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THE MASS SPECTROMETER AS A SUBSTANCE-SELECTIVE DETECTOR IN CHROMATOGRAPHY

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SUMMARY

The computerized mass spectrometer has several modes of data acquisition when applied as a detector in chromatographic systems. These and several modes of data reduction are defined with examples from the field of environmental measurements of organic pollutants. The overall advantages and disadvantages of the various techniques are discussed.

INTRODUCTION

The computer-controlled mass spectrometer has two general modes of operation as a continuous detector in chromatographic systems. One mode is to acquire conventional mass spectra of components as they emerge from the chromatographic system. These mass spectra are used to identify the individual components. The alternative mode is to apply the mass spectrometer as a substance-selective detector. This mode is called selected ion monitoring (SIM), which is defined as the dedication of a mass spectrometer to the acquisition of ion abundance data at only selected masses in real time as components emerge from the chromatographic system.

SIM is not a new principle in mass spectrometry (MS). A technique called peak stepping or peak switching has been used for decades in the precise measurement of isotope ratios. In this classical application, the ionic abundances measured were usually limited to those separated by just a few atomic mass units, for example ^{16}O and ^{18}O. In recent years there has been a very significant increase in the applications of SIM. This was brought about largely by the development of the computer-controlled quadrupole mass spectrometer as vapor phase chromatography detector. The presence of the gas chromatography (GC) inlet system permitted the introduction into the mass spectrometer of very small samples of complex mixtures in an easily handled liquid form. The computer-controlled quadrupole mass spectrometer provided a method for high speed and very accurate and precise ion monitoring over a very wide mass range. Finally, the dedicated minicomputer and its related peripherals gave the experimentalist access to a wide range of control functions and real-time ion

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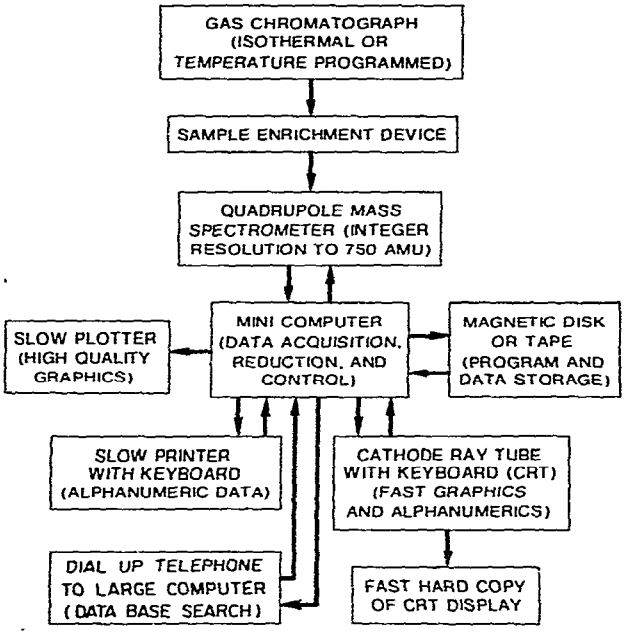


Fig. 1. Block diagram for the GC–MS system used in this work.

monitoring techniques. It is the purpose of this paper to present some of the basic concepts involved in the application of the computer-controlled mass spectrometer as a substance-selective detector. The examples presented are taken from current research in the measurement of organic compounds in environmental samples. Fig. 1 is a block diagram of the type of GC–MS system used in this work.

EXPERIMENTAL

Mass spectra were measured with a Finnigan Model 1015 quadrupole mass spectrometer. The inlet system was a Varian Series 1400 gas chromatograph. The chromatograph was interfaced to the spectrometer by an all-glass jet-type enrichment device and an all-glass transfer line. Control of the quadrupole rod mass set voltages, data acquisition, data reduction, and data output was accomplished with a System Industries data system which employed a Digital Equipment Corporation PDP-8/E mini-computer and a 1.6-million word Diablo disk drive. The gas chromatograms and mass spectra were displayed on a Tektronix Model 4010 cathode ray tube or a Houston Instruments Model DP-1 flat bed plotter. The mass spectrometer operating parameters were adjusted to give the standard ion abundances for the reference compound decafluorotriphenylphosphine[1]. All measurements were made with electron-impact ionization at a nominal electron energy of 70 eV.

