

Identifying Compounds Despite Chromatography Limitations: Organophosphates in Treated Sewage



Identifying compounds in complex mixtures can be difficult when coelution occurs, clean analyte mass spectra are unobtainable, or plausible library mass spectral matches cannot be found. The authors discuss a novel high resolution mass spectral technique — ion composition elucidation — that acquires data to determine the compositions of ions in mass spectra as the compounds are eluted into the mass spectrometer. This information often leads to the identification of compounds that otherwise would be neglected.

Highly concentrated extracts of sewage treatment plant effluents contain detectable levels of dozens of compounds that result from human activities. Recent concern about the use and disposal of pharmaceuticals and personal care products has stimulated interest in detecting, identifying, and quantifying these and related compounds; in determining their toxicities; and in assessing the risks they pose to ecosystems and to humans (1). Scientists also must investigate the sources, degradation rates, and degradation products of any compounds that pose such risks before they develop remediation strategies.

Gas chromatography coupled with mass spectrometry (GC–MS) provides the most effective analytical method for identifying trace and ultratrace levels of analytes in complex extracts that are eluted or coeluted from a GC column. When coelution occurs, MS provides distinct patterns of ion abundances from each compound that can be correlated with the individual compounds. In contrast, Fourier transform infrared and nuclear magnetic resonance spectroscopy require larger amounts of well-separated compounds for analyses than does MS. Rather than devise techniques to isolate and con-

centrate coeluted compounds before analysis by other analytical techniques, we developed a high-resolution MS technique to identify compounds in complex environmental extracts based upon GC–MS data alone.

The latest version of the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland) or Wiley (John Wiley & Sons, New York) mass spectral library usually included within mass spectrometer data systems for automated comparison of analyte and library mass spectra provides entries for 147,198 compounds (see the NIST web site at <http://www.nist.gov/srd/nist1a.htm>) or 440,000 spectra (see the Wiley web site at <http://www.wileyregistry.com>). These comparisons have three outcomes: a good match can provide a tentative identification; multiple plausible matches can be obtained (2); or no matches are observed because of the absence of the analyte mass spectrum in the library or because a poor-quality background-subtracted mass spectrum for the analyte arises from coelution of more-concentrated compounds or a high level of chemical noise relative to the analyte ion abundances.

The U.S. Environmental Protection Agency (EPA) lists 2800 high-production-volume chemicals (each at least 1 million

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lb/yr) (see http://www.epa.gov/opptintr/chemrtk/hpv_1990.pdf) and estimates 87,000 compounds are used in commerce (<http://www.epa.gov/scipoly/oscpendo/>). These compounds, their production by-products, and their degradation products can be found in sewage treatment plant effluents. Many of these compounds are absent from mass spectral libraries, and most are omitted from lists of target compounds (see <http://www.epa.gov/nerlesd1/chemistry/pharma/critical.htm>).

To assess the risk posed by sewage treatment plant effluents flowing into water supplies, researchers need analytical tools to identify compounds that do not yield tentative identifications based upon mass spectral library searches. *Ion composition elucidation*, a high resolution MS technique developed during the past decade by EPA is such a tool. Ion composition elucidation provides the number of atoms of each element that compose the ions observed in mass spectra. Ion composition elucidation consists of two parts: mass peak profiling from selected-ion recording data for data acquisition and a profile-generation model for automated data interpretation.

Organophosphate compounds found in sewage treatment plant effluent present a range of difficulty for compound identification. Identification is simple when analysts observe distinctive mass spectra with unique library matches, but it is very difficult when only the molecular ion is discernable above the chemical noise.

We have found that ion composition elucidation provided confirmation for each compound and was essential for identifying two sets of isomeric compounds.

Experimental

Sample collection, extraction, and cleanup:

We will describe the sample collection, extraction, and cleanup aspects of our work only briefly in this article, but they are described fully in reference 3. During a 6-h period, we pumped 60 L of sewage treatment plant effluent through a 5- μm pore-diameter filter (Whatman, Clifton, New Jersey) to remove particulates and through 6 g of 1:1 (w/w) polystyrene highly cross-linked with polystyrene cross-linked with 50% divinylbenzene-poly(methyl methacrylate) sorbent within an Absolut Nexus polypropylene cartridge (Varian, Harbor City, California).

The retained organic compounds were eluted from the sorbent with 20 mL of *n*-hexane and then 20 mL of ethyl acetate. We poured the combined eluate through a

column of anhydrous sodium sulfate to remove water and then rinsed the column with methylene chloride to elute any remaining analytes.

After evaporation to 1 mL, we passed this extract through two Envirogel gel permeation columns (Waters, Milford, Massachusetts) in series using methylene chloride as the eluting solvent. The lipid-containing fraction was discarded. The fraction that contained analytes was evaporated to 400 μL , reconstituted in 1 mL of *n*-hexane, and further cleaned on a silica-gel column. We combined three eluates from this column and reconstituted them in 400 μL of toluene. The concentration factor was 1.5×10^5 for this procedure.

Standards: We purchased tributyl phosphate (2000 $\mu\text{g}/\text{mL}$ in acetone) from Chem Services (West Chester, Pennsylvania) and tris(2-chloroethyl) phosphate (97%), tris(2-butoxyethyl) phosphate (94%), triphenyl phosphate (99+%), and tritolyl phosphate (90% mixture of isomers, technical grade) from Aldrich (Milwaukee, Wisconsin) to provide comparison mass spectra and retention times for confirmation of tentative compound identifications.

