained due to changing ion source conditions as the sample volatilized from a heated probe. Summation of KVE scans for 1.5 min to average signals degraded the resolution to <10,000 due to calibration drift. Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) with a 0.8-sec cycle time and recalibration against a lock mass each cycle provided isotopic patterns in excellent agreement with theoretical patterns without loss of mass resolution.

2 INTRODUCTION

The high isotopic abundances of ^{37}Cl (24.2%) and ^{81}Br (49.3%) simplify interpretation of mass spectra when an ion contains either Cl or Br. Isotopic patterns are observed with the dominant members of each pattern separated by 2 Da as illustrated in Figure 1 for an ion containing 3 to 7 Cl atoms. These patterns are not changed significantly by the presence of C, H, F, O, N, or P atoms in an ion, but the presence of one or more S atoms has a small effect due to the relative abundance of ^{34}S (4.2%). Generally, for pure compounds, the number of Cl or Br atoms can be determined from the isotopic pattern observed in the low resolution mass spectrum. However, mass spectra for mixtures of polychloro- or polybromo-compounds display composite isotopic patterns and interpretation becomes problematic. Multiple polychlorinated compounds can be found in synthetic product mixtures and in sites contaminated with PCBs, PCDFs, PCDDs, or PCNs. Although clean-up and chromatographic techniques are used to separate such mixtures into individual components, HRMS can be used to rapidly deconvolute overlapping isotopic patterns with probe introduction of the raw sample.

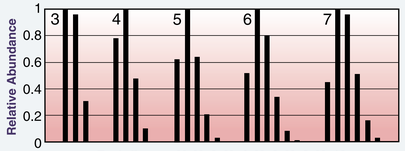

Figure 1. *Isotopic abundance patterns for 3-7 Cl atoms.*

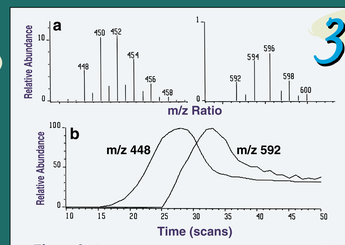

Figure 2. *Portions of a low resolution mass spectrum (a) and ion chromatograms for m/z 448 and m/z 592 for the synthetic mixture (b).*

A synthesis was performed by the Human Exposure Research Branch (EPA) to develop a chlorine-containing hapten for an immunoassay method.[1] Before purification, the product mixture was analyzed to determine if the desired product was present. A methanol solution of the product (1 μL) was injected into the capillary at the end of a probe before its insertion into a VG70-250SE mass spectrometer. The methanol was evaporated in a roughing pumped region before insertion against the block of the ion source, which was at 250 °C. The probe was heated ballistically to 200 °C to volatilize compounds from the probe.

3 A SYNTHESIS PRODUCT

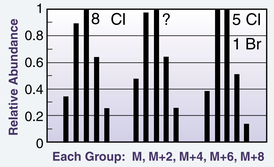

Figure 3. *Isotopic abundance patterns for 8 Cl atoms, the sample (?), and 5 Cl and 1 Br atoms.*

In Figure 2a are shown portions of a low resolution mass spectrum corresponding to the maximum in the m/z 592 ion chromatogram shown in Figure 2b. Two isotopic patterns were observed. The desired synthetic product contained 4 Cl atoms and would be expected to display the second isotopic pattern in Figure 1 starting at m/z 448. In Figure 3 are shown the observed isotopic pattern and the most similar patterns based on Cl atoms alone and on both Cl and Br atoms. Br atoms were considered, because both Cl and Br atoms were present in the reaction vessel. The isotopic pattern of the sample best matched that for 8 Cl atoms. Did this result indicate a failed synthesis?

4 PATTERN VARIABILITY

As illustrated by Figure 4, the isotopic pattern starting at m/z 448 changed considerably as compounds volatilized from the probe. This pattern variability indicated interferences were present that probably arose either from another compound with a molecular ion having an even m/z ratio equal to or larger than 448 or from a series of fragment ions from a much higher-mass polychlorinated compound. Less likely was the presence of multiple interferences from different compounds for each of the ions in the pattern.

The abundance of these ions in the isotopic pattern starting at m/z 592 was too low to assess how many Cl atoms were present in the molecular ion. The ion chromatograms in Figure 2b indicated that this pattern arose from a different compound that volatilized from the probe after most of the compound yielding the m/z 448 ion.