All of the GC reported in this paper was carried out using a 6 ft. × 2 mm I.D. glass column packed with 1.95% QF-1–1.5% OV-17 on 80–100 mesh Supelcoport. The flow-rate was *ca.* 30 ml/min, the injector temperature 270°, and the interface oven–transfer line temperature 135–140°. Polychlorinated biphenyl analyses were

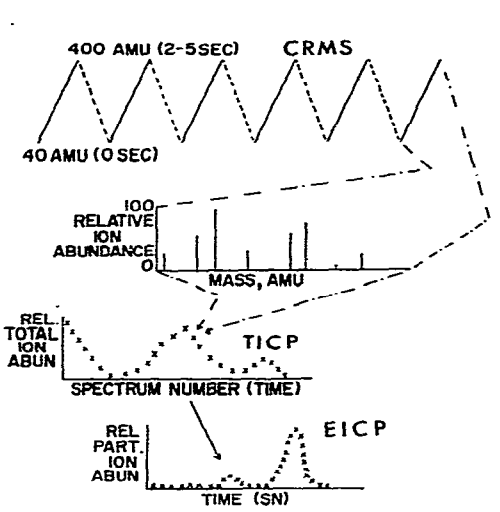
conducted isothermally at 180°. For toxaphene, temperature programming from 140 to 220° at 8°/min was employed.

DEFINITIONS OF TERMS

There is usually a period of confusion in terminology, concepts, etc., any time that technology is rapidly advanced by a number of individuals and organizations in a relatively short time period. The application of a mass spectrometer as a substance-selective detector in chromatography is no exception. The terms accelerating voltage alternation, mass fragmentography, single ion detection, and multiple single ion detection are among a number of terms that have been used to describe this technique. An analysis of this terminology by Watson *et al.*[2] led to the recommendation of a standard term, selected ion monitoring (SIM), because it best conveys to the reader the significant information about the technique that sets it apart from other techniques. The term SIM is general in that it does not imply a particular type of spectrometer, the number of ions measured, or the type of ions measured. It must be recognized, however, that SIM is a real-time measurement technique and that a designation is also required for the output obtained from SIM. Watson *et al.*[2] suggested the name selected ion current profile (SICP) as the most appropriate. Consistant with the definition of SIM, a SICP is then a plot of the change in ion abundance as a function of time, using abundances measured by SIM. We strongly recommend these terms be adopted as the standard designations and they will be used throughout this presentation.

Clear precise terminology is particularly important in computerized GC–MS work because there are several other widely used techniques that may be confused with SIM and the SICP. It is important to understand these techniques to appreciate the overall advantages and disadvantages of SIM. Perhaps the most widely used real-time data acquisition technique in GC–MS is the continuous repetitive measurement of spectra (CRMS). Fig. 2 contains a schematic diagram of CRMS and two types of data reduction that are in common use. The sawtooth diagram in the top of Fig. 2 is a representation of CRMS during the elution of components from a gas chromatograph. Each solid line represents a sweep of the mass spectrometer from an arbitrary starting mass, *e.g.*, 40 a.m.u., to an arbitrary ending mass, *e.g.*, 400 a.m.u. Each dotted line represents the resetting of the mass spectrometer sweep control to the starting mass. In a typical GC–MS run, several hundred to over a thousand mass spectra may be acquired in this way. Each sweep of the mass range usually requires a time in the range of 2–5 sec, but faster or slower scans may be used in some cases. The second diagram from the top in Fig. 2 merely shows that each solid line of the sawtooth represents a mass spectrum as displayed in the standard histogram format. The most important idea is that CRMS produces a set of mass spectra that are more or less complete, depending on the selection of the mass range. Each integer mass between the starting and ending masses is measured and recorded. This is in sharp contrast to SIM where measurements are made at only a few masses in real time.

The third diagram from the top in Fig. 2 illustrates a widely used data reduction process that uses data acquired by CRMS. Each point on the ordinate is the normalized sum of all the ion abundance data in a single mass spectrum, and each point on the abscissa represents the spectrum number or a corresponding unit of time. This plot is referred to as a total ion current profile (TICP) which is defined as a normalized



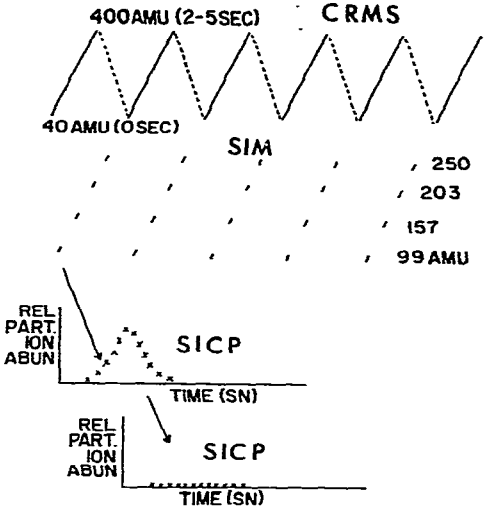


Fig. 2. Schematic diagram of CRMS, a TICP, and an EICP.