Gas chromatography: We used an Agilent 6890 gas chromatograph (Agilent Technologies, San Jose, California) interfaced with a mass spectrometer. We made 1- μL splitless injections of the extract onto a 30 m \times 0.25 mm, 0.25- μm d_f Rtx-5MS capillary column (Restek, Bellefonte, Pennsylvania). The temperature program was 90–300 $^\circ\text{C}$ at 7 $^\circ\text{C}/\text{min}$ for full-scan acquisitions. The injector, transfer line, and ion source temperatures were 250 $^\circ\text{C}$, 300 $^\circ\text{C}$, and 250 $^\circ\text{C}$, respectively. The helium flow was 0.1 mL/min or 1 mL/min when the mass range scanned was 50–500 amu or 150–450 amu, respectively.

Mass spectrometry: We used the first stage of a Finnigan MAT 900S ion-trap hybrid mass spectrometer (Thermo Electron, San Jose, California) to acquire all mass spectral data. We used 0.5- or 1.5-s magnetic scans to obtain total ion chromatograms for various ranges. The accelerating potential was 5 kV, the tungsten filament current was 1.0 mA, and the electron impact energy was 70 eV. The secondary electron multiplier voltage was 1.6–2.2 kV — it was higher within this range for greater resolving power or when we investigated low analyte levels.

Mass peak profiling from selected-ion

recording data: The data-acquisition facet of ion composition elucidation is mass peak profiling from selected-ion recording data

(4,5). As many as 31 individual mass-to-charge ratio (m/z) values are monitored for 20 ms during each 1-s selected-ion recording cycle as analytes are eluted from a gas chromatograph into a double-focusing mass spectrometer. The m/z values are monitored across individual mass peak profiles. The areas under the chromatographic peaks in the m/z traces then are plotted to provide full or partial mass peak profiles.

Figure 1a shows six ion chromatograms. The top and bottom traces correspond to the maxima in the partial profiles for the lock mass and calibration mass that bracket the analyte mass shown in Figure 1c. The calibrant — perfluorokerosene — is ever-present in the ion source; thus, we have observed a steady signal. The two baseline excursions in the traces resulted from reversing the voltage polarity of the draw plates for 5 s before and after the analyte eluted. The shaded areas between the excursions are integrated by the data system. The four intervening traces in Figure 1a display chromatographic peaks as the analyte eluted. The areas of these peaks were plotted as the top four points on the full mass peak profile shown in Figure 1c.

Determining the composition of an ion with mass greater than 150 amu usually requires three steps. First, users must monitor a broad mass range by 21 m/z values with a mass interval of 100 ppm using a resolving power of 3000 (10% valley definition) to determine if multiple ions with the same nominal (integer) mass are present. Figure 1b shows a single profile delineated by three m/z values for the nominal mass of 299 amu. A coarse estimate of the exact mass was calculated as the weighted average of the three points. Second, analysts must use a resolving power of 10,000 (10% valley definition) to monitor a narrower mass range close to the estimated exact mass, again using 21 m/z values but with a 10-ppm mass interval (see Figure 1c). As Figure 1c shows, the weighted average of the top eight points provided an exact mass within an error limit of 6 ppm, which corresponded to 46 possible compositions as calculated by the profile-generation model discussed below. Third, operators must monitor the top portions of the m/z 299 profile and profiles greater in mass by +1 and +2 amu each by seven m/z values with a 10-ppm mass interval (see Figure 1d). The latter two profiles arose from ions that contained one or two atoms of heavier isotopes (^{13}C , ^2H , ^{17}O , or ^{18}O). For each of these three experiments, the lock mass and calibration mass partial profiles shown in Figure 1c were monitored by five m/z values.

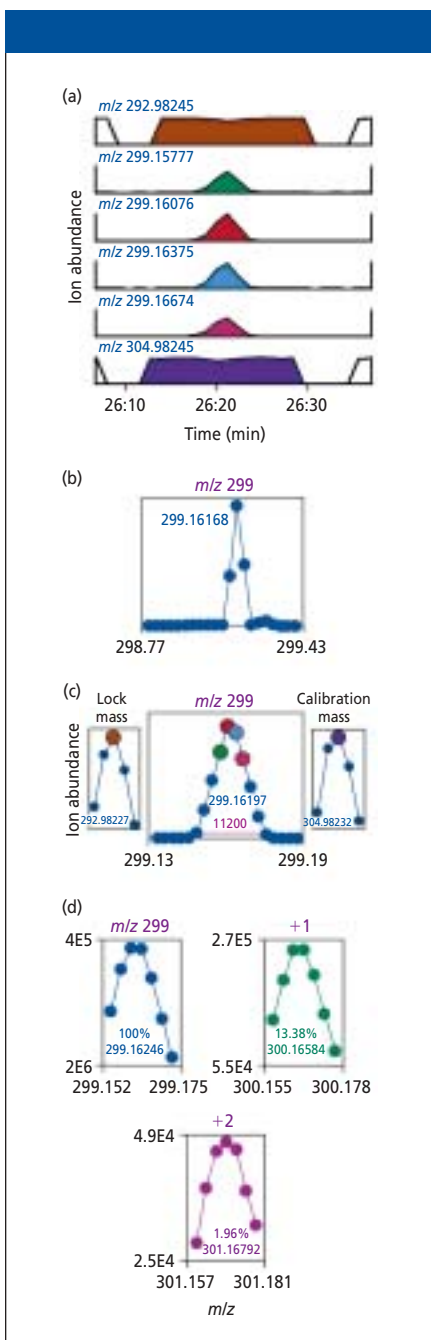


Figure 1: (a) Ion chromatograms acquired with 10,000 resolving power for m/z values corresponding to the maxima for two calibrant ions (top and bottom traces) and for the top four points on the full profile in Figure 1c (middle four traces). (b) A full mass peak profile plotted from chromatographic peak areas for 3 of 21 m/z values monitored across a wide mass range at 100-ppm (0.030 amu) intervals. (c) A full mass peak profile acquired with 10,000 resolving power plotted from chromatographic peak areas from 10 of 21 ion chromatograms for m/z values that monitored masses separated by 10 ppm (0.0030 amu). Also shown are two partial profiles for a lock mass ion and a calibration ion, which bracket the analyte ion mass. Each is delineated by peak areas from ion chromatograms for five m/z values. (d) Three partial profiles for an analyte ion and its +1 and +2 profiles. Each was plotted from chromatographic peak areas from seven m/z values with 10-ppm mass increments. The resolving power was 10,000.