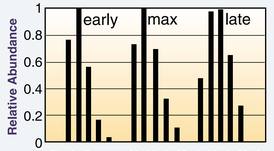

Figure 4. *The isotopic pattern starting as m/z 448 as the compounds volatilized. Mass Resolution = 1000.*

5 MASS RESOLUTION REQUIRED

Because loss of 2 Cl atoms from polychlorinated aromatic compounds is common, the ions contributing to the relative abundances in the low resolution (1000) mass spectrum could differ from each other by the mass difference between a ^{37}Cl atom and a ^{35}Cl atom (1.997 Da) and that of 2 H atoms (2.0157 Da). For ions with a m/z ratio of 450, a resolution of 24,100 (450/0.0187) would be required to observe a 10% valley between a pair of mass peak profiles of equal heights. The isotopic patterns were investigated using 20,000 mass resolution, the maximum resolution routinely used with our instrument, to separate the interfering isotopic patterns.

6 KVE SCANS

During each KVE scan, significant changes in the amount of analytes volatilized from the probe and in sensitivity due to the detuning that accompanied the changing source pressure distorted measurement of the relative abundances of the ions in each pattern. To minimize the influence of these factors, the KVE scan that provided the largest signal for the largest m/z ratio in each isotopic pattern was used to estimate the relative abundances of the ions in each series. The patterns suggested that the lower-mass series of ions contained 6 Cl atoms, and the other series of ions 4 Cl atoms. However, the differences between the relative abundances observed in different scans was so great, that confidence in the data was limited. Hence, a more accurate way to determine these values was sought.

KVE scanning from m/z 447 to m/z 461, during which the electrostatic sector voltage is scanned linearly with a constant magnet current, was used to estimate the relative abundances of the ions in the first isotopic pattern. This range included a calibration ion from perfluorokerosene, which established the nominal mass for the ions in each isotopic pattern. Each scan required 6.4 sec due to the limited data acquisition speed of the data system. A portion of one KVE scan is shown in Figure 5. Two series of ions were observed with baseline resolution. The mass difference between each pair of ions was much greater than that suggested above.

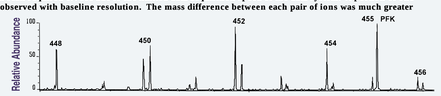

Figure 5. *A portion of a KVE scan from m/z 448 to m/z 456 acquired with 20,000 resolution as sample volatilized from the heated probe.*

To compensate for this variability through signal averaging over a time span during which conditions returned to normal, KVE scans were summed for the 1.5 min required to volatilize the sample. As illustrated by Figure 6, the width at half height of the profiles increased by a factor of 2.1 due to calibration drift and degraded the resolution to less than 10,000.

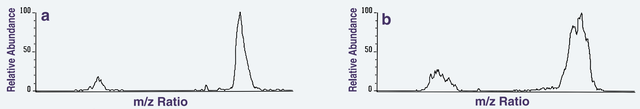

Figure 6. *A portion of a single KVE scan near m/z 452 (a) and for KVE scans summed over about 1.5 min (b).*

7 MPPSIRD

A new data acquisition method developed by the Environmental Sciences Division of the U.S. EPA, Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD),[2,3] overcame the limitations of slow scan speed and calibration drift associated with KVE scans. MPPSIRD provided a cycle time of 0.8 sec and recalibration against a lock mass occurred during every cycle. Hence, integration of data across the volatilization peak provided an improved S/N ratio without loss of mass resolution. Further advantages of MPPSIRD over KVE scanning included more than 100 times greater sensitivity[4], automated data interpretation, and use of 10-fold less computer memory. The disadvantage was the requirement that the exact masses to be monitored must be accurately known before relative abundances can be determined.

The first partial profile in Figure 7a was plotted from the chromatographic peak areas under the ion chromatograms in Figure 7b for six m/z ratios across 60% of the mass range of the M+4 profile. The mass increment between m/z ratios was 5 ppm. The abundances of the M+6 and M+8 partial profiles relative to the M+4 partial profile were obtained as the appropriate ratio of the sums of the six areas used to plot each partial profile. Because a single SIR descriptor monitors only three partial profiles, two SIR descriptors were prepared to monitor the partial profiles, one for the M, M+2, and M+4 ions and the other for the M+4, M+6, and M+8 ions. Monitoring of the M+4 partial profile by both SIR descriptors permitted the ratios to be determined for a series of five ions in each series.

