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THE MASS SPECTROMETER AS A CHLORINE-SELECTIVE CHROMATOGRAPHIC DETECTOR

II. APPLICATIONS TO CHEMICAL SYSTEMS

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SUMMARY

A chlorine-selective chromatographic detector based on the computer analysis of gas chromatographic–mass spectrometric data was described in the previous paper. The presence of chlorine is inferred from isotope cluster patterns in the mass spectra; the computer is instructed to locate these patterns and indicate which spectra are most likely to arise from chlorinated compounds. The application of this detector to a drug metabolism study and the analysis of chlorinated species in an environmental matrix are presented. The performance of the detector in these systems is quite good, even for compounds of low abundance in a mixture.

INTRODUCTION

The analysis of chlorinated organic species continues to present a challenge to analytical chemistry. The widespread past use of chlorinated pesticides, the transfer of pesticides into the food chain, and the environmental toxicity and persistence of these pesticides have made their analysis extremely important. To complicate the analysis, the compounds of interest are most often found as trace constituents in very complex matrices such as soils, sediments, or body fluids and tissues. In order to simplify the analysis, many chlorine-selective detectors have been described for use in conjunction with gas chromatography (GC) (for some recent examples, see refs. 1–5); somewhat fewer for use with liquid chromatography[6–8]. In all cases, the information obtained from the analysis is (a) the chromatographic retention time, and (b) the probable presence of chlorine atoms. No other structural information is collected.

Chromatographic mass spectrometry (MS), on the other hand, often provides a wealth of structural information about a molecule in the form of a set of fragment ions. At the very least, a "fingerprint" is generated that can be used as further evidence in identification via library search methods. The mass spectrometer is relatively non-selective in its data collection when operated in a full-scanning mode; all com-

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pounds entering the ion source are ionized and detected. However, chlorinated compounds, because of their unique stable isotope patterns, are often easily recognized. Methods have been developed for determining the halogen content of an isotope cluster in a spectrum[9], and for determining if chlorine atoms are present in a molecule by pattern recognition analysis of its mass spectrum[10], but these methods are time consuming; and they have not been applied to large sets of data.

Other workers have proposed non-chromatographic analysis of chlorinated compounds using direct probe techniques[11,12]. The mass deficiency of chlorine, particularly evident in high-resolution MS, can be used to indicate ions containing chlorine. Since these analyses are generally done in a chemical ionization mode, the molecular weight of the compound can be deduced; and the number of chlorine atoms present is determined from the isotope pattern. These methods suffer in that they eliminate the chromatographic retention information useful in distinguishing among isomers[13,14], and that they provide no fragmentation of the molecule which would be useful for structure determination. We believe full-scanning chromatographic MS is the method of choice for many types of analyses; and we are attempting to utilize the computer to sort through the tremendously large sets of data that are generated, improving the efficiency of the analyst. If a GC–MS analysis produces 250 mass spectra, but only 5 of those are of chlorinated compounds, the analyst can virtually ignore 98% of the collected data, provided that the important 2% can be located easily.

To that end, we have been involved in the development of computer methods for the selective reduction of GC–MS data, particularly by automatically searching for mass spectra containing isotope cluster patterns from chlorine and bromine atoms[15]. The previous paper[16] describes and evaluates a "chlorine-selective detector" based on such a search. We report here that the technique can be used in a variety of chemical systems to quickly locate the mass spectra of chlorinated compounds, even if the structures of the molecules in question are unknown. Compounds containing only carbon, hydrogen, nitrogen, and oxygen cause little interference because the isotope patterns of these elements bear no resemblance to that of chlorine. Since the full mass spectra are collected and retained, the investigator can manually examine spectra of interest and conduct library searches or use other computerized "element searches." The sensitivity of a full-scan mass spectrometer depends very much on the instrument and the conditions used in the experiment, but is generally poorer than, for example, the electron-capture detector. However, our chlorine-selective "detector" is more selective than the electron-capture detector toward many types of compounds and provides better structural evidence than any other chlorine-selective detector.

EXPERIMENTAL

Computer programs were written in BASIC with FORTRAN subroutines; and listings are available from one of the authors (R.J.A.). The program is identical to that described elsewhere[16]. Execution time varies considerably with the number of spectra to be searched and the number of chlorine atoms to be considered. The plot in Fig. 2a was generated in 26 min (ca. 3.5 sec per scan) which includes the generation of the data for the plots in Fig. 1a–d. The plot in Fig. 3a was generated in 7.2 min. (ca. 2.1 sec per scan).