Linear interpolation between the mass errors for the two calibration ions estimated the errors in the exact masses for the analyte profiles, which then were corrected accordingly.

In addition to the exact masses shown in the three analyte profiles, Figure 1d also shows the abundances of the +1 and +2 profiles relative to the m/z 299 profile. These relative abundances are calculated as the sum of the areas used to plot the +1 or +2 profile divided by the sum of areas for the m/z 299 profile multiplied by 100%.

Mass peak profiling from selected-ion recording data is performed by macro language programs within two Lotus 1-2-3, version 9.0 spreadsheets (Lotus Development Corp., Cambridge, Massachusetts). No hardware modifications of the double-focusing mass spectrometer were required. For each experiment, a user enters centroid masses of target profiles, start and end times that bracket the chromatographic peak for each analyte-specific time window (as many as 32), the resolving power, and data file name. The first program prepares ASCII files that are executed by the data system to prepare for data acquisition and to process the data afterward. Entries into selected-ion recording descriptors are typed automatically, and instrument parameters, including the mass resolving power, are set. After data are acquired, the software displays the ion chromatograms, integrates the peak areas, and prepares an ASCII report file listing the m/z values and peak areas. In the second spreadsheet, the software plots the profiles and calculates the exact masses and relative abundances. The software can be modified for use with double-focusing mass spectrometers that provide a macro language for instrument control and for display, processing, and ASCII file outputs of masses and peak areas.

Mass peak profiling from selected-ion recording data provides a 1 cycle/s data-acquisition speed, which is sufficient to delineate chromatographic peaks, the 100-fold sensitivity advantage of selected-ion recording relative to full scanning, the high discrimination against mass interferences realized by using a resolving power of as much as 20,000 (10% valley) routinely, and continuous recalibration because the lock mass maximum is located at the start of each selected-ion recording cycle.

Profile-generation model: The automated data-interpretation facet of ion composition elucidation is the profile-generation model, which is written in QuickBASIC, version 4.50 (Microsoft Corp., Bellevue, Washington) (6). The three exact masses and two relative abundances determined from Figure 1d were entered into the model. The model compared these measured values with the calculated values for the 46 possible compositions determined from the full profile. Table I lists six compositions from the profile-generation model output table. Some values are marked to indicate that measured and calculated values were inconsistent, and the compositions in those rows were rejected. Two compositions, including the fragment ion containing the PO_4 moiety, passed all five comparisons.

Multiple isomers: Figure 2a shows mass peak profiles calculated for m/z 410, 411, and 412. The m/z 410 ion contains only atoms of the most abundant isotopes, and the +1 and +2 profiles are composites from multiple ions that contain one or two atoms of higher isotopes. Figure 2b shows the same partial profiles plotted from selected-ion recording data. The ion chromatograms in Figure 2c were obtained with a mass resolving power of 10,000 (10% valley definition) and correspond to the maxima for

Table I: Possible compositions corresponding to m/z 299.16246 \pm 6 ppm (0.0018 amu) based upon comparison of three exact masses and two relative abundances

Composition	Mass Defects*			Relative Abundances (%)	
	0	+1	+2	+1	+2
$\text{C}_{16}\text{H}_{24}\text{NPF}_2$	0.16144 (-3.4)	0.16473 (-3.7)	0.16801 (+0.3)	18.36†	1.59†
$\text{C}_{17}\text{H}_{22}\text{OF}_3$	0.16228 (-0.6)	0.16568 (-0.5)	0.16883 (+3.0)	19.10†	1.92
$\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_2$	0.16338 (+3.1)	0.16644 (+2.0)	0.16916 (+4.1)	20.23†	2.34
$\text{C}_{20}\text{H}_{21}\text{F}_2$	0.16113 (-4.4)	0.16453 (-4.4)	0.16793 (0.0)	22.36†	2.38†
$\text{C}_{11}\text{H}_{24}\text{N}_2\text{O}_6\text{F}$	0.16184 (-2.1)	0.16494 (-3.0)	0.16688 (-3.5)	13.45	2.04
$\text{C}_{12}\text{H}_{28}\text{O}_6\text{P}$	0.16235 (-0.4)	0.16581 (-0.1)	0.16774 (-0.6)	13.87	2.09
Measured values	0.16246	0.16584	0.16792	13.38	1.96

*Numbers in parentheses represent error in parts per million.

†Indicates inconsistency between the measured and calculated values.