Fig. 3. Schematic diagram of CRMS, SIM, and two SICPs.

plot of the sum of the ion abundance measurements in each member of a series of mass spectra as a function of the serially indexed spectrum number. This same plot is often referred to as a reconstructed gas chromatogram (RGC), but this nomenclature is not preferred as it does not accurately define a TICP. An RGC could just as well be the output of a flame-ionization detector, as redrawn by a draftsman. An important point to recognize is that the TICP in Fig. 2 is the result of a computer data reduction and not a real-time display. With magnetic-deflection spectrometers it is common to monitor continuously the unresolved ion beam and produce a total ion current plot in real time. This plot should be similar to the TICP in Fig. 2, but clearly it will differ in that it will contain contributions from ions below mass 40 and above mass 400.

There is one more very valuable data reduction technique whose output is most often confused with an SICP. In this technique data acquired by CRMS, and perhaps displayed in a TICP, are further reduced by plotting the change in relative abundance of one or several ions as a function of time. This plot is illustrated at the bottom of Fig. 2. The plot appears very similar to an SICP but the data used are quite different. The significance of this difference is explained below, but first a clear precise name for this output is required. The original name suggested by Hites and Biemann[3] was mass chromatogram. Unfortunately this is not a very descriptive name and, as pointed out by Watson *et al.*[2], it may be confused with the output from a gas-density GC detector[4]. The name extracted ion current profile (EICP) was suggested[5] because the data for the few ions used in the plot are extracted from the larger set used to generate a TICP. The terms limited mass output and limited mass search are often used to describe this same plot. However, they are less meaningful than EICP since the nature of a limited mass is not clear.

The significant difference between an SICP and an EICP is that SIM produces a real increase in the signal-to-noise ratio by time-averaging random noise. The EICP produces an apparent increase in sensitivity by removing from the TICP the ion abun-

dance data from background, unresolved components, and other irrelevant ions. The contrast between continuous repetitive measurement of spectra and SIM is illustrated in Fig. 3. If the sweep of the complete spectrum is made in 3.6 sec (3600 msec), the data system may integrate the ion current at each mass for 10 msec (3600 msec/360 a.m.u.). If the same total time is allowed for the SIM measurements at the selected masses 99, 157, 203, and 250 a.m.u. in real time, then integration of signal intensity at each may proceed for 900 msec (3600 msec/4 a.m.u.). The longer integration time during SIM permits enhancement of the signal-to-noise ratio by averaging of random noise. Therefore there is a substantial improvement in the detection limit by SIM. This is in contrast to the EICP, which still uses the 10-msec data with their inherently lower signal-to-noise ratio.

The SICP illustrated in the third diagram from the top of Fig. 3 was generated by summing the abundances of all four ions measured during SIM. Clearly, one could also plot the change in abundance of each ion separately, and we make no distinction between various types of SICP plots. However, as illustrated in the bottom of Fig. 3, a SICP for mass 125 would yield no peak since mass 125 was not measured during SIM.

Fig. 4 is a display of a TICP, an SICP, and an EICP. The TICP was generated from CRMS over the mass range from 40 to 400 a.m.u. during chromatography of seven chlorobiphenyl isomers. Five nanograms of each isomer were injected and an 11-msec integration time was applied at each mass. The total time for a sweep from 40 to 400 a.m.u. was about 5 sec. The EICP was obtained from the TICP using seven masses characteristic of chlorobiphenyls[6]. The SICP is the result of SIM using the same seven masses, but an integration time of 540 msec on each. The SICP is the sum of the data from the seven masses. The signal-to-noise contrast between the SICP and

