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TARGET COMPOUND ANALYSIS BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The use of two-dimensional gas chromatography* for the analysis of specific target compounds in complex matrices in combination with mass spectrometry has been investigated. The combination of a high capacity, high polarity packed first gas chromatography column followed with a low capacity, low polarity, high resolution second column has been found useful. A component of interest is switched from the first column into a cold trap and then flash-evaporated into the second column. This combination allows part per billion analyses in complex mixtures such as soil extracts, crude oils, and biological extracts without any prior sample cleanup whatever. Two important advantages arise naturally therefore from this approach: a significant reduction in analysis time and a major improvement in the specificity of the analysis. The method offers a relatively inexpensive yet powerful alternative to mass spectrometry–mass spectrometry.

INTRODUCTION

In recent years considerable interest has been focused on the development of analytical methods for part per billion (10^9) and even part per trillion (10^{12}) analyses of selected organic species. Quantification of such materials in unusually complex matrices such as soil and biological extracts has been especially widely studied. The analyses which have been devised for 2,3,7,8-tetrachlorodibenzodioxin in environmental samples are representative of the current analytical approach to such problems[1,2]. Typically a method will involve an arduous multistep purification procedure, including, for example, liquid chromatography and gel permeation chromatography. These preliminary isolation steps are then usually followed by mass spectrometry or gas chromatography–mass spectrometry (GC–MS) for detection and quantification. In the present paper, we show that two-dimensional GC can eliminate much and in many cases all requirements for sample cleanup even in very complex matrices.

* Note by the Editor: The authors insisted on calling this "two dimensional" against the recommendations of our referees (see also I. M. Hais, *J. Chromatogr.*, 187 (1980) 466) and against the judgement of the Editor.

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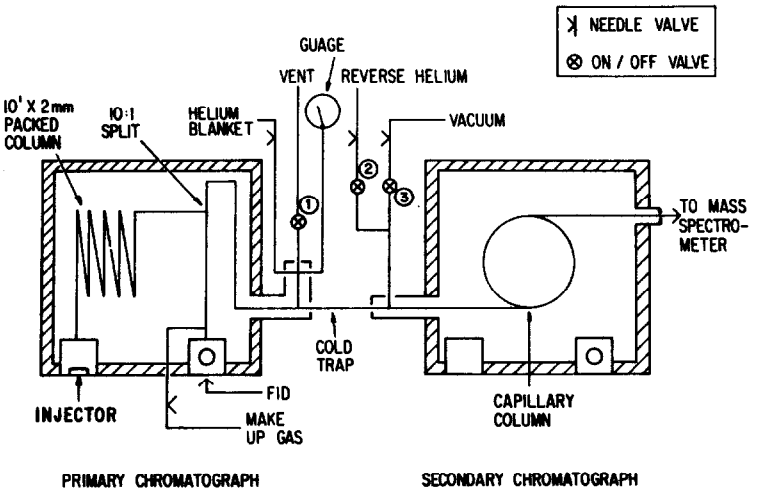
Two-dimensional GC is not a new concept and the reader is directed to a recent review article for a general background treatment[3]. The application of multi-stage GC to the analysis of complex environmental and biological samples has been discussed previously[4–7]. None of these workers combined their chromatographs with mass spectrometers. The present work is an extension of our previously reported experiments[8] in which the GC–GC apparatus is interfaced to a mass spectrometer and in which a vacuum pump is used to sample the effluent of the primary column. The use of vacuum sampling differs from traditional switching schemes such as "Deans switching"[9]. A detailed description of the most recent version of our apparatus is provided together with a number of examples illustrating its particular utility for environmental analysis.

EXPERIMENTAL

An overall diagram of the GC–GC apparatus used in these experiments is shown in Fig. 1. The apparatus is composed of two Varian-Aerograph Model 1440 gas chromatographs. The first (or primary) chromatograph is equipped with a flame ionization detector. The second chromatograph is directly coupled to the electron-impact ion source of a high resolution mass spectrometer such that the secondary chromatography column itself extends to the ion source. The mass spectrometer is the Vg Analytical Model ZAB 2F equipped with a laminated magnet and operated exclusively in electron-impact mode for these experiments.