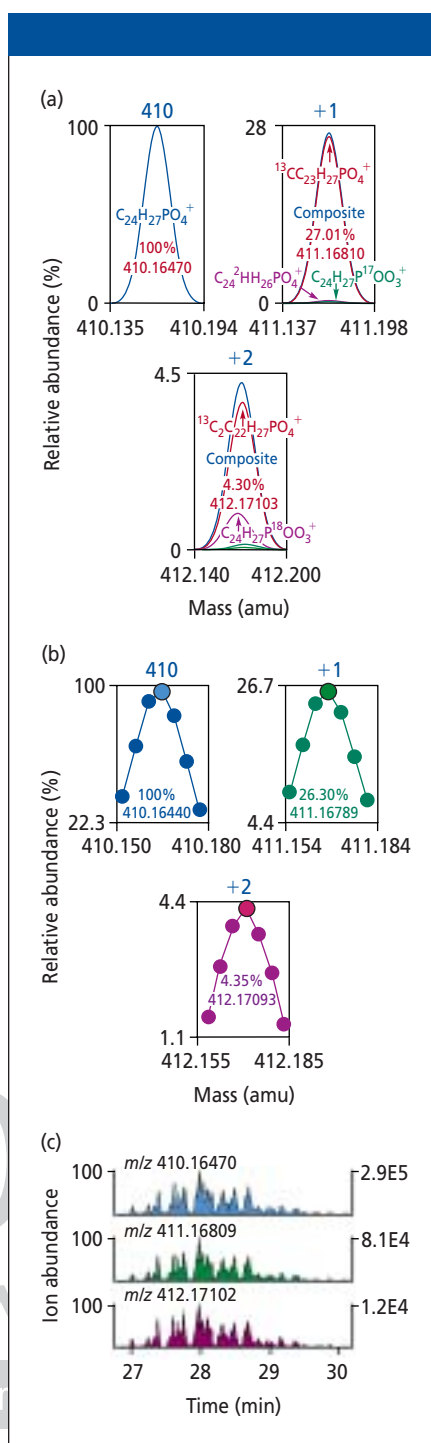


Figure 2: (a) Calculated mass peak profiles for the monoisotopic, +1, and +2 mass peak profiles corresponding to $C_{24}H_{27}PO_4^+$. Plotted are the three +1 ion contributions to the composite +1 profile and the four largest contributions to the composite +2 profile. The profiles are labeled with the calculated exact masses and relative abundances. (b) The corresponding partial profiles plotted from mass peak profiling from selected-ion recording data labeled with the measured exact masses and relative abundances. (c) The ion chromatograms correspond to the m/z value at the maximum of each of the three partial profiles. At least 25 isomers are evident.

the three partial profiles in Figure 2b. More than 25 isomers were present. The summed areas under these isomers were plotted as the maximum point for each partial profile.

Results and Discussion

The total ion chromatogram (150–450 amu) in Figure 3 displays dozens of peaks, many of which are composites caused by coelution of multiple compounds. We used 0.5-s magnetic scans throughout the 150–450 amu range to provide good chromatographic resolution for the total ion chromatogram in Figure 3. We found a series of organophosphate compounds used as flame retardants and plasticizers in the extract. The structure of each compound is shown near its colored chromatographic peak in the total ion chromatogram. The figure also shows the ion chromatograms in the same colors for the prominent ion of highest mass in each compound's mass spectrum. We made tentative identifications easily for five organophosphate compounds based upon matches of their mass spectra with spectra in the NIST library; however, we needed to use ion composition elucidation for five other compounds to have confidence in their tentative identifications.

Compelling library matches: Figures 4a and 4b show background-subtracted mass spectra and the best NIST mass spectral library matches for the first eight of these compounds to be eluted. We used 1.5-s scans throughout the 50–500 amu range to provide full mass spectra (see Figure 4). We found compelling matches for tris(2-chloroethyl) phosphate (Figure 4c), tris(3-chloropropyl) phosphate (Figure 4e), and tris(1,3-dichloropropyl) phosphate (Figure 4f). A dozen or more mass peaks were present in both the background-subtracted and library mass spectra, few mass interferences were observed, and characteristic chlorine isotope patterns indicated the number of chlorine atoms present in each ion. The differences between the analyte and library mass spectra in relative mass peak heights for low- and high-mass ions did not necessarily refute these tentative identifications. These differences often result from lower sensitivity for high-mass ions provided by quadrupole mass spectrometers, which usually are the source of the library mass spectra, and from skewing in the mass spectra obtained using the double-focusing mass spectrometer with a 1.5-s scan cycle to monitor chromatographic peaks only 6–8 s wide.

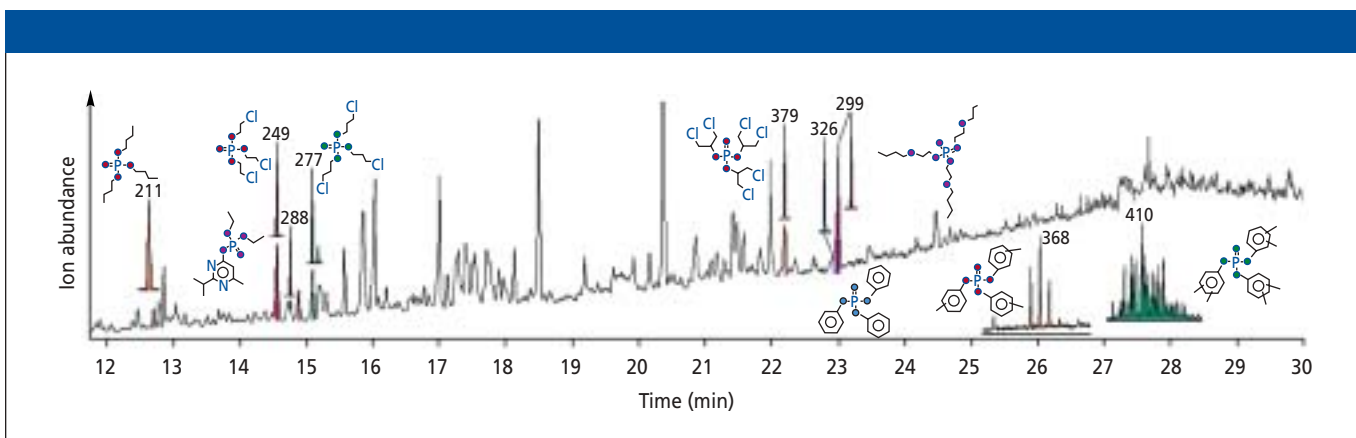


Figure 3: A total ion chromatogram (150–450 Da) for an extract of sewage treatment plant effluent. Superimposed are individual ion chromatograms for the highest mass, prominent ions for organophosphate compounds having nine different molecular formulas, and their structures obtained from the NIST library of mass spectra.