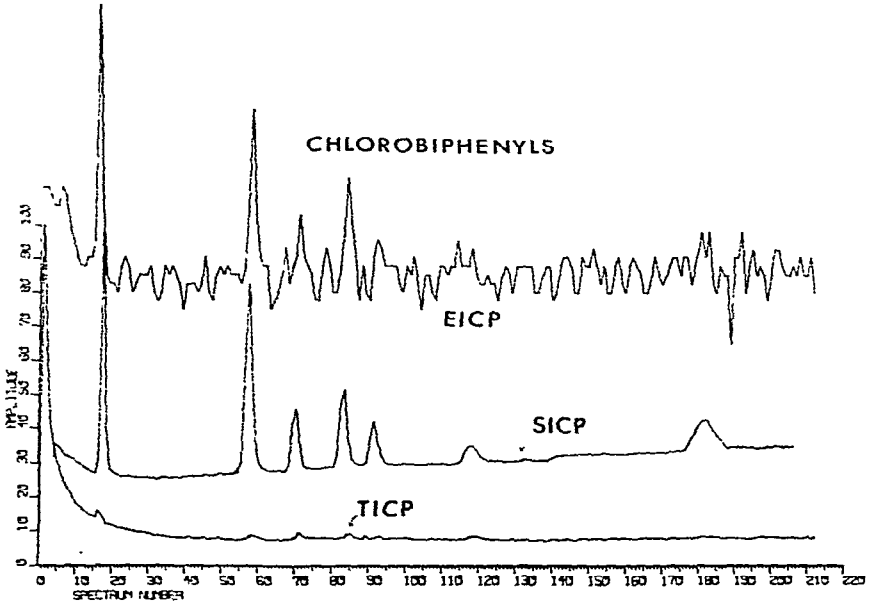


Fig. 4. TICP, SICP, and EICP from the chromatography of seven chlorobiphenyl isomers.

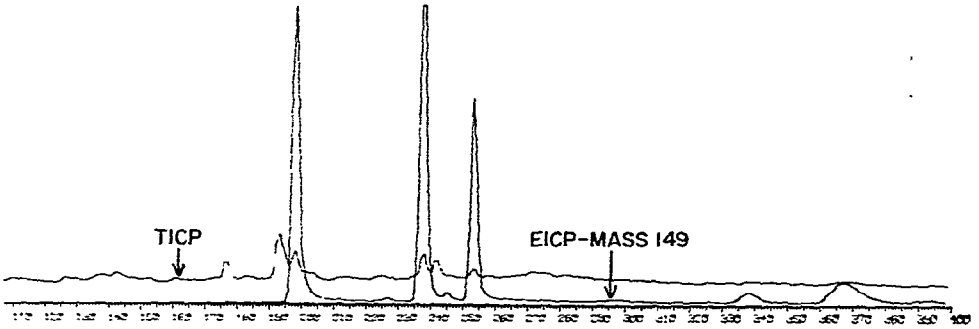


Fig. 5. EICP for mass 149 and the corresponding TICP.

the EICP is clearly demonstrated. This illustration is not meant to imply that EICP is not a valuable technique, but to show the differences in the methodology.

A routine application of an EICP is shown in Fig. 5. In this example the TICP data have an adequate signal-to-noise ratio and the EICP was used to highlight effectively those areas of the chromatogram having abundant mass 149 measurements.

ENVIRONMENTAL SAMPLES

In the application of SIM to environmental samples our emphasis was on problems of current high interest that are not readily amenable to other methods of analysis.

Toxaphene is a widely used chlorinated hydrocarbon pesticide that is not a single compound, but a mixture of at least 177 polychlorinated C_{10} compounds[7]. The mixture is prepared by chlorinating camphene and the vast majority of the components of the mixture are probably closely related isomers having compositions in the range of $C_{10}H_{10}Cl_6$ to $C_{10}H_8Cl_{10}$. The center chromatogram in Fig. 6 is a TICP of 2 μg of the toxaphene mixture. The major components probably make up no more than about 50 ng of the mixture and few, if any, components were resolved on the packed column. This result suggests a lower detection limit of the order of 20 μg/l for toxaphene in water. However, this would be dependent on the absence of interfering substances and extensive extract purification could be required for most environmental samples.

A SIM method for toxaphene was sought to lower the GC–MS detection limit and to preclude the need for some or all of the extract purification. However, it was important to retain in the SIM method as much as possible of the qualitative reliability inherent in the CRMS method. Clearly it would be desirable to retain in the SICP the characteristic pattern of peaks present in the TICP. The principle problem in developing a SIM method is to select masses that not only give good sensitivity, but also are selective for the desired analyte. All ions less than mass 150 were eliminated from consideration because their abundances are generally higher than heavier ions in numerous potentially interfering substances. The masses were selected with the aid of a standard computer program that analyzed the toxaphene TICP data and printed the masses of all ions heavier than mass 150 and greater than 20% relative abundance. All mass spectra between spectrum numbers 46 and 164 were analyzed in this way.

