Journal of Chromatography, 294 (1984) 87–98
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,672

ISOMER SPECIFIC ANALYSIS OF SELECTED CHLORODIBENZOFURANS

WOODFIN V. LIGON, Jr.* and RALPH J. MAY
General Electric Company, Corporate Research and Development, Schenectady, NY 12301 (U.S.A.)
(First received December 13th, 1983; revised manuscript received February 16th, 1984)

SUMMARY

Two-dimensional gas chromatography has been used to provide full chromatographic resolution of three chlorodibenzofuran isomers. The materials studied were: 2,3,7,8-tetrachlorodibenzofuran, 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,7,8,9-hexachlorodibenzofuran. The materials were detected using medium resolution mass spectrometry.

INTRODUCTION

Chlorination of dibenzofuran can potentially yield a total of 135 unique products. These products vary both in degree of chlorination and in the positional substitution of chlorine atoms on the basic ring structure. Certain members of this group which have a 2,3,7,8 substitution pattern are known to have measurable physiological effects in a number of animal species. Other members of the group show little biological activity. For this reason, any attempted evaluation of the potential physiological activity of a given chlorination mixture which is based solely on chemical analysis must utilize techniques which allow quantification of the active components.

Analyses of these mixtures by gas chromatography–mass spectrometry (GC–MS) is the method of choice because of the ability of this technique to deal with complex mixtures and small amounts. Such analyses have been reported by a number of workers[1,2]. Such work has failed, however, to provide unambiguous chromatographic resolution of the most interesting species. Without effective separations, it is not possible to ensure that the observed signals accurately reflect the amounts present. If reliable quantification cannot be ensured correlations of analytical data with clinical or epidemiological data are meaningless.

In this paper, we report the chromatographic resolution of three important components of the set: 2,3,7,8-tetrachlorodibenzofuran, (2378-TCDF); 2,3,4,7,8-pentachlorodibenzofuran, (23478-PenCDF); and 1,2,3,7,8,9-hexachlorodibenzofuran, (123789-HexCDF). Two-dimensional GC methods were used to obtain the separations. Analysis was by mass spectrometry.

0021-9673/84/$03.00

EXPERIMENTAL

The two-dimensional GC–MS apparatus used here was constructed in this laboratory and is described in an accompanying paper[3]. Briefly, the apparatus consists of two sequential chromatographs interfaced to a high resolution mass spectrometer. Specific components eluting from the first chromatograph can be switched into a cold trap and then transferred to the second chromatograph. A portion of the primary effluent (10%) is split to a flame ionization detector in order to allow monitoring of the primary chromatogram. Peak-switching is carried out with reference to the flame ionization detector signal which is undisturbed by the switching procedure. Ultralow (pg) level samples do not give detectable signals from the flame ionization detector. For this reason such samples are co-injected with index compounds which elute just before the compound of interest thereby facilitating accurate peak-switching. In the present work the primary separations were carried out on a 10 ft by 2 mm I.D. glass column packed with 3% Silar 10C on 100–120 mesh Gas-Chrom Q obtained from Applied Science Labs., State College, PA, U.S.A. The carrier gas was helium at a flow-rate of 40 ml/min. Primary separations were carried out under isothermal conditions. Tetrachloro congeners were analyzed at 230°C. Pentachloro and hexachloro congeners were analyzed at 240°C.

Secondary separations were carried out using high resolution open tubular capillary columns. The secondary separation of 2378-TCDF has been accomplished in two different ways using combinations of capillaries in series. In the first method a 30-m bonded DB-1701 fused-silica capillary obtained from J & W Scientific was combined with a 21-m C-87 glass capillary obtained from Quadrex, New Haven, CT, U.S.A. DB-1701 is a bonded phase consisting of 86% dimethylpolysiloxane and 14% cyanopropylphenylpolysiloxane. This system was programmed from 100°C to 240°C at 20°C/min and held at 240°C. In the second method a 12.5-m bonded DB-17 capillary obtained from J & W Scientific was combined with a 30-m SP-2330 capillary obtained from Supelco, Bellefonte, PA, U.S.A. DB-17 is a bonded phase consisting of methylphenylpolysiloxane. This system was programmed from 100°C to 230°C at 20°C/min and held at 230°C. Analysis of 23478-PenCDF and 123789-HexCDF were carried out either on the C-87 capillary or on the DB-1701, each used independently. For these analyses the column was programmed from 150°C to 250°C at 20°C/min and held at 250°C.