We found compelling matches for tris(2-butoxyethyl) phosphate (Figure 4g) and triphenyl phosphate (Figure 4h), both flame retardant plasticizers. Ten or more mass peaks are common to both the background-subtracted and the NIST mass spectra, and relatively few mass interferences cloud the issue. The isotopic contributions from atoms that contain ^{37}Cl atoms were gone. Even so, the observed matches provide

strong tentative identifications for these two compounds.

Less-certain library matches: The matches between the NIST mass spectra and the background-subtracted mass spectra for tributyl phosphate isomers used as plasticizers (Figures 4a and 4b) and diazoxon (Figure 4d), a metabolite of the pesticide diazinon, are less certain. Fewer than 10 ions are in common, not all prominent

ions in the reference spectra are present in the analyte mass spectra, and conversely not all prominent ions in the analyte mass spectra are present in the reference mass spectra. Fewer common ions, major mass interferences, and the low level of the analytes in the extract yielded less-certain tentative identifications than those discussed previously. However, the mass spectral matches in Figures 4b and 4d provide strong, if

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not compelling, evidence for tentative identifications, and prominent interferences in Figure 4a led to a questionable match.

Two mass spectra with few analyte mass peaks: We found the raw mass spectra in Figures 5a and 5b (top spectra) by paging through a data file, one scan at a time. Background subtraction for scans that showed maxima in m/z 368 and m/z 410 ion chromatograms provided the middle mass spectra in Figures 5a and 5b. The m/z 367–369 ions and m/z 409–411 ions provided two

sets of three ion chromatograms that tracked each other well. The high level of chemical noise, low levels of analytes, and low abundance of fragment ions prevented observation of the fragment ions produced from these compounds. By entering the two sets of three ions into the NIST library and eliminating hits with prominent fragment ions in the mass range monitored, we found six possibilities with two compositions for the lower mass set and seven possibilities with seven compositions for the higher mass

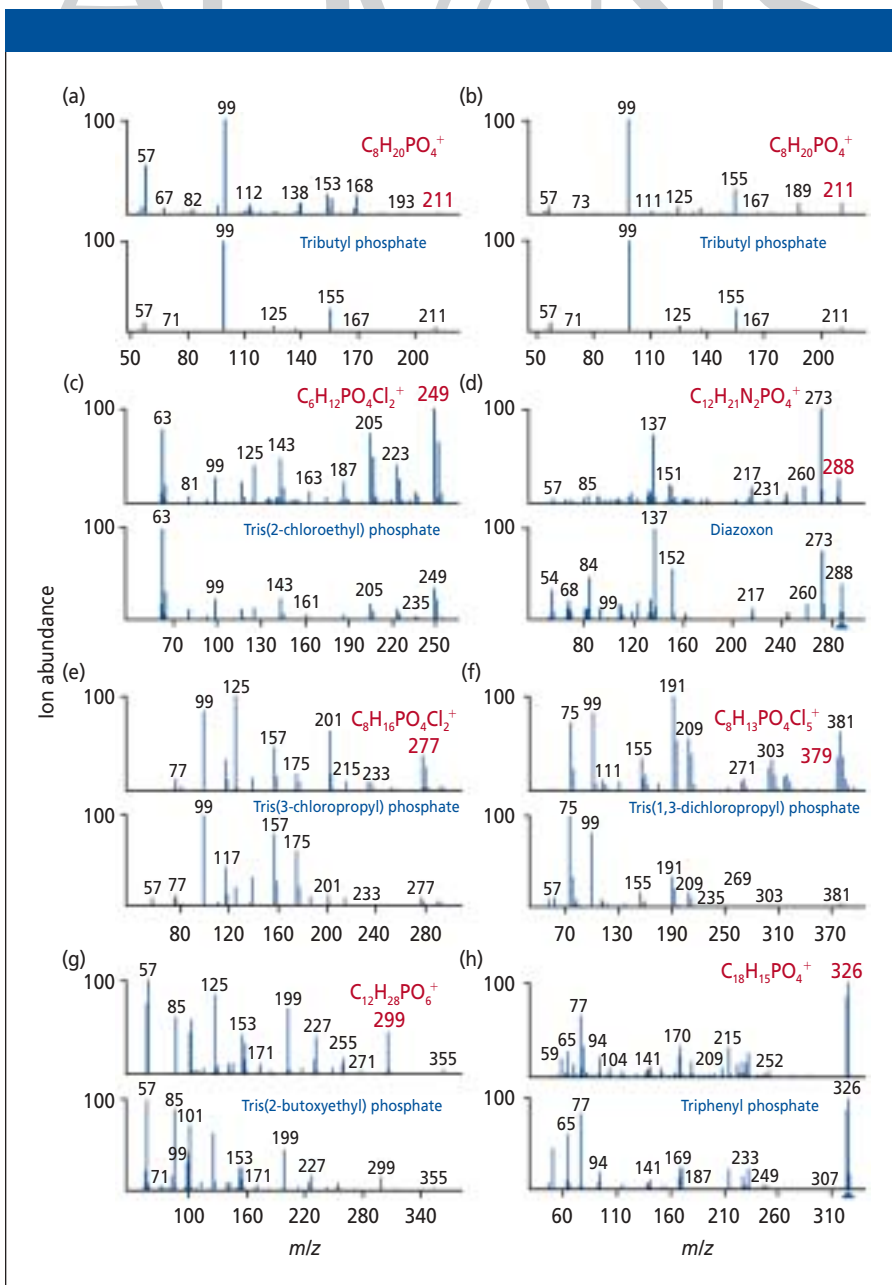


Figure 4: Pairs of background-subtracted mass spectra (top mass spectrum of each pair) and the best matches of NIST library mass spectra (bottom mass spectrum of each pair). Ions below m/z 50 have been removed. The spectra are presented from (a) through (h) in the order of each compound's elution. The compositions of the ions in red were determined using ion composition elucidation. The wide mass range for (f) caused differences of 2 amu to appear as only 1 amu. Hence, the mass scale has been expanded around each chlorine isotopic cluster to show a gap between the ions separated by 2 amu.

ions. No tentative identifications based upon the *full* mass spectra — in this case, the molecular ion, the +1 profile, and the $M - H$ ion — were possible.