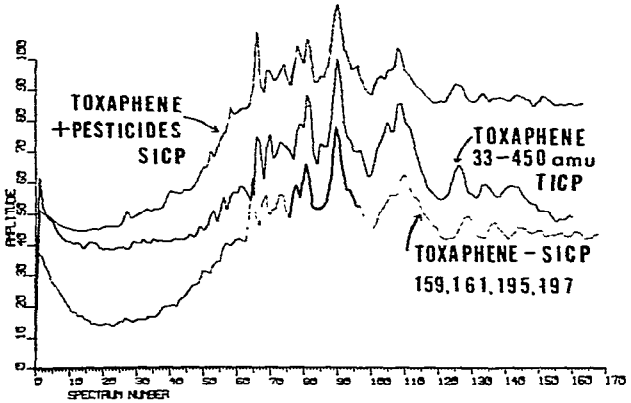


Fig. 6. TICP and two SICPs for toxaphene.

Mass 159 was observed in 72 spectra and masses 195 or 197 in 16 spectra. Also present in 14 spectra was a mass 161 ion. The ions at masses 159 and 161 probably contain three chlorine atoms in most components and probably have the composition $C_4H_6Cl_3$. Several major components of toxaphene have been isolated[7], and in the spectra of these the ions at masses 159 and 161 display chlorine isotope distribution patterns corresponding to the presence of three chlorine atoms. The ions at masses 195 and 197 are more difficult to judge and appear to contain three chlorines in one of the isolated components and four in the other. Thus the compositions could be $C_7H_6Cl_3$ or $C_4H_7Cl_4$. In the toxaphene mixture the chlorine isotope distribution ratios vary widely because of the multicomponent contributions to a given mass. In the spectra of the isolated components the ions of masses 159, 161, 195, and 197 are the most abundant above mass 150. The bottom chromatogram in Fig. 6 is an SICP of 200 ng of the toxaphene mixture. This was generated from the sum of the abundances of the four selected masses and reveals an approximate ten fold increase in signal intensity compared to the TICP. The integration time used in the CRMS was 24 msec and in the SIM 1609 msec. Also apparent in the SICP is the retention of the characteristic pattern of peaks present in the TICP. The application of SIM to toxaphene measurements has the potential of lowering the detection limit to the 0.1–2 μg/l range in environmental samples.

In order to test the selectivity of the toxaphene masses, a mixture was prepared consisting of 200 ng of toxaphene mixture, 25 ng of dichlorodiphenyltrichloroethane (DDT), 20 ng of dichlorodiphenyldichloroethylene (DDE), and 20 ng of dieldrin. The top chromatogram in Fig. 6 is an SICP of this mixture, again using the sum of the abundances of the four toxaphene masses. Comparison of this with the SICP of toxaphene alone indicates the excellent selectivity of the four masses for the toxaphene components. The compounds DDT, DDE, and dieldrin were chosen for the comparison because these materials are the ones most likely to interfere with toxaphene measurements in an environmental sample, even after some preliminary purification of the extract.

Another measure of selectivity is the occurrence of the four masses in approximately 39,000 mass spectra contained in the data base of the international mass spectral search system (MSSS)[8]. Table I shows the results of a computer-assisted

TABLE I

SPECTRA IN THE MSSS DATA BASE HAVING MASSES 159, 161, 195, AND 197 IN THE 10–100% RELATIVE ABUNDANCE RANGE

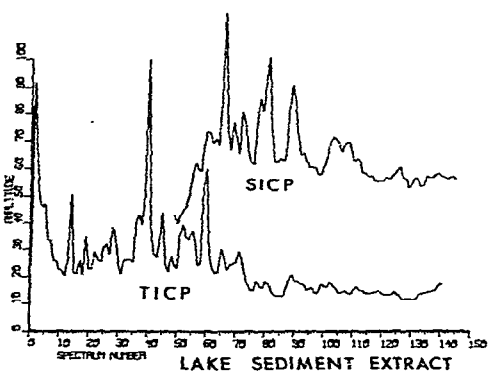
Masses searched	*Occurrences in non-toxaphene spectra*
159	1568
159 and 161	417
159 and 195	37
159, 161 and 195	8
159, 161, 195 and 197	3

search of this data base, with each search requiring several seconds on a commercial time-sharing system. Clearly the four masses taken together show outstanding selectivity, but combinations of fewer than four masses were also selective. No chlorinated hydrocarbon pesticide spectra were included in the eight spectra containing masses 159, 161 and 195. It is these chlorinated hydrocarbons that are the most likely interferences in the toxaphene analysis.