The mass spectrometer used in this work was a Vg Analytical, Model Zab. Mass spectral data was acquired using electron impact ionization at a mass resolution $M/\Delta M$ of 3000 (10% valley definition). Electrostatic selected ion monitoring was employed. The selected ion data was acquired using an Incos data system modified as described previously[3]. Perfluoro-4,4′-diaminobiphenyl was used to generate the "lock mass" signal. This material is introduced using a heated reservoir inlet and can be used at very low levels because it carries almost all of its ion current on the molecular ion.

RESULTS AND DISCUSSION

As noted in the introduction only certain chlorine substitution patterns have been associated with physiological effects. For each level of chlorination there are,

however, many possible substitution patterns, all of which give very similar mass spectra and in many cases near identical chromatographic behavior. Previously reported studies of the 38 possible tetrachlorinated congeners[2] found no single chromatographic system which cleanly resolved 2378-TCDF.

In work which has been reported elsewhere[4], Bell and Gara of this laboratory have re-synthesized the 38 tetra species and in addition have synthesized the 28 penta species and the 17 hexachlorinated species. The availability of these standard materials has allowed a detailed investigation of their chromatographic behavior. This has led to the development of unambiguous chromatographic separations of three materials having the 2,3,7,8 substitution pattern.

The unusual complexity of these mixtures has required the use of two-dimensional GC to obtain complete chromatographic resolution. The two-dimensional system employed a high capacity, high polarity packed first column for the primary separation followed by a much less polar high resolution capillary or combination of capillaries for the secondary separation. Selected components from the primary column were collected in a cold trap and subsequently transferred to the secondary column or column system.

A packed primary column was used to obtain relatively high capacity and a relatively high tolerance for samples which contain involatile residues. A high polarity liquid phase is used for the primary separation in order to generate a large separation between polar and non-polar species. This is useful because the most common sample contaminants are largely aliphatic in nature and therefore relatively non-polar. Collection of a given peak from the polar primary column gives a mixture which contains the relatively polar aromatic species of interest together with co-eluting aliphatic species with much higher boiling points. In the subsequent secondary separation, using relatively non-polar columns, these aliphatic components elute after the components of interest because of their higher boiling points. Since these materials elute after the species of interest, they are less likely to cause interference.

Tables I, II and III show the relative retention data for the complete sets of tetra, penta, and hexa chlorinated species. In every case the species of interest has been assigned a relative retention time (RRT) value of 1.000 and all other components of the same chlorination level have been reported relative to this species. In Tables II and III a single compound from the next lower level of chlorination is also included in order to allow an evaluation of the overlap of the various families of materials. In each table values describing the natural peak width of the analyte at baseline in terms of RRT are provided. This range of RRT values represents the elution window which must be transferred from the primary column into the cold trap in order to ensure collection of the component of interest. In addition, the natural peak width has been used to determine the range of RRT values within which leading edges or tails of other congener peaks could potentially contribute to the elution window of the species of interest. These interference ranges are reported in Tables I–III. As an example, note in Table I that six congeners have RRT values which fall within the RRT interference window (0.875–1.125) surrounding 2378-TCDF. It is these six congeners which must be resolved cleanly from 2378-TCDF in the secondary separation. Likewise for the pentas, there are two congeners in the interference window for 23478-PenCDF. Similarly for the hexas, there is a single congener in the 123789-HexCDF RRT interference window.