Searching for organophosphate compounds by monitoring a characteristic ion:

The compounds that produced the mass spectra in Figures 4a–4g have at least one alkyl chain attached to an oxygen atom of the phosphate moiety. All seven reference mass spectra display an m/z 99 ion, namely $H_4PO_4^+$. The ion chromatograms for a full profile centered on m/z 98.98472 (the exact mass of this characteristic ion for alkyl phosphates) were obtained with 10,000 resolving power. No mass interferences in the eight m/z 99 full profiles were observed.

The first chromatographic peak revealed the presence of a compound that had been overlooked, an isomer of tributyl phosphate. This compound was coeluted partially with a larger amount of a second compound, which contributed to the observed composite mass spectrum. The background-subtracted mass spectrum in Figure 4a for this isomer is of lower quality than that of the second isomer in Figure 4b. Seven of the eight chromatographic peaks observed in the m/z 98.98472 ion chromatogram corresponded to compounds that provided the mass spectra in Figures 4a–4g. The smallest peak corresponded to a smaller amount of a second tris(chloropropyl) phosphate isomer (shown in the m/z 277 ion chromatogram in Figure 3).

Application of ion composition elucidation:

We acquired full mass peak profiles with 10,000 resolving power to measure the exact masses of the monoisotopic ion for each of the ions labeled in red in the 10 analyte mass spectra in Figures 4 and 5. Next we acquired partial profile data with the same resolving power to determine the exact masses and relative abundances of the +1 and +2 profiles. For cases in which the mass interferences distorted the shape of the +1 or +2 partial profiles, we measured fewer than three exact masses and two relative abundances.

When we could not determine unique compositions for the largest mass ion observed, we determined compositions for lower-mass ions, which have many fewer possible compositions (7). Figure 6 is an example. The exact masses of the neutral losses between ions provided the neutral loss compositions. Adding a neutral loss composition to that of a smaller ion for which the composition was known provided a unique composition for the next larger mass ion. Although 47 compositions were possible for the m/z 355.18875 (± 3 ppm) ion based upon consideration of carbon, hydrogen, nitrogen, oxygen, fluorine, phosphorus, and sulfur atoms, the correct composition was determined through this sequential approach because the neutral losses from each ion left behind the phosphate core as a part of all ions studied. Ion composition elucidation provided confirmatory evidence

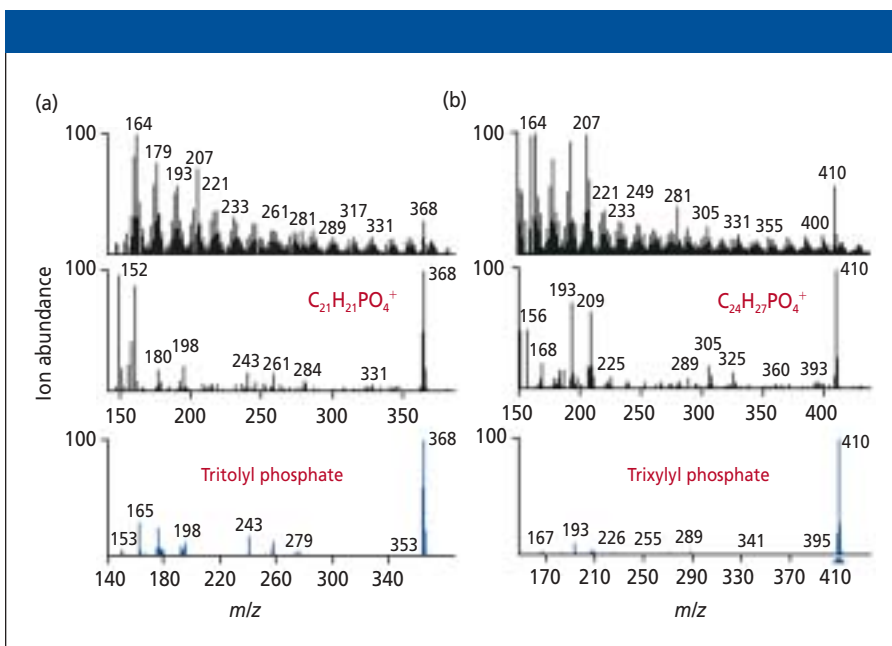


Figure 5: Raw (top), background-subtracted (middle), and NIST library (bottom) mass spectra for compounds that provided easily observed (a) m/z 368 and (b) m/z 410 ions. The composition of the m/z 410 ion was determined using ion composition elucidation. Multiple compositions remained possible for the m/z 368 ion.

in these compounds for the tentative identifications. Only the last two compositions listed in Table I were possible for the m/z 299.16246 (± 6 ppm) ion in Figure 6 as determined by the profile-generation model, based upon the three exact masses and two relative abundances from Figure 1d.

Figure 7 illustrates that mass interferences are still possible, even with 10,000 resolving power. Although the m/z 368 ion chromatogram (Figure 7a) revealed the presence of five isomers, the chromatograms for m/z 369 and 370 (Figures 7b and 7c) showed a gradually increasing signal as the temperature in the GC oven increased. We used ion composition elucidation to determine that the column bleed ions responsible for most of these signals were $C_{11}H_{33}O_4Si_5^+$ and its +1 ions. A resolving power of 267,000 would be necessary to provide a 10% valley between mass peak profiles of equal height from the analyte +1 profile and the monoisotopic ion of the column bleed component (m/z 369.12115 and m/z 369.12253, respectively). Five isomers produced the m/z 368 ion.