All GC–MS analyses and programming were conducted on a Hewlett-Packard 5985 B GC–MS system. Fused-silica capillary columns, 30 m × 0.32 mm, coated with Durabond DB-1 (J&W Scientific, Rancho Cordova, CA, U.S.A.) were used throughout except as noted. Splitless injection (250°C) was employed. GC oven conditions are reported in figure legends. The transfer line to the mass spectrometer carried the entire GC effluent to the ion source, and was maintained at 275°C. MS was performed in the electron impact mode at 70 eV. The ion source temperature was 200°C.

All solvents and standards were purchased from commercial sources and were used without further purification. Urine extracts from Lysodren-treated rats were generously donated by Dr. Bruce Jensen, Department of Chemistry, University of Maine at Orono, Orono, ME, U.S.A.
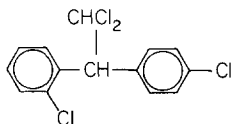
River water was collected in a 1-gallon, acid-washed, hexane-rinsed, amber bottle. The sample was taken near the surface at the eastern shore of the Stillwater River, just north of Stillwater, Maine, U.S.A. (The Ledges). The water was spiked with lead-free gasoline to a concentration of about 40 ppm (55 μl gasoline in 1.0 l water) and with practical-grade DDT to a level of 0.9 ppm (900 μg/l). The 1.0-l sample was extracted with three 50-ml portions of hexane (pesticide-grade), and the combined hexane extracts were dried over anhydrous, hexane-washed sodium sulphate. The extract was concentrated to about 300 μl. Aliquots of 1–2 μl were injected into the gas chromatograph.

RESULTS AND DISCUSSION

The use of any chlorine-selective detector is likely to be most beneficial in those systems in which only a few chlorinated compounds occur in a complex matrix of non-chlorinated species. Over the past two years we have encountered several such systems, and describe the use of the chlorine-selective program in two of them.

Body fluids are complex mixtures of chemical species which, under normal circumstances, contain few or no chlorinated organic compounds. The study of the metabolism of chlorinated compounds is therefore faciliated, because any chlorinated species appearing in the body fluid after administration of the chlorinated compound may be assumed to be a metabolite. If the metabolic pathway of the chloro compound is unknown and the structure of the metabolites is uncertain, standards of the metabolites would be unavailable and their mass spectra would have never been recorded. It would therefore be impossible to locate all of the chlorinated species in the data set by use of mass chromatograms. Proper choice of mass chromatograms can only be made if some knowledge of the mass spectrum is assumed. Since structure determination of the metabolites is desired, the use of a conventional chlorine-selective detector (e.g., an electron-capture detector) is not particularly helpful.

The metabolism of the anti-cancer drug Lysodren [1,1-dichloro-2-(o-chloro-

phenyl)-2-(p-chlorophenyl)-ethane(I)] and its synthetic analogues has been under investigation at the University of Maine for the past several years[17]. Lysodren is an Food and Drugs Administration (FDA)-approved chemotherapeutic agent for treatment of adrenal cortical carcinoma, but is almost always accompanied by unfortunate side effects[18]. Studies at UMO have been directed toward finding suitable analogues of Lysodren which are equally as effective but which lack the side effects. In these studies, the elucidation of the metabolic pathways of the parent drug or analogue is important, since it is believed that a metabolite of Lysodren is responsible for its toxicity[17].