TABLE I

RELATIVE RETENTION DATA FOR THE 38 TETRACHLORODIBENZOFURANS

Natural peak width of 2378-TCDF at baseline reported as an RRT range: 0.937–1.063. RRT interference window for 2378-TCDF: 0.875–1.125.

Congener	*RRT*
Primary separation on Silar 10C	
1368	0.388
1379	0.474
1378	0.482
1347	0.486
1468	0.494
1367	0.513
1247	0.525
1348	0.537
1248	0.559
1346	0.583
1246	0.588
1369	0.601
1268	0.625
1478	0.632
1237	0.649
2468, 1238	0.656
1467, 2349	0.663
1234	0.678
1236	0.689
Index*	0.690
1249	0.701
1349	0.709
1278	0.740
1267	0.765
1469	0.788
2368	0.800
1279	0.812
2467	0.872
2347	0.937
1239	0.973
2378	1.000 (477 sec)
2348	1.005
1269	1.016
2367	1.062
2346	1.077
3467	1.237
1289	1.349
Secondary separation on DB1701/C-87	
2346	0.962
2347	0.978
2348	0.985
2378	1.000 (2043 sec)
1269	1.014
2367	1.014
1239	1.034

TABLE I (*continued*)

Congener	*RRT*
Secondary separation on DB-17/SP-2330	
2347	0.983
2378	1.000 (1566 sec)
2348	1.018
2346	1.033
1239	1.037
2367	1.056
1269	1.066

* Index compound was the tripropyl ester of 1,3,5-benzene tricarboxylic acid.

It should be clear that it is necessary for the region collected from the primary column to be selected very accurately. If an area larger than the natural peak width at baseline is collected, a larger number of congeners can potentially be collected in the cold trap and must then be resolved in the secondary separation. Conversely, collection of too narrow an elution window may reduce the interference from other congeners but will definitely reduce the sensitivity because part of the sample is being rejected. In this work, very accurate selection of the elution window has been assured by always operating the primary column under isothermal conditions and by collecting the primary effluent relative to a co-injected internal standard which elutes just before the region of interest. The relevant standards or "index compounds" and their RRTs are reported in the respective tables. Careful collection of the appropriate elution window from the primary column ensures that only a finite, relatively small number of congeners must be resolved in the secondary separation.

The secondary separation requires, therefore, a high resolution chromatography system which will resolve the component of interest from the congeners which co-elute from the primary column. In the case of the tetras, it was clear from the work of Mazer *et al.*[2] that a number of the more common liquid phases would not be satisfactory. Components which co-eluted from our primary column corresponded exactly to the components which were reported to be unresolved in this earlier work. Accordingly, a number of other liquid phases were examined including Kováts' C-87 hydrocarbon, DB-1701, DB-17, and OV-17. None of these capillaries used alone was capable of resolving this mixture using experimentally accessible numbers of theoretical plates.

Careful examination of retention times and elution orders suggested however that particular combinations of the phases might provide the desired separations. Accordingly, very careful measurements of the partition coefficients (K_r) for 2378-TCDF and the six interfering congeners were made for all of the phases noted above and for SP-2330. A computer program was then employed to calculate the separations which could be expected from linear combinations of the phases taken two at a time according to the method of Laub and Purnell[5]. The computer program employed examined all combinations of phase volume fraction (φ) in increments of 0.1 (*e.g.* 0.1 phase A plus 0.9 phase B then 0.2 phase A plus 0.8 phase B, etc.). Combinations of phases for which the calculations predicted separations of 2378-TCDF

TABLE II

RELATIVE RETENTION DATA FOR THE 28 PENTACHLORODIBENZOFURANS

Natural peak width of 23478-PenCDF at baseline reported as an RRT range: 0.921–1.079. RRT interference window for 23478-pentachlorodibenzofuran: 0.961–1.039.