The primary chromatograph is fitted with a 1/4 in. O.D. by 2 mm I.D. by 10 ft. glass column packed with 3% Silar 10c on Gas-Chrom Q. The packing was ob-



VALVE	OPERATING MODE		
	DIVERT	SAVE	SECONDARY SEP.
1	OPEN	OPEN	CLOSED (SEE TEXT)
2	OPEN	CLOSED	CLOSED
3	CLOSED	OPEN	CLOSED

Fig. 1. GC–GC apparatus constructed from two Varian-Aerograph 1400 chromatographs.

tained from Applied Science Labs., State College, PA, U.S.A. The secondary chromatograph is equipped with a 30-m fused-silica capillary (J & W Scientific, Model DB-1). This is a bonded methyl silicone phase. All transfer lines which contact the sample are glass-lined stainless steel and interconnections are made by butt-joining this tubing inside 1/16 in. stainless steel unions sealed by graphite ferrules. The cold trap is merely a liquid nitrogen cooled segment of the glass-lined stainless steel tubing which connects the two chromatographs. All flow-switching is accomplished remotely using solenoid valves none of which is in a heated region. Details of the flow system are provided in the Discussion section. Helium carrier gas for the system was purified using a carrier gas purifier supplied by Supelco, Bellefont, PA, U.S.A., Model 2-3800.

Mass spectral data were acquired in two modes. In the first mode spectra were acquired only from selected ions by electrostatic or magnetic peak-switching (selected ion monitoring). In the second mode full mass spectra were acquired by scanning the magnetic field (full scan acquisition). In both modes data were acquired using a Finnigan MAT Incos Model 2400 data system. In the full scan acquisition mode, the data were obtained using the data system in a completely unmodified condition. Selected ion data was acquired using a special modification of the data system which allows it to communicate with the hardware multiple ion detection equipment (DIG-MID) integral with the Vg ZAB mass spectrometer. This modification (which is available from the authors on request) causes the computer to suspend acquisition until the mass spectrometer reaches the selected mass and also maintains the use of the "mass lock" function of the DIG-MID apparatus. A mass lock signal is generated by adding a reference substance to the mass spectrometer ion source continuously using a heated inlet system. The DIG-MID uses this signal to adjust the mass calibration for drift. Selected ion monitoring at low or high resolution for very long periods of time (more than 1 h) is possible as a direct result of this lock mass correction. Harvan *et al.*[10] have reported a related approach to interfacing the DIG-MID and an Incos data system.

DISCUSSION

Minimum detectable concentrations in experiments designed to analyze complex organic substrates by GC–MS are determined by a number of factors. The most fundamental and relevant of these factors is frequently the amount of sample which is available to the analyst. In general if more sample is available then a lower detection limit can be obtained. This relationship arises because a mass spectrometer responds to a given weight of substance and because at a given concentration a larger sample will contain a larger absolute amount of the substance of interest. In a conventional GC–MS experiment, therefore, exceedingly low detection limits can be obtained simply by using very large samples and many steps of purification and concentration before attempting mass spectrometric analysis. In experiments which do not involve such preliminary purification steps, the detection limit is given to a first approximation by the gram-sensitivity of the mass spectrometer to the substance of interest divided by the number of grams of sample which can be injected into the system. In practice (under the best conditions) only a few milligrams of sample can be accommodated by an analytical GC system, and the average base sensitivity of the spectrometer described herein is in the vicinity of a few picograms for a 10:1 signal-to-

noise ratio. This indicates that detection limits of a few parts per billion should be possible using samples of a few milligrams. This calculation assumes, of course, that the material of interest will be pure when it reaches the mass spectrometer or that any materials which arrive simultaneously will not interfere with the detection of the substance of interest.

For a complex environmental sample this assumption is generally invalid. In order for the sample to have a reasonable chance of being resolved from possible interferences a high resolution chromatography system must be used. Such high resolution chromatography systems have capacities far below a milligram and further are not tolerant of involatile residues which may be present in unpurified samples. This fundamental difficulty results in realistic detection limits in crude samples which are at least a factor of 1000 higher than the low parts per billion calculated above. In addition the problem of involatile residues has generally rendered such a direct approach impractical. In this paper we show that the calculated low detection limits can be approached for crude samples if the high capacity and tolerance of involatile residues characteristic of relatively low resolution packed columns is combined in a series arrangement with the high resolution capability of fused silica capillary columns.