It is emphasized that the current SICP plotting program does not treat the selected masses as a unit, but simply sums abundances measured at each mass and plots the result. Significant additional selectivity is anticipated from a program that plots an abundance sum at a spectrum number if and only if all the selected ions are present. This algorithm was recognized by Kuehl[9] during studies of polychlorinated biphenyls and programs using it will be available in the near future.

An environmental application of the toxaphene SIM analysis is shown in Fig. 7. The TICP of an unpurified lake sediment extract (dichloromethane–acetone) contained a number of peaks, but no recognizable pesticide spectra. The analysis was repeated using the toxaphene SIM masses, and the characteristic toxaphene pattern was readily recognized. Fig. 8 shows the SICPs from the lake sediment extract and a toxaphene standard. The patterns match rather well, except for components eluting after spectrum 100. This suggests either some selective natural degradation or less efficient extraction of the late-eluting components of toxaphene.

A problem currently under study is concerned with toxic organics in wastes



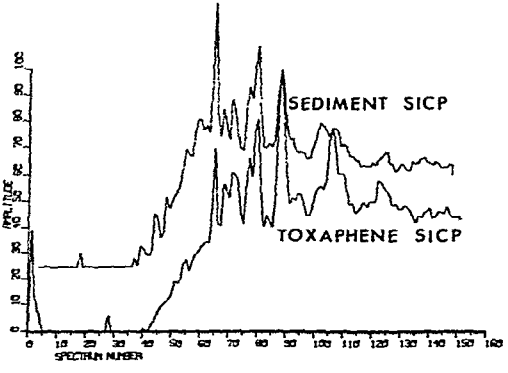


Fig. 7. TICP and SICP from a lake sediment extract.

Fig. 8. The SICP from the lake sediment extract and a SICP of a toxaphene standard.

that enter sewage treatment plants. Raw sewage is one of the most difficult of all environmental samples because it is rich in compounds that cause serious emulsions during liquid–liquid extraction procedures. Therefore, extraction efficiency is relatively low and analyses are complicated by the enormous variety of compounds present. SIM procedures were tested with dichloromethane extracts of raw sewage to evaluate the technique with this type of sample. In one experiment one liter of raw sewage from a treatment plant that processes primarily domestic waste was dosed with 50 μg of the polychlorinated biphenyl (PCB) mixture Aroclor 1254, 5 μg of dieldrin and 5 μg of pyrene. The bottom chromatogram in Fig. 9 is a TICP of a portion of the unpurified extract which, assuming 100% recovery, should contain 300 ng of the PCB mixture and 30 ng each of dieldrin and pyrene. There was a great deal of MS information contained at each spectrum number, but no well defined peaks were observed because the extract was a very rich mixture of a many unresolved compounds. For example, spectrum number 60 contained a total ion current count in excess of 1.5 million, but no clearly discernible mass spectrum of any specific compound. A typical spectrum of a pure compound at the 20-ng level gives a total ion current count of approximately 0.5 million. The second chromatogram from the bottom is an SICP of the same extract using the sum of the abundances of seven masses characteristic of chlorobiphenyls[6]. Comparison of this with the second chromatogram from the top, an SICP of 200 ng of an Aroclor 1254 standard, gives a good example of the improved detection limit and selectivity of SIM. Integration of the respective areas led to the conclusion that the dichloromethane-extraction efficiency from raw sewage was about 50%. The top chromatogram is an SICP from the sum of the abundances of the masses 79, 101, 202, 261, 263, and 265. The compounds dieldrin and pyrene are clearly recognizable.

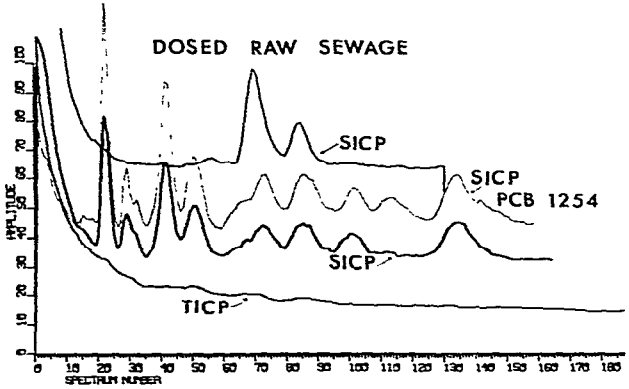


Fig. 9. TICP and several SICPs from a dosed raw sewage extract.