Congener	*RRT*
Primary separation on Silar 10C	
13468	0.410
12468	0.425
23479	0.469
13479	0.480
12368	0.499
13478	0.502
12478	0.517
13467	0.536
12479	0.544
12467	0.558
12347	0.572
23469	0.577
13469	0.599
12348, (2378)	0.623
12378	0.625
12379	0.643
12346	0.646
12367	0.655
23489	0.674
12469	0.685
13489	0.766
12369	0.790
23468	0.814
12349	0.844
12489	0.852
Index*	0.853
23478	1.000 (774 sec)
12389	1.042
23467	1.046
Secondary separation on C-87	
23467	0.955
23478	1.000 (583 sec)
12389	1.132
Secondary separation on DB-1701	
23478	1.000 (584 sec)
23467	1.038
12389	1.070

* Index compound was the dibutyl, hexyl ester of 1,3,5-benzenetricarboxylic acid.

requiring less than 100,000 theoretical plates were examined experimentally. As a first step the two columns in question were simply combined using zero dead volume fittings with no attempt being made to optimize the relative lengths. The separation obtained using this preliminary combination was compared to the separation which had been calculated for that particular phase ratio considering both the actual column length and the film thickness. In general the separation observed did not fit the

TABLE III

RELATIVE RETENTION DATA FOR THE 17 HEXACHLORODIBENZOFURANS

Natural peak width of 123789-HexCDF at baseline reported as an RRT range: 0.962–1.037. RRT interference window for 123789-HexCDF: 0.925–1.074.

Congener	*RRT*
Primary separation on Silar 10C	
123468	0.521
134678	0.538
134679	0.547
124678	0.554
124679	0.598
(23478)	0.631
123478	0.658
123479	0.662
123678	0.667
124689	0.680
123467	0.703
123679	0.716
123469	0.805
123689	0.809
Index*	0.840
123789	1.000 (810 sec)
123489	1.044
234678	1.123
Secondary separation on C-87	
123489	0.977
123789	1.000 (1326 sec)
Secondary separation on DB-1701	
123789	1.000 (1004 sec)
123489	1.024

* Index compound was the butyl, dihexyl ester of 1,3,5-benzenetricarboxylic acid.

model exactly. Two columns of equal length and film thickness did not necessarily provide separations which fit the predicted values for a 50:50 phase ratio. This inconsistency arises in part from the very considerable uncertainty in the film thickness which is an important component of the partition coefficient determination. It probably also involves other factors such as the inherent compressibility of the carrier gas (see below). In this work the film thickness was taken to be that value provided by the manufacturer. Although the separation predicted for a particular combination was not observed, the separation which was observed could always be found among the calculated values. For example, the experimental separation observed for a 50:50 phase ratio might be found to correspond very closely to the separation calculated for a 60:40 phase ratio. Such comparisons of the calculated and experimental data allowed the alignment of the experimental data with the calculations. Once this alignment was established the calculated values could be used to predict how changes in the relative lengths of the two columns would change the separation. Appropriate values for the relative lengths for the two columns were selected on this basis and then verified experimentally.

The calculated values from the model were also used to calculate values of relative retention (α) for 2378-TCDF with respect to its immediate neighbors. This value was then used to calculate the minimum required number of theoretical plates which would provide baseline resolution of 2378-TCDF. The preliminary experimental data from the simple combination of the two candidate columns was used to calculate plates per meter. This value, in combination with the total required plates calculated from the model, allowed calculation of the minimum required overall column length. Both the required phase ratio and the required length were therefore determined with reasonable precision. Readers unfamiliar with the mathematical manipulations necessary to obtain these fundamental chromatographic parameters are referred to the monograph of Jennings[6]. The nomenclature and symbols utilized herein correspond exactly to those used by Jennings.

A referee of this paper has pointed out a recent paper by Krupčík *et al.*[7] describing the derivation of new equations which allow accurate predictions of the separations which will be obtained from combinations of capillary columns. These equations take into account the compressibility of the carrier gas and calculate separations based on capacity ratio (partition ratio) data. This approach eliminates any need to know film thickness accurately and requires only that the film thickness be uniform throughout the column. Having been advised of this new work, the current authors have subsequently tested the Krupčík equations in the present system. It was found that these new equations do indeed more accurately predict the separations observed experimentally than does the approach described herein based on partition coefficient data. It is recommended, therefore, that future workers follow the Krupčík approach.