    The parent drug and all of its interesting synthetic analogues contain four chlorine atoms per molecule. Two of these chlorine atoms are readily lost in metabolism, but two remain on the aromatic rings. Identifying metabolites of these compounds involves GC–MS analysis of body fluids and a subsequent search for compounds with two, three, or four chlorine atoms. Since the isotope patterns that would result from these combinations of chlorine atoms are predictable, the system is ideal for a computerized search of the data set for mass spectra exhibiting these clusters. Fig. 1a, b, c and d shows the individual isotope cluster chromatograms[15] for four, three, two and one chlorine atoms, respectively. A weighted summation of these results is the chlorine-selective chromatogram in Fig. 2a. This latter should indicate any and all regions in the data set where the mass spectra display chlorine-isotope patterns. Fig. 2b is the total ion current plot for the same data set and indicates where all compounds, chlorinated or not, appear. A comparison of Fig. 2a and Fig.
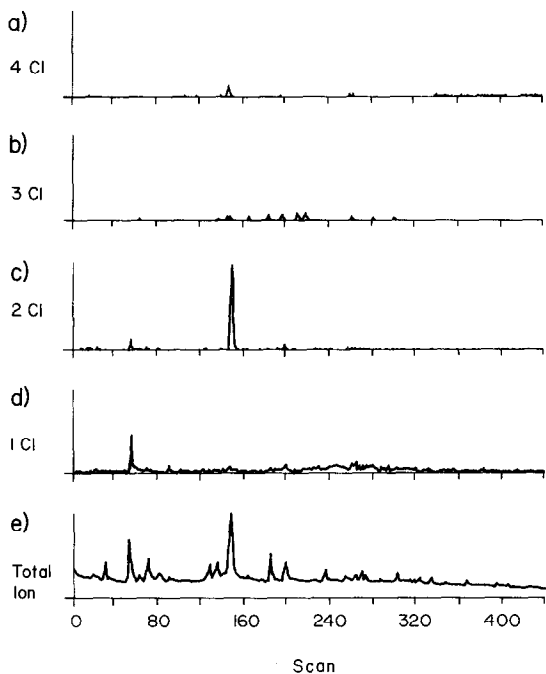

Fig. 1. Isotope cluster chromatograms for (a) four, (b) three, (c) two, and (d) one chlorine atom patterns and (e) total ion profile for a GC–MS analysis of an extract of urine from a rat fed Lysodren. GC oven temperature programmed from 160 to 280°C at 8°C/min.
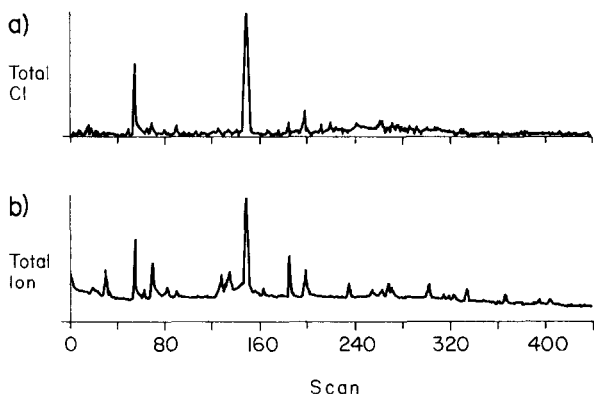
Fig. 2. (a) Total chlorine chromatogram and (b) total ion profile for the data set in Fig. 1.

2b illustrates the usefulness of the technique. Chlorinated compounds are readily located (*e.g.*, at scans 55, 149 and 199), while non-chlorinated compounds are, for the most part, ignored (*e.g.*, scans 31, 71, 128, 135, and 185). The mass spectrum of *o,p'*-dichlorobenzophenone, an identified metabolite of Lysodren appears in Fig. 3. The isotope patterns which resulted in the relatively high score are evident. In addition, the overall baseline in the chlorine-selective chromatogram is considerably less than in the total ion current plot. The background noise is non-chlorinated, and so is eliminated. The result is an improved signal-to-noise ratio for the chlorinated compounds. The analyst can concentrate interpretive efforts on those regions of the chromatogram that the computer has indicated are most likely to contain chlorinated species. The full mass spectral data remain in storage and are not changed by the computer search, so the data are available for library searching, manual or automated interpretation, or other data manipulation methods.

A rather unexpected advantage of our chlorine-selective program was observed when samples were dissolved in chlorinated solvents such as dichloromethane. With conventional chlorine-selective detectors, such solvents produce tremendously large signals and high background, and for these reasons, they are usually avoided. By simply instructing our computer to ignore any isotope patterns below m/z 84 (the molecular weight of dichloromethane), our chlorine-selective program can block out the signal (and background) from dichloromethane without significantly affecting its performance in locating larger chlorinated compounds. Fig. 4 demonstrates this feature. A solution of *o,α*-dichlorotoluene in dichloromethane was chromatographed under conditions which caused the solute to elute on the tail of the large solvent peak. The total ion current profile (Fig. 4b) shows both solvent and solute. The chlorine-selective program, upon modification to detect only isotope clusters greater than m/z 84, locates only the dichlorotoluene (Fig. 4a), dramatically increasing signal-to-noise ratio for this analysis.