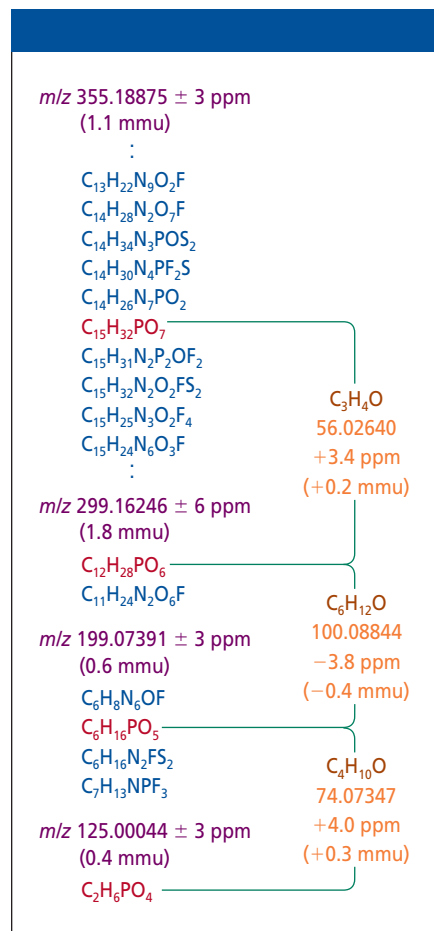


Figure 6: Possible compositions for four fragment ions from an organophosphate compound.

The standard deviation for multiple measurements is inversely proportional to $(N - 1)^{0.5}$. Statistically, monitoring five isomers is similar to monitoring a single isomer five times. The mass error limit is 6 ppm for a single-exact mass determination made with a resolving power of 10,000 (7). Dividing the error limit of 6 ppm by the square root of $(5 - 1)$ provides 3 ppm as a more appropriate error limit. We determined that 45 compositions still were possible based upon the measured exact mass of the m/z 368 ion (368.11731 amu), the error limit, and considering only carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur as possible elements, with at least one-third of the ion's mass due to carbon atoms.

For the m/z 410 ion, 10,000 resolving power provided the partial profiles shown in Figure 2b. Table II is a partial output from

the profile-generation model that shows four compositions that passed all five comparisons of measured and calculated values. However, the ion chromatograms in Figure 2c reveal that at least 25 isomers were present. Using an error limit of 1.2 ppm (6 ppm divided by the square root of 24), only the composition $C_{24}H_{27}PO_4$ passed all five tests. We entered this composition into the NIST library search option and found the single reference mass spectrum in Figure 5b for trixylyl phosphate. The large number of possible positional isomers for this structure account for the isomers in Figure 2c.

One of the possible compositions for the m/z 368 ion was $C_{21}H_{21}PO_4^+$. We entered this composition into the NIST library search option and found four matches for tritolylyl phosphate. One library match is shown in Figure 5a. For three of the four

NIST library matches, the methyl group was located at the same position on each of the three rings (tri-para, tri-meta, or tri-ortho). For the fourth library match, a CH_2 group was located between the oxygen atoms of the phosphate group and the aromatic rings. These structures might account for four of the five isomers in Figure 7a.

Comparison with standards: When standards are commercially available, confirming tentative identifications is straightforward. Analysts purchase a standard and confirm identification by obtaining a retention time and mass spectrum identical to those of the analyte. We purchased five standards: tributyl phosphate, tris(2-chloroethyl) phosphate, tris(2-butoxyethyl) phosphate, triphenyl phosphate, and tritolylyl phosphate. The first four standards to be eluted provided identical retention times and very similar mass spectra — any interferences in the analyte mass spectra were, of course, absent from each standard's mass spectrum. This evidence and the ion compositions determined using ion composition elucidation for the prominent ions of highest mass in the analyte mass spectra confirmed the tentative identifications of these compounds.

The tributyl phosphate standard provided the same retention time as the second, more-abundant isomer. Figure 7a shows the m/z 368 ion chromatogram for the analyte isomers and tritolylyl phosphate standard. Four of the five isomers provided the same mass spectra and retention times, and similar relative abundances were observed among the isomers.

Although we did not obtain a unique composition using ion composition elucidation for this ion, our determination of the composition of the molecular ion of the related compound, trixylyl phosphate, suggested the correct composition and led to purchase of the correct standard to confirm its very tentative identification. The three aryl phosphates found in the sewage treatment plant effluent are used as flame-retardant plasticizers.

We obtained only 5 of 10 standards. Still, the tentative identifications based upon sometimes-dubious library matches for the remaining five analytes were made more certain by confirmation of the composition of a prominent, high-mass ion in each compound's mass spectrum.

Further supporting these organophosphate compound identifications are reports of tributyl phosphate (8–11), tris(2-chloroethyl) phosphate (9–16), tris(3-chloropropyl) phosphate (11–14), tris(1,3-