GC–GC system description

The design philosophy of the GC–GC system involves three inter-related concepts. First is the use of a high capacity, high polarity packed column to provide a preliminary separation. Such columns allow milligram level injections of volatile (and non-volatile) organics without significant loss of chromatographic resolution. Such columns are relatively resistant to deposition of non-volatile residues in the first few inches and are inexpensive and simple to replace. Such columns generate at any given point in the chromatogram a mixture of species in which the non-polar components are far higher boiling than the polar components.

The second design concept involves the trapping of a component of interest as it elutes from the primary chromatograph and the subsequent injection of this component (which will almost certainly be contaminated with other co-eluting materials) onto the second chromatograph. The total amount of material trapped from a narrow elution window of the first column is generally acceptable for routine injection onto the second column.

The final feature of the design is the use of a high resolution, low capacity, low polarity GC column for the secondary separation. The high resolution of the secondary column optimizes the likelihood that the component of interest will be resolved. It also improves the signal to noise ratio at the mass spectrometer by compressing sample introduction into as small a time window as possible thereby maximizing sample flow as measured in grams per second. The low polarity of the second column ensures that the separations obtained will be largely dependent on boiling point and will therefore take advantage of the fact that many materials which co-elute from the first highly polar column will have very different boiling points.

Description of detailed function

The overall system is shown in Fig. 1. This system can be configured in three separate modes: the divert mode, the save mode and the secondary separation mode.

Valve positions in each mode are shown in the figure. None of the valves are in a heated region.

Two modes of operation are possible during the course of the primary separation. In the first of these, called the divert mode, unwanted materials eluting from the first column are vented out of the instrument. With reference to the figure, the various relevant flows in this mode can be traced as follows: flow from the primary column proceeds to a point where 10% of the gas is split to a flame ionization detector. This detector allows the operator to monitor the progress of the primary separation and to locate regions of interest using index compounds specifically added to the sample as retention index markers. The remaining 90% of the primary effluent proceeds to a tee junction where it joins with a clean helium flow proceeding backward ("reverse helium") out of the cold trap. The reverse helium serves two functions. First it forms a barrier preventing unwanted primary effluent from reaching the cold trap. Second it provides helium to the secondary column during the divert mode thereby maintaining flow in this column. Flow in the secondary column prevents the development of a vacuum at the cold trap which would otherwise be generated by the mass spectrometer's vacuum system. Such a vacuum could induce flow of unwanted materials into the cold trap. The combined flows (reverse helium and primary effluent) proceed in the direction of the vent where they are joined by a third flow of clean helium (the "helium blanket") which purges the unwanted primary effluent out of the system. The system operates in the divert mode at all times when unwanted materials are eluting.

The second mode of operation possible during the primary separation is called the "save" mode. It is useful to again trace the flow patterns in detail. As before 10% of the flow is diverted to a flame ionization detector and the remaining 90% proceeds to a tee junction where it can flow either to the vent or to the cold trap. In this mode a solenoid valve controlling the reverse helium flow is closed and a second solenoid valve connecting the outlet of the cold trap to a vacuum pump through a needle valve is open. The needle valve is used to set the flow to the vacuum pump to a value equal to the flow of primary effluent reaching the inlet of the cold trap. This vacuum-initiated flow moves the primary effluent into the cold trap where the components eluting are trapped at liquid nitrogen temperature. It is not practical to balance the vacuum-initiated flow exactly with the primary effluent flow and therefore the vacuum-initiated flow is usually set to be slightly higher than the primary effluent flow. This situation could potentially result in atmosphere being pulled into the trap through the vent line. Alternatively, if the vent line was closed a vacuum could be developed on the outlet of the primary column which could shift the flame ionization detector split ratio and ultimately even shift primary chromatographic retention times. In order to avoid this problem entirely, the helium blanket mentioned earlier is enabled in this mode and continues to flow during this phase of operation. Any imbalance between the vacuum-induced flow and the primary effluent flow is compensated by the helium blanket. The vent remains open thereby ensuring that the outlet of the primary column and flame ionization detector split operate near atmospheric pressure and are therefore undisturbed by the sampling procedure. The cold trap itself is simply a liquid nitrogen cooled section of the glass-lined stainless-steel transfer line between the two gas chromatographs.