Of the more than 180 possible combinations of phases examined using the

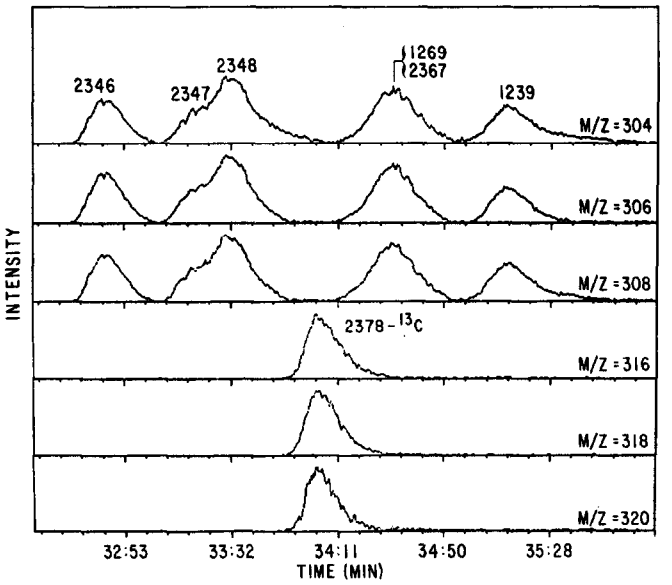


Fig. 1. Separation of 2378-TCDF from the six congeners which co-elute from the primary column using a DB-1701 (30 m) in series with a C-87 capillary (21 m).

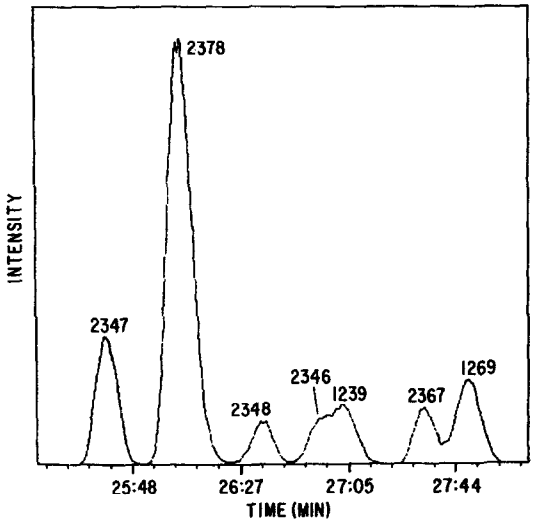


Fig. 2. Separation of 2378-TCDF from the six congeners which co-elute from the primary column using a DB-17 (12.5 m) in series with an SP-2330 capillary (30 m).

computer program described above, two combinations were optimized experimentally both for overall length and phase ratio. The combinations selected were first a 30-m DB-1701 followed by a 21-m C-87 and second a 12.5-m DB-17 followed by a 30-m SP-2330. All film thicknesses were 0.2 μm except DB-1701 which was 0.15 μm.

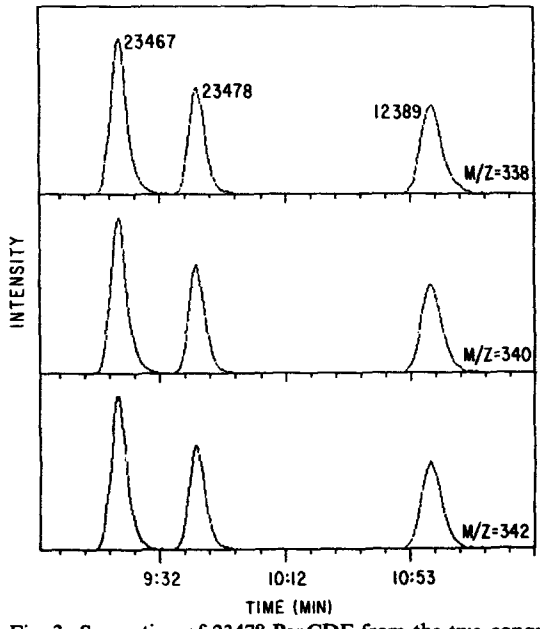


Fig. 3. Separation of 23478-PenCDF from the two congeners which co-elute from the primary column using a C-87 capillary.