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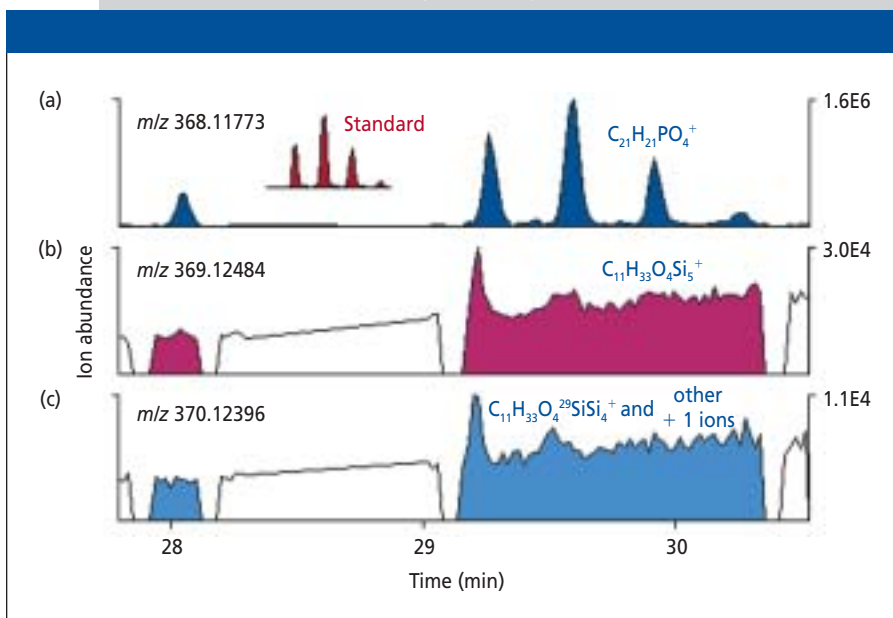


Figure 7: Ion chromatograms for the center mass of the m/z 368, +1, and +2 partial profiles. The increasing signal for the lower two chromatograms indicated a column bleed interference was present.

Table II: Possible compositions corresponding to m/z 410.16440 \pm 1.2 ppm (0.00050 amu) based upon comparison of three exact masses and two relative abundances

Composition	Mass Defects*			Relative Abundances (%)	
	0	+1	+2	+1	+2
$C_{22}H_{25}N_3O_3P$	0.16335 (−2.6)†	0.16651 (−3.4)†	0.16932 (−3.9)†	25.93	3.76
$C_{25}H_{23}N_4P$	0.16603 (+4.0)†	0.16913 (+3.0)†	0.17222 (+3.1)†	29.88	4.24
$C_{26}H_{22}N_2O_3$	0.16304 (−3.3)†	0.16629 (−3.9)†	0.16926 (−4.1)†	29.57	4.86
$C_{24}H_{27}O_4P$	0.16470 (+0.7)	0.16810 (+0.5)	0.17104 (+0.3)	27.24	4.36
Measured values	0.16440	0.16789	0.17093	26.30	4.35

*Numbers in parentheses represent error in parts per million.

†Indicates inconsistency between the measured and calculated values.

dichloropropyl) phosphate (18–21), tris(2-butoxyethyl) phosphate (9,11), triphenyl phosphate (10,11,15,16), and tritoyl phosphate (10) found or quantified in surface water (11), numerous streams (15), rivers, groundwater, rainwater, sewage treatment plant effluents (9), a large drinking water reservoir (13), indoor air (10,11), and on pine needles in the Sierra Nevada mountains (12).

Compounds not in mass spectral libraries: At least one isomer was found in the NIST mass spectral library for each of the nine organophosphate molecular formulas. However, reference 17 describes identification of several nonorganophosphate isomers found in a municipal well near Toms River, New Jersey, where researchers had observed an increased incidence of childhood cancer. These compounds were not in the NIST or Wiley mass spectral libraries.

We determined the compositions of the molecular ion and 10 fragment ions. Interpretation of this information limited possible isomers to a number sufficiently small to make a library search of the commercial and chemical literature practical. We made tentative identifications as 1:2 (w/w) styrene-acrylonitrile adducts based upon a literature search, and we confirmed three of five isomer identities after obtaining a standard from the current industrial process used to make styrene-acrylonitrile polymers.

Conclusion

Sewage treatment plant effluents can contain hundreds of compounds, which result in coelution of components from GC columns. Because each coeluted compound provides a different mass spectrum, GC-MS provides the best single analytical technique for identifying and quantifying trace and ultratrace levels of analytes that traverse a GC column. After identifying these compounds, researchers can perform the toxicological studies necessary to assess the risk to ecosystems and humans from these chemicals.

Many compounds in commerce are found in mass spectral libraries, and one or even a few library matches can lead to tentative identification of the compounds, at which point analysts can purchase standards to compare their mass spectra and retention times with those of the analytes. However, no library mass spectral matches, very poor matches, or many matches can stymie attempts to identify compounds. Ion composition elucidation provides a powerful tool for advancing beyond this impasse.

Determining the molecular ion or fragment ion compositions provides knowledge that can be used to hypothesize tentative identifications before standards are obtained for confirmation.

Analysts specifically targeting the organophosphate compounds identified in this article using a mixture of standards would find them. However, our approach using ion composition elucidation found these compounds without targeting them. Likewise, we have identified many other compounds without any prior knowledge or suspicion of their presence in complex extracts.

Ion composition elucidation software uses the selected-ion recording mode of double-focusing mass spectrometers to realize the potential of high mass resolving power for identifying compounds. Mass peak profiles are constructed from chromatographic peak areas under ion chromatograms acquired for as many as 10 m/z values across individual mass peak profiles. The weighted average of the top several points that delineate the profile provides its exact mass, and ratios of the areas used to plot partial profiles provide their relative abundances. The profile-generation model automatically provides a list of compositions that correspond to the exact mass of an ion within the error limit of its mass determination. This list is usually reduced to one composition when the exact masses of the ion and the profiles greater in mass by +1 and +2 amu, which arise from heavier isotopes, are entered into the model along with the relative abundances of the +1 and +2 profiles.

EPA reports several applications of ion composition elucidation to analytical problems in 12 articles and 12 posters (see <http://www.epa.gov/nerlesd1/chemistry/ice/default.htm>). These include characterization of a Superfund site, identification of isomeric compounds in municipal well water and of compounds in a reservoir, confirmation or rejection of tentative compound identifications for regulatory purposes, and measurement of the exact mass of an α -hemoglobin adduct to within 0.2 amu.

Note

The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and performed the research described. This manuscript has been subjected to EPA's peer and administrative review and has been approved for publication.

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