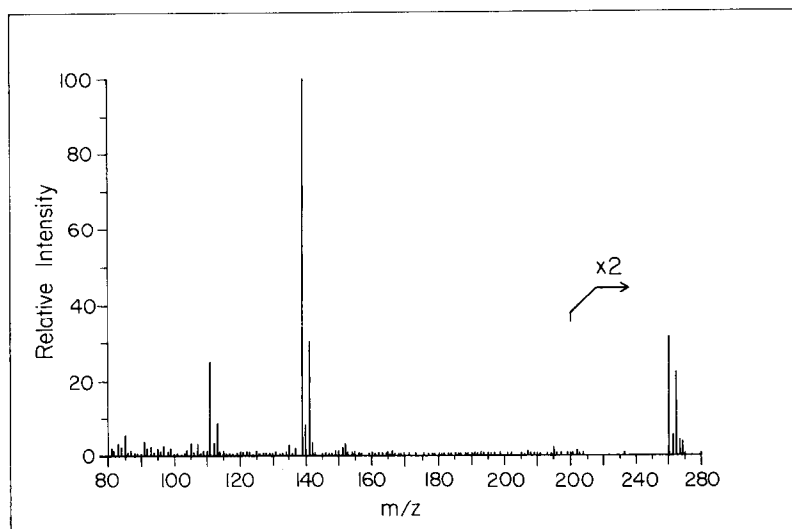In other, similar experiments, we observed an even more striking result. Vol-

Fig. 3. Mass spectrum of *o,p'*-dichlorobenzophenone from scan 55 of the data set in Figs. 1 and 2.

atile impurities such as trichloroethylene in the dichloromethane solvent co-eluted with the solvent and were completely masked in the total ion current profile. The presence of these impurities was not even suspected until the data was searched using the chlorine-selective detector modified to ignore isotope clusters less than m/z 84. The trichloroethylene, having chlorine-containing ions greater than m/z 84 was de-

✳✳ CHLORINE SELECTIVE CHROMATOGRAM ✳✳

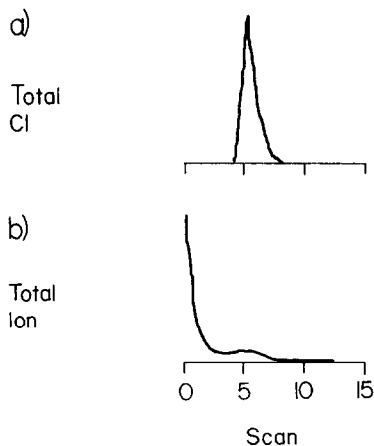Data File #7002   FRN 11131   1-2 Cl   Scan 1 to 15

Fig. 4. (a) Total chlorine chromatogram and (b) total ion profile for GC–MS analysis of *o,α*-dichlorotoluene in dichloromethane. Program was modified to ignore all isotope clusters below m/z 90. Packed column GC on 2 m × 2 mm I.D. glass column, 3% OV-17 on Gas Chrom Q; helium flow-rate 30 ml/min; isothermal at 210°C.

tected; the dichloromethane was not (data not shown).

Environmental analyses represent another area where selective detection of chlorinated compounds is extremely valuable. Chlorinated pesticides and pollutants occurring as trace constituents in water, soil, or sediment samples are likely to be accompanied by a host of non-chlorinated compounds present in the matrix. If a general screen is being performed, one does not know in advance what compounds to expect, and one would like structural information on any chlorinated compounds detected. Chromatographic MS followed by selective data reduction as described above is again indicated. To evaluate the performance of the detector on samples of this type, the analysis of a river water sample was undertaken. A sample of river water was collected and spiked with gasoline (a non-chlorinated pollutant) and DDT (a chlorinated pollutant). After extraction, the sample was analyzed by GC-MS. A portion of that chromatogram is shown in Fig. 5. As in Fig. 1, a range of 1-4 chlorine atoms was searched, any compounds exhibiting Cl_1, Cl_2, Cl_3, or Cl_4 isotope cluster patterns would be detected. No Cl_3 or Cl_4 isotope clusters were detected in this