DISCUSSION

The principal potential advantages of selected ion monitoring may be summarized as follows: high selectivity, tunable selectivity, qualitative reliability, high sensitivity, reduced need for sample purification, and quantitative accuracy.

In practice, it may not be possible to achieve all of the advantages simulta-

neously. There is a general tendency to select the most abundant ion or ions in a spectrum for SIM. In certain cases this may have a significant effect on the selectivity and therefore the reliability of the measurement. For example, the most abundant ion in the electron-impact spectrum of 2-methylbenzothiazole is the molecular ion, mass 149. However selection of this ion for SIM could result in measurement errors due to the ubiquitous phthalate esters, which display intense mass 149 fragment ions (Fig. 5). The selection of a less abundant but more selective ion may provide adequate detection limits and preserve the reliability of the measurement. We recommend the use of the MSSS[8] to estimate the selectivity at a given mass or group of masses and to evaluate potential interferences.

Another mechanism to assure reliability is to monitor several ions that have an established abundance relationship. The molecular ion and its corresponding isotope-containing species have abundance relationships that are well known. If the compound of interest contains chlorine, bromine, or other elements with several abundant isotopes, an excellent approach to qualitative reliability is to monitor several ions and compare the observed and expected abundance ratios. Fig. 10 shows a number of calculated chlorine/bromine isotope distribution patterns normalized to the most abundant ion of the group. Within these patterns are numerous possibilities for comparisons of ratios. For compounds that do not contain readily measurable isotopic species, a method has been recommended[10] in which several ions of known relative abundance are monitored and their abundance ratios compared.

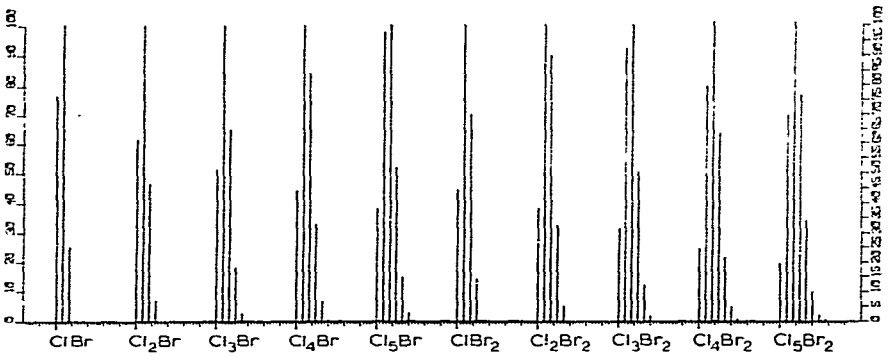


Fig. 10. Some calculated chlorine–bromine isotope distribution patterns.

The reduction in sample preparation as a result of SIM will depend on the nature of the sample. In the environmental field, air and relatively clean water samples offer the best possibility for elimination of all extract purification. For fatty tissue, sediment, and sewage samples some reduction in extract purification is usually possible.

There have been a relatively large number of publications, including several in this symposium, that have emphasized the high sensitivity and quantitative accuracy of the SIM method. It is our conclusion that SIM is at least the equivalent of the most well known detectors. It appears that the ultimate in quantitative accuracy is possible with SIM and a stable isotope labelled internal standard that is the same compound as the measured analyte[11].

One difficulty with SIM is the simultaneous measurement of two or more components with significantly different concentrations. Selection of a long integration time will enhance the signal-to-noise ratio of less abundant ions, but abundant ions will saturate the detection system. Alternatively, a short integration time may avoid saturation of the detector but preclude clear observation of the less abundant ions.