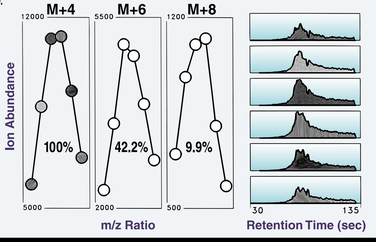

Figure 7. *M+4, M+6, and M+8 partial profiles (a). The M+4 partial profile was plotted from the chromatographic peaks in (b).*

8 ELEMENTAL COMPOSITION OF THE FIRST ION IN EACH PATTERN

The elemental composition of the first ion in each isotopic pattern at m/z 448, 450, and 592 was important for determining if the desired product was synthesized and for indicating what other compound was made. These compositions were also needed to determine the exact masses to be used as center masses in SIR descriptors used to monitor the ions in each isotopic pattern. Data acquired using MPPSIRD were interpreted using a Profile Generation Model (PGM)[4] to determine these elemental compositions.

The number of possible compositions for a given exact mass and error limit increases rapidly with mass. To reduce the number of possible compositions, the exact mass of 4 or 6 Cl atoms, as tentatively determined from the KVE scans, was subtracted from the exact masses of the ions and the PGM then calculated possible compositions for the remaining masses based on an error limit of 3 ppm for the experimental exact masses and the elements present in the reaction vessel, except for Cl and Br, which were accounted for from the isotopic patterns. For the m/z 448 and m/z 450 ions, the observed exact masses and relative abundances of the M+1 partial profiles were used to reject all but the correct compositions.

For the m/z 592 ion, triplicate determinations of the exact mass of the M+1 partial profile would have rejected all but the correct composition, but the $C_{13}F_{23}^+$ ion of PFK interfered. The correct composition was chosen from 6 possibilities based on organic chemistry, but excluding compositions on chemically based arguments provides less certainty than exclusion based on the physical properties of atomic mass and isotopic abundance.

9 ISOTOPIC PATTERNS FROM PARTIAL PROFILES

Shown in Figure 8 are the theoretical and experimental patterns from triplicate determinations. The accuracy and reproducibility of the patterns obtained using MPPSIRD was much superior to those for KVE scans. MPPSIRD provided isotopic patterns that were compared with theoretical patterns to confidently determine the number of Cl or Br atoms that were present in ions.

For the isotopic pattern starting at m/z 592, background interferences, the low relative concentration of the compound in the sample, and the low relative abundance of the largest m/z ions in the series (<1% of the base peak) permitted determination of only the first two ion ratios. However, these two ratios alone were sufficient to conclude that 6 Cl atoms were present in the ion.

The first compound had the elemental composition, including 4 Cl atoms, of the desired product. The exact mass difference between the m/z 592 and m/z 450 ions corresponded to a neutral loss that contained no Cl atoms. This loss, a convincing isotopic pattern for 6 Cl atoms in both ions, and organic chemistry provided the information needed to identify the additional compound that was synthesized.

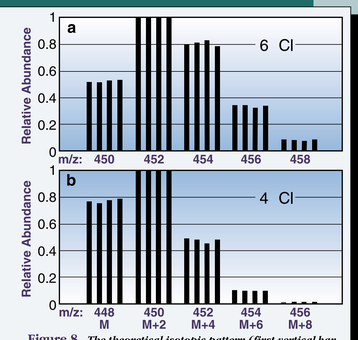

Figure 8. *The theoretical isotopic pattern (first vertical bar for each ion) and patterns determined using MPPSIRD (last 3 vertical bars). Mass Resolution = 20,000.*

10 CONCLUSION

With low mass resolution and probe introduction, interferences from other components in mixtures limit the amount of useful information obtained from mass spectra. Thus, chromatographic interfaces are used to separate compounds in the time domain before ionization. An alternative solution is to separate ions by mass using high mass resolution. An advantage is that more compounds can be volatilized from a heated probe in a vacuum than the number that elute as peaks from a GC column, and more difficult liquid sample introduction techniques can be avoided. Unfortunately, full scan data acquisition rates were too slow to obtain accurate relative abundances, and calibration drift degraded the resolution established by the slits and tuning parameters when scans were summed. Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) provided a faster scan speed and continuous calibration to overcome these limitations. MPPSIRD, which is used routinely in our lab with 20,000 mass resolution to determine relative abundances of M+1 and M+2 mass peak profiles, was used to confidently establish isotopic abundance patterns for polychlorinated synthesis products.

11 REFERENCES

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Acknowledgment: The authors thank Kenneth R. Keeper for performing the synthesis and Dr. Jeffrey C. Johnson and Dr. Jeanette Van Emon for providing a sample of the product.

Note: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved this abstract as a basis for a poster presentation. The actual presentation has not been peer reviewed by EPA.