✶✶ CHLORINE SELECTIVE CHROMATOGRAM ✶✶

Data File # 7122   FRN 12104   1 - 4 Cl   Scan 1 to 200

a)
3 Cl
                        NOT FOUND

b)
2 Cl

c)
1 Cl

d)
Total
Cl

e)
Total
Ion
     0   40   80   120   160   200
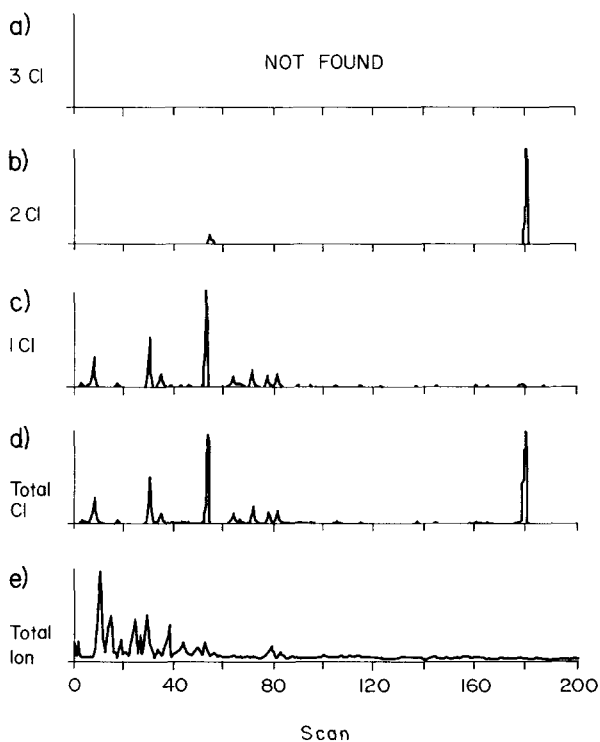
                        Scan

Fig. 5. Isotope cluster chromatograms for (a) three, (b) two, and (c) one chlorine atoms; (d) total chlorine chromatogram, and (e) total ion profile for a GC-MS analysis of an extract of contaminated water. GC oven temperature programmed from 70 to 300°C at 8°C/min.
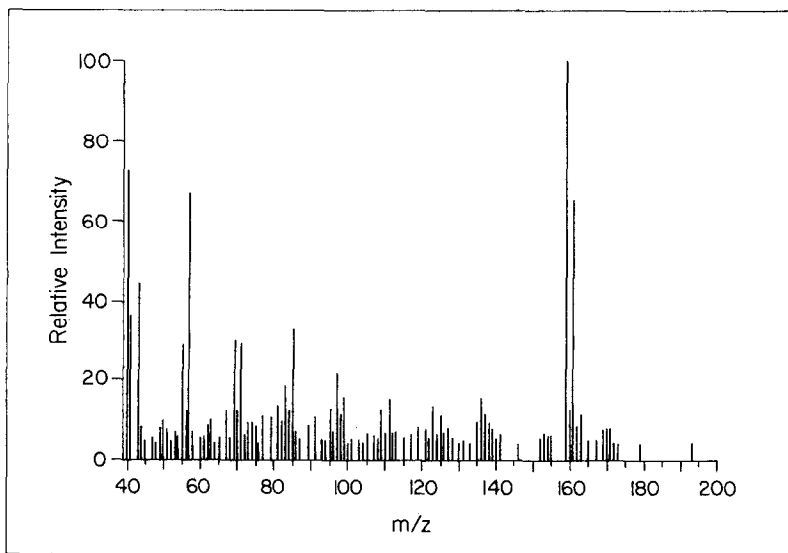
Fig. 6. Mass spectrum number 181 from the data set shown in Fig. 5.