After the primary column separation is completed and all components of in-

terest are stored in the cold trap, the objective becomes to transfer these trapped components to the secondary chromatographic column for further separation. This mode of operation is called the secondary separation. The detailed configuration of the system is as follows: the solenoid valves controlling the reverse helium and the vacuum-induced flow are both set in the off position. The vent valve is partially closed so that a pressure of *ca.* 8 p.s.i. (or whatever pressure is appropriate for the second column) is produced in the cold trap region as a result of the primary flow and the helium blanket flow. When this pressure has stabilized and the secondary oven is equilibrated at an appropriate temperature, the cold trap is rapidly heated electrically using two co-axial 1/16 in. O.D. heaters. This raises the temperature of the cold trap to *ca.* 300°C. in less than 10 sec. After firing the trap, the secondary separation proceeds as any normal chromatographic analysis using whatever oven temperature profile is appropriate.

The system allows, therefore, in a facile manner the rapid transfer and high resolution analysis of components trapped from a preliminary separation.

RESULTS

The usefulness of two-dimensional GC for the analysis of target compounds has been evaluated for three different systems. In two of these systems Prudhoe Bay crude oil was chosen as a model matrix. This material like all crude oils is an especially complex mixture of aliphatic, aromatic, and heterocyclic compounds with a distribution of boiling points from near room temperature to greater than 400°C. Involatile materials are also present. Figs. 2 and 3 show the results of the analysis of *ca.* 3 ng of dibutylnitrosoamine and *ca.* 3 ng of β-naphthylamine both co-injected in independent experiments with *ca.* 1 mg of Prudhoe Bay crude oil. Both of these species

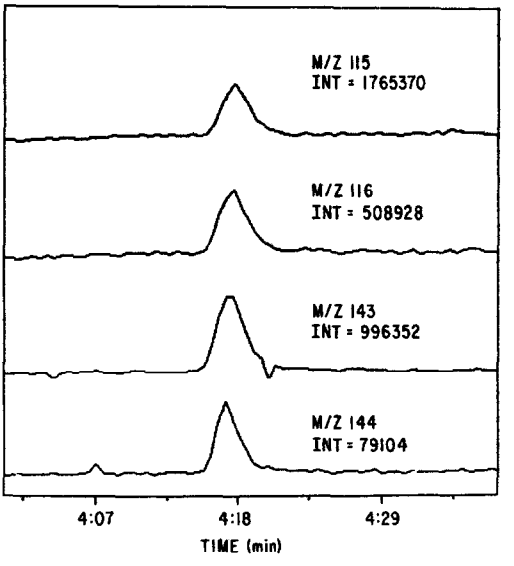


Fig. 2. Signal obtained from the GC–GC–MS analysis of 3 ng of β-naphthylamine in a Prudhoe Bay crude oil matrix.

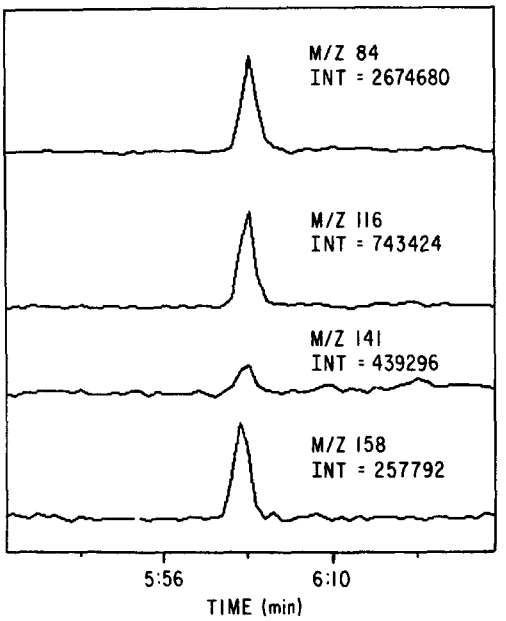


Fig. 3. Signal obtained from the GC–GC–MS analysis of 3 ng of dibutylnitrosoamine in a Prudhoe Bay crude oil matrix.

were trapped from the primary column as described previously and then rechromatographed on the secondary column with subsequent direct transfer into the mass spectrometer. In these two cases the known relationship between the chromatographic pattern for the oil matrix and the retention time of the subject compound was used to locate the region of interest for trapping. (In the general case an index compound which elutes just before the species of interest is co-injected with the sample to improve the accuracy of peak selection.) For the case of β-naphthylamine, mass spectral data were acquired by selected ion methods using electrostatic switching. Four characteristic ions (m/z = 115, 116, 143, 144) were monitored using a mass resolution of *ca.* 1000.