In each case the bonded phase column was made to precede the non-bonded phase in order to minimize possible modification of the downstream column by bleed from the upstream column. The separations obtained are shown in Figs. 1 and 2 and are tabulated in Table I. The values of the partition coefficients for these four phases are available from the authors. It is recommended, however, that these values (or capacity ratios for the Krupčík method) be determined for every new capillary column. It should be noted that the Krupčík equations predict that reversing the order of elution for a series of capillaries will change the separations obtained. It is also interesting that C-87 glass capillaries from two different vendors provide partition coefficient values which are different in both the relative and the absolute sense. For example, 2378-TCDF and 1269-TCDF are resolved with an alpha value of 1.017 on a Chromosorb brand C-87 capillary whereas these compounds are completely convoluted on a Quadrex brand C-87 capillary. Given that this phase is generally considered among the best characterized, it is especially unlikely that the other phases used here can be reproduced exactly.

Although both of the separations described above have been used to analyze actual samples, routine use is now limited to the second combination because both phases are available in fused silica whereas C-87 is available only in glass. Moreover, the separation obtained using the second combination is superior.

The mixtures collected in the elution windows for 23478-PenCDF and 123789-HexCDF are both well resolved on the C-87 capillary alone and on the DB-1701 alone. These separations are reported in Tables II and III and are shown in Figs. 3–6.

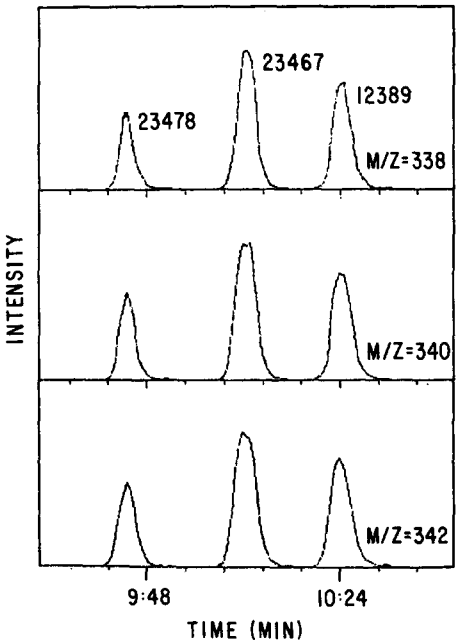
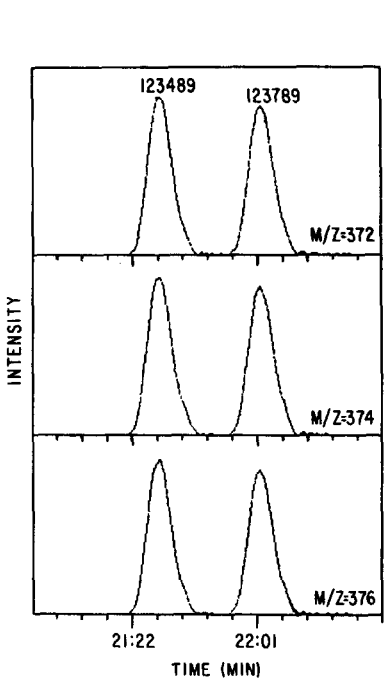


Fig. 4. Separation of 23478-PenCDF from the two congeners which co-elute from the primary column using a DB-1701 capillary.