portion of the data set (scans 1–200). The weighted sum of the individual isotope cluster chromatograms produces the "total chlorine chromatogram" in Fig. 5d. The DDT eluted around scan 350 and is not shown in the figure. The majority of the peaks in the chromatogram are aliphatic and aromatic hydrocarbons, as would be expected from the gasoline contamination. The total chlorine chromatogram provides a much simpler display. Two major peaks and several minor peaks are indicated; inspection of the mass spectra at these points allows the analyst to decide whether or not chlorinated compounds are, in fact, present. The mass spectrum from scan 181 of this data set is shown in Fig. 6. A two-chlorine isotope cluster beginning at m/z 159 verifies the presence of a chlorinated molecule. This fragment ion is most probably a dichlorobenzyl ion and arises from an impurity in the DDT. The mass spectra at the remaining peaks in the chlorine-selective chromatogram probably do not represent chlorinated compounds, although the spectra do show patterns of ions which could be mistaken for chlorine isotope clusters. Since these ions are isotopically unrelated, these would be classified as Type III interference[16]. The detection has performed its function, however, in reducing the amount of data the analyst must examine. Instead of 200 mass spectra, only a handful contain potential isotope clusters. The find at scan 181 is particularly important, since the peak in the total ion profile is very small; and a weary analyst might have completely neglected it. It is a major peak in the chlorine selective chromatogram and would have been detected right away.

    The selectivity of the program for chlorinated compounds relative to non-chlorinated compounds was substantially better in these analyses of "real" samples than described in the evaluation of the program on the U.S. Environmental Protection Agency (EPA)/National Institutes of Health (NIH) Mass Spectral Data Base[16]. If one compares the overall average score for mass spectra which represent chlorinated compounds (80,200) to the overall average of all other mass spectra in the data set

(3580), for the data set illustrated in Fig. 5, one can calculate a selectivity factor of 80,200/3580 ≈ 22. This does not take into account that the compound at scan 181 is present in only about 5% of the amount of the most abundant compound in the mixture, so another factor of 20 in the selectivity factor is probably justified. A similar calculation based on the results of the mass spectral data base evaluation gave a selectivity factor of 18 (ref. 16). In part, this improvement is due to the nature of the instrument and the nature of the sample matrix. For samples run on our instrument under controlled conditions, we obtain a more consistent quality of spectra than is found in the large and varied data base. In the examples cited above, the chlorinated compounds under investigation did not contain other elements with interfering isotope patterns. (Type II interferences[16]). In addition, the matrix did not contain compounds of elements with unusual and potentially interfering isotope patterns such as silicon, bromine, tin, germanium, zinc, etc. The mass spectral data base contains examples of all of these.

As in our previous evaluation, the most serious interference resulted from a few non-chlorinated compounds whose mass spectra exhibit a pattern of isotopically unrelated ions that by coincidence is very similar to a chlorine isotope pattern. This type of interference we have designated as Type III interferences[16]. They are, in most cases, unavoidable since the computer is asked to seek a specific pattern of ions and the spectra do, in fact, display that pattern of ions. However, an experienced spectrometrist can usually detect and eliminate these interferences by a quick visual inspection of the spectrum.

We are currently investigating the feasibility of using the chlorine-selective scores to quantitate chlorinated compounds. Our initial results (not shown) indicate that the quantitation based on the area under the chlorine-selective plot (relative to an internal standard) approximates the results of quantitation based on the area under a particular mass chromatogram (relative to an internal standard). A detailed report on that study will be the subject of a future communication.

The sensitivity of our detector is determined by the sensitivity of the GC–MS system at the time of data collection. Because our method is a selective data *reduction* technique and not a selective data *collection* technique, there is no sensitivity enhancement as there would be, for example, in a single-ion monitoring GC–MS experiment. However, improved signal-to-noise ratios in the chlorine selective chromatograms should lead to improved sensitivity.

Any techniques which improves the sensitivity of MS in general will improve the sensitivity of the selective data reduction methods. Negative ion chemical ionization MS, for example, is reported to improve sensitivity by as much as a factor of 1000 for certain electrophilic compounds[19]. Fourier-transform MS[20], which can collect signals from all ions simultaneously, also promises to improve the sensitivity of MS and therefore of this chlorine-selective detection.

CONCLUSION

A computer program to selectively locate the mass spectra of chlorinated compounds in complex samples is useful in a variety of circumstances. Based on the search for appropriate isotope clusters, the "detector" rapidly screens large GC–MS data sets and reduces the amount of data the analyst must interpret. The full mass

spectra are retained for future reference. The program is useful even with complex sample matrices such as biomedical and environmental samples. Electrophilic compounds such as phthalate esters, which interfere with electron-capture detection, cause no interference in the computer search, since these compounds do not display isotope clusters diagnostic for chlorine.

ACKNOWLEDGEMENTS

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