For the case of dibutylnitrosoamine mass spectral data were again acquired by selected ion monitoring. Four ions (m/z = 84, 116, 141, 158) were monitored. Such a wide mass range cannot be covered using electrostatic switching and therefore magnetic peak switching was utilized. A mass resolution of *ca.* 1000 was also used for this experiment. The signal-to-noise ratio obtained was very satisfactory (see figures) in both of these experiments and clearly indicates that much smaller amounts of material could have been detected. No attempt has been made to rigorously establish limits of detection or limits of quantification. It is interesting however that even this unusually difficult matrix provides no significant interferences following a two-dimensional separation. It should be noted that both of these species are relatively polar and therefore elute very early from the secondary GC column relative to the matrix components. A large and persistent signal arising from this complex matrix does occur later in the secondary chromatogram but does not interfere with the

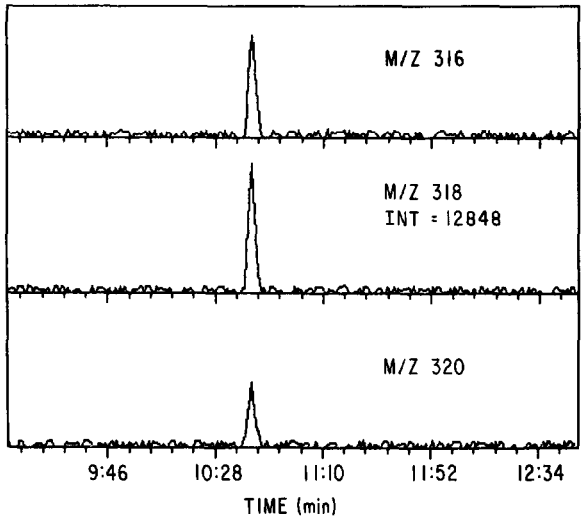


Fig. 4. Signal obtained for 2,3,7,8-tetrachlorodibenzofuran (99 atom-% ^{13}C) analysed from a combined hydrocarbon and polychlorobiphenyl transformer fluid matrix.

analysis of these species. In order to analyze non-polar species in a more polar matrix it would be appropriate to select a non-polar primary column and a relatively polar secondary column.

A third experiment involved the analysis of hydrocarbon-based transformer dielectric fluid for 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF). In this experiment a sample of new transformer fluid (10 C oil) was spiked with *ca.* 370 ppm of polychlorinated biphenyl (Arochlor 1254) and then 1 mg of this solution was co-injected onto the primary column with 20 pg of 2,3,7,8-TCDF enriched in ^{13}C at 99 atom-%. (Samples of ^{13}C-enriched 2,3,7,8-tetrachlorodibenzofuran were prepared at General Electric by R. Bell and A. Gara. This work is described in ref. 11.) The material was trapped from the primary column using di-*n*-octylphthalate as an index compound. The molecular ion and its isotopomers (m/z = 316, 318, 320) were monitored at 3000 resolution using electrostatic selected ion monitoring as the compound eluted from the secondary chromatograph. Fig. 4 shows the result of this analysis. The signal-to-noise ratio is greater than 10:1. This represents the detection of a concentration of 20 ppb based on the grams of matrix injected. No interference whatever can be detected under these conditions. This result indicates that this approach could be used to screen quickly transformers which have at some point contained PCBs for the presence of 2,3,7,8-TCDF. An isomer-specific analysis of this species is reported in an accompanying paper[12]. It should be noted that the possibility inherent in this system for the choice of a wide variety of primary and secondary columns greatly facilitates the design of an isomer-specific analysis. It is interesting that attempts by a vendor (Sciex) to analyze samples of this nature at these levels using triple quadrupole MS–MS provided no useful data whatever. Metastable decompositions arising from the oil matrix provided interfering signals at almost every mass in the daughter ion spectrum.

The apparatus described here is not limited to analysis of target compounds

in complex matrices. Another very useful application involves the analysis of trace impurities in relatively pure organic substances. In this case the system can be used to divert only the major component from the primary chromatogram while storing all of the minor components in the cold trap for secondary separation.