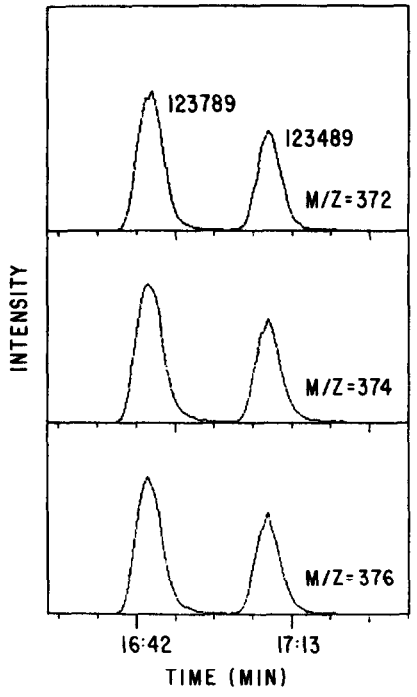


Fig. 5. Separation of 123789-HexCDF from the congener which co-elutes with it on the primary column using a C-87 capillary.

Fig. 6. Separation of 123789-HexCDF from the congener which co-elutes with it on the primary column using a DB-1701 capillary.

The full analytical scheme developed for chlorodibenzofurans will not be described here. It is useful to note, however, that all samples are spiked before analysis with ^{13}C labeled analogs of the species of interest to allow quantification by isotope dilution methods. In the two-dimensional experiment, the labeled internal standard serves an additional function in that, if it is found in the GC–MS analysis at the proper level, there can be no question whatsoever that the correct region was collected from the primary chromatograph. In this way the labeled standard provides, for every sample, positive verification of proper operation throughout the chromatographic procedure.

CONCLUSION

This method of analysis has a number of advantages over conventional GC–MS.

(1) The method allows separations which, at present, are simply not possible using single-dimensional GC.

(2) The method requires that the specific component of interest have two unique retention times on very different GC liquid phases. This greatly improves the specificity of the analysis.

(3) The method can deal with samples which have had minimal cleanup. Even

samples of neat hydrocarbon oil can be analyzed without interference[3]. The method does not require special injection techniques. Solvent volumes of at least 10 μl can be accommodated.

(5) The sample which reaches the secondary column is especially clean in that it contains only volatile species. Accordingly, the lifetime of expensive capillary columns is considerably extended relative to columns used with direct injection techniques.

(6) Finally, the analyte which reaches the mass spectrometer is much purer than with single dimensional separations. For this reason we have been able to routinely use a mass resolution of 3000 rather than 10,000, thereby gaining greatly in mass spectral sensitivity without significantly raising the level of interference. The authors feel that in terms of specificity, the recording of two independent retention times more than compensates for any loss of specificity inherent in using a lower mass resolution.

The authors emphasize that detection of the subject species in a given sample by the methods described herein does not by itself have implications regarding human health. Such findings may be relevant to human health only when specific congeners can be quantitatively measured and when toxicological and/or epidemiological data permit inference of a valid dose/response relationship.

REFERENCES

1 H. R. Buser and C. Rappe, *Chemosphere*, 3 (1979) 157.
2 T. Mazer, F. D. Hileman, R. W. Noble and J. J. Brooks, *Anal. Chem.*, 55 (1983) 104.
3 W. V. Ligon, Jr. and R. J. May, *J. Chromatogr.*, 294 (1984) 77.
4 R. A. Bell and A. Gara, *Proceedings of the Symposium on Chlorinated Dioxins and Dibenzofurans in the Total Environment, American Chemical Society, 186th National Meeting, Washington D.C. August 8-September 2, 1983*, American Chemical Society, Washington, DC, paper No. 6.
5 R. J. Laub and J. H. Purnell, *Anal. Chem.*, 48 (1976) 1720.
6 W. Jennings, *Gas Chromatography with Glass Capillary Columns*, Academic Press, New York, 1980.
7 J. Krupčik, G. Guiochon and J. M. Schmitter, *J. Chromatogr.*, 213 (1981) 189.