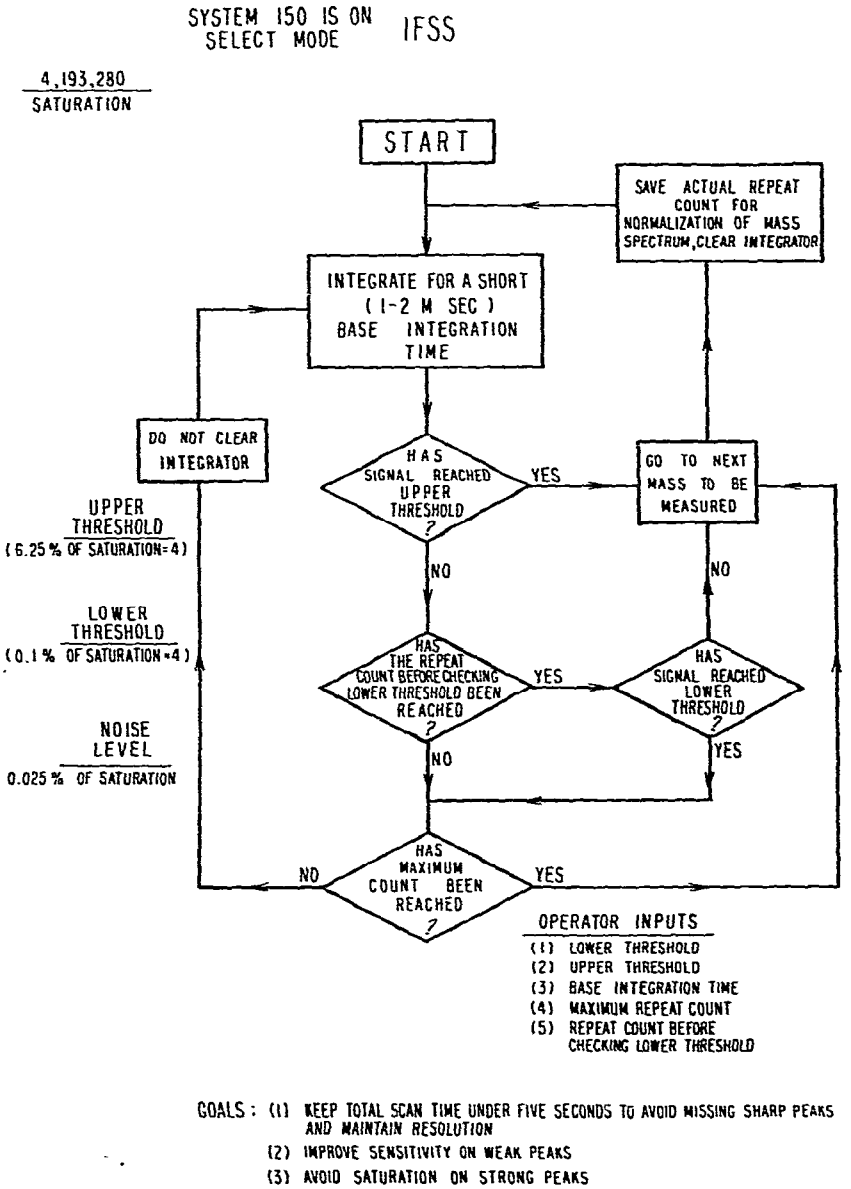


Fig. 11. Flow chart for an algorithm to dynamically determine integration times as a function of signal strength.

Ion-counting detection systems may be one solution to this problem. Another solution is a method for the dynamic selection of integration time as a function of signal strength (IFSS).

The logic of an IFSS algorithm is shown in Fig. 11. The approach is based on the concepts of user defined upper and lower signal thresholds and a base integration time that is repeated a variable number of times depending on the signal level at a given mass. If a measurement of a given ion abundance reaches the upper threshold before completion of the maximum number of repeats of the base integration time, integration is terminated. Therefore the relatively strong signals from abundant ions are integrated briefly before saturation of the detector. On the other hand, relatively weak signals from the less abundant ions may be integrated for the maximum number of repeats of the base integration time and therefore the signal-to-noise ratio is improved. However, if a signal has not reached the lower threshold within a specified number of repeats of the base integration time, integration is terminated and little time is wasted on non-existent ions. All data are, of course, normalized with respect to integration time before storage, which permits applications of IFSS to quantitative measurements. Using IFSS, measurements have been made of compounds that differ in concentration by a factor of 1000 with no saturation of the abundant ions and a good signal-to-noise ratio on the less abundant ions.

Finally, a continuing problem with most computerized data systems is the absence of truly flexible software that not only permits the types of data acquisition, reduction, and output discussed here, but also is structured to facilitate modifications by the experimentalist seeking new methods. Documentation in the form of good user manuals, flow charts describing how programs work, and clear concise explanations of algorithms is usually not available. Program source listings and instructions on how to make program changes are even more secluded. This sometimes leaves the user with the feeling of helpless wonder about what the software is really doing.

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