The result of a single such analysis is shown here to demonstrate this additional capability of this system. For this analysis 0.5 mg of a commercial sample of biphenyl was co-injected with 800 pg of dibenzofuran enriched in ^{13}C to a level of 90 atom-%. The addition of this internal standard allowed the quantification of dibenzofuran in the sample. The biphenyl sample was obtained from Alpha Chemical Company (Lot No. 013079). The results of this analysis are shown in Fig. 5.

Full mass spectra were obtained of each component. As can be seen from the figure a large number of trace hydrocarbons were detected and identified. A large biphenyl peak is obtained because such large amounts tend to tail on the primary column. The dibenzofuran signal was quantified using the internal standard and found to represent a concentration of *ca.* 300 ppb based on the biphenyl matrix. Many of the hydrocarbons were present at much higher levels than the dibenzofuran. Assuming that the biphenyl used historically to prepare chlorinated biphenyls had a similar spectrum of impurities, it is reasonable to anticipate that chlorinated analogues of many of these species exist in commercial polychlorinated biphenyls. The presence of chlorinated dibenzofurans in many commercial chlorobiphenyls is, of course, well established and this work suggests that they may arise from dibenzofuran impurities in the original biphenyl.

Crude extracts of soil and of animal tissue have also been examined using this equipment. For the analysis of 2,3,7,8-TCDF, these matrices were found to provide less interference than the model crude oil matrix described here. This occurs because in these extracts a relatively much smaller fraction of the total matrix components is actually volatile. Degradation of primary column performance resulting from in-

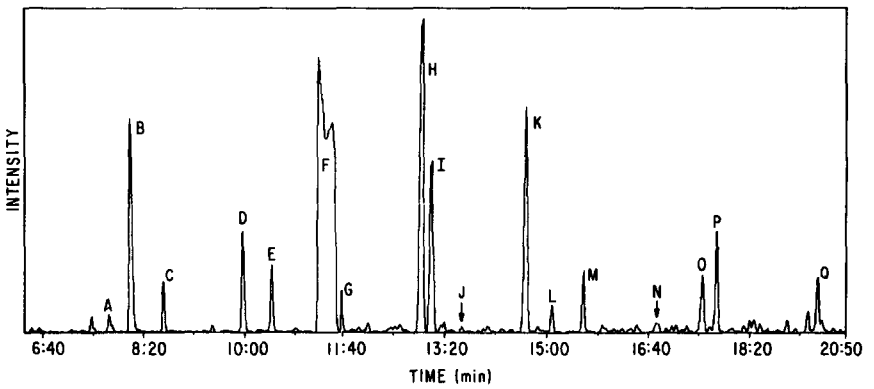


Fig. 5. Reconstructed gas chromatogram obtained from the GC–GC–MS analysis of a commercial biphenyl sample where the major component (biphenyl) was diverted and all minor components were saved. Components in the chromatogram are identified as follows: A = tetrahydronaphthalene; B = naphthalene; C = methyltetrahydronaphthalene; D = methylnaphthalene; E = dimethylnaphthalene; F = biphenyl; G = methylbiphenyl isomer; H = methylbiphenyl isomer; I = methylbiphenyl isomer; J = dibenzofuran (quantified by isotope dilution at 300 ppb); K = fluorene; L, M = dimethyldiphenylmethane or trimethylbiphenyl isomers; N = fluorenone; O = anthracene or phenanthrene; P = artifact (integrated bleed from primary column); Q = possibly methyl-substituted quaterphenyl.

jection of crude extracts occurs slowly but should be evaluated on a daily basis. The analyst should be aware that in addition to losses in resolution, such degradation can also change the relationship between analyte and index compound.

It should be noted that each of the analyses described herein was completed in less than 45 minutes. This time was measured starting with injection in the primary chromatograph and ending with a completed hard copy (equivalent to Figs. 2, 3 and 4) of the computer-acquired mass spectral data.

CONCLUSIONS

Two-dimensional GC combined with MS provides a valuable tool for the analysis of environmental samples. Frequently little sample preparation is necessary. The determination of two retention times is a natural part of the experiment and adds increased specificity to a GC–MS analysis. Further the elimination of solvents and high level contaminants before they reach the mass spectrometer substantially reduces both sample-induced variations in spectrometer response (matrix effects) and spectrometer maintenance. This technique should be considered a powerful and relatively inexpensive alternative to MS–MS.

The authors specifically do not intend to imply by their choice of model systems that the compounds studied have clinically significant physiological effects at the levels employed here. Such determinations are correctly left to toxicologists.

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