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**MICROBORE COLUMNS vs. OPEN TUBULAR COLUMNS  
FOR SUPERCRITICAL FLUID CHROMATOGRAPHY IN  
ENVIRONMENTAL ANALYSIS: SEPARATION OF  
POLYCHLORINATED BIPHENYLS AND TERPHENYLS**

by

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## MANAGEMENT PERSPECTIVE

This manuscript describes an analytical method for the detection and quantitation of polychlorinated biphenyls and polychlorinated terphenyls in spiked sediment samples at ppm levels. Results indicate that microbore columns could be a better alternative to open tubular columns because they can handle a greater loading of analytes. UV-detector has no advantage over the flame ionization detector as far as sensitivity is concerned but can eliminate some interfering analytes that do not absorb at 208 nm.

The study was undertaken at the request of Prof. H. Hatano, Kyoto University, Department of Chemistry, Kyoto, and supported through grants from the Japan Society for the Promotion of Sciences and the Natural Sciences and Engineering Research Council of Canada awarded to Dr. F.I. Onuska in 1986.

Dr. J. Lawrence  
Director  
Research and Applications Branch

## PERSPECTIVE-GESTION

Ce rapport manuscrit décrit une méthode analytique de détection et de quantification de biphényles polychlorés et de terphényles polychlorés dans des échantillons de sédiments enrichis en concentrations de l'ordre des ppm. Les résultats indiquent que les colonnes "microbore" pourraient constituer une meilleure solution que les colonnes capillaires parce qu'elles peuvent supporter un taux supérieur de charge stationnaire d'analytes. Le détecteur à UV ne présente pas d'avantage sur la détection à ionisation de flamme en termes de sensibilité, mais il peut éliminer certains analytes interférants qui ne sont pas absorbés à 208 nm.

L'étude a été faite à la demande de M. H. Hatano, université de Kyoto, département de chimie, Kyoto, et a été financée grâce à des subventions de la société japonaise pour la promotion des sciences exactes et des sciences naturelles ainsi que par le Conseil de recherches en génie du Canada; ces subventions ont été accordées à M. F.I. Onuska en 1986.

M. J. Lawrence

Directeur

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## ABSTRACT

The supercritical fluid chromatographic behavior of polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs) is described. The method uses a microbore C-18 column and an open tubular capillary column coated with SE-52 and carbon dioxide as the supercritical fluid. Factors affecting the separation and retention of these compounds are discussed.

## RÉSUMÉ

Le comportement chromatographique en fluide sous pression supercritique des biphényles polychlorés (BPC) et des terphényles polychlorés (TPC) est décrit. La méthode nécessite le recours à une colonne "microbore" C-18 et une colonne capillaire enduite de SE-52; le gaz carbonique sert de fluide supercritique. Dans ce rapport, on analyse les facteurs qui agissent sur la séparation et la rétention de ces composés.

## 1.0 INTRODUCTION

Supercritical fluid chromatography (SFC) has been focused on the application of open tubular columns since 1981 (1). The application of supercritical fluid chromatography for the separation of thermally labile compounds, non-volatile solutes or high molecular weight analytes is now a widely utilized practice. However, there are significant problems for similar application in environmental trace analysis because sensitivity of currently available detectors and capacity of narrow bore columns are not sufficient to generate a quantifiable signal corresponding to concentration levels of pollutants.

Recently, interest has grown in microbore columns of 1 mm i.d. packed with 5  $\mu$ m particles (2). Although still in the development stage in SFC, packed microbore columns are destined to play a very important role in environmental trace analysis in the near future. When solvent viscosity and analyte's molecular weight are relatively large, microbore packed columns appear to have a distinct advantage over open tubular columns appear to have distinct advantage over open tubular columns with regard to the number of plates generated per unit time and also to the loading capacity and speed of analysis (3).

In 1985, we critically evaluated a complete methodology for the quantitation of PCBs by gas chromatography (4). Packed and open tubular columns were compared. In this case, open tubular columns

showed distinct advantages over packed columns. It is well known that polychlorinated biphenyls (PCBs) and polychlorinated terphenyls (PCTs) are structurally and toxicologically related classes of anthropogenic chemicals that have been identified as potentially serious environmental hazards. Polychlorinated terphenyls have been used for similar applications as are PCBs. However, at an early stage of open tubular column gas chromatography, column thermal stability problems, very long retention times and poor resolution under a packed column operating conditions were encountered. These technical problems detracted environmental analytical chemists from further research to confirm presence of these pollutants in all environmental compartments.

The polychlorinated terphenyls have always been a part of technical grade Aroclors, Clophen-Harz W. Kaneclors, Delorens and other trade name technical products, especially those containing over 54 percent chlorine in their molecules. Technical PCBs were manufactured by chlorination of technical biphenyl. This biphenyl contained between 7 and 15 percent by weight of higher polyphenyls. The majority of higher polyphenyls were ortho-, meta and para-terphenyls that underwent chlorination together with biphenyl. The final reaction mixture was distilled over to achieve a certain density, which is directly proportional to the content of chlorine in the chlorinated mixture. Usually, the product obtained was not characterized for the content of higher chlorinated polyphenyls (5).

As early as in the 1971 Zitko et al (6) found PCTs in subcutaneous fat and herring gull eggs (7). Another Canadian contribution by Thomas and Reynold (8) described the detection, esti-



mation and confirmation of identity of PCTs in paperboard and food packaging material. Freudenthal and Greve (9) indicated presence of the PCTs in water, oysters, eel and even human fat in samples from Germany. Concentrations as low as 80 ng/kg and as high as 1 mg/kg were reported.

Techniques used for the quantitation of PCBs and PCTs are based on perchlorination of all individual congeners of PCBs and PCTs to fully chlorinated decachlorobiphenyl and tetradecachlorinated terphenyls (10, 11). Dechlorination of PCBs and PCTs has also been used as an alternative to perchlorination (11). The advantages of these approaches are characterized by their conversion to one single derivative - decachlorobiphenyl for PCBs and tetradecachloroterphenyls for PCTs for each positional perchlorinated isomer. Sensitivity is increased further since response to an electron capture or mass spectrometric detectors usually increases with chlorine content. On the other hand, a specific presence of the most toxic congeners cannot be confirmed and only the total amount of PCBs or PCTs can be calculated.

## 2.0 EXPERIMENTAL

### 2.1 Chemicals

The commercial Aroclors 1221, 1232, 1242, 1248, 1254, 1260, 1268, and polychlorinated terphenyls such as Aroclor 5432, 5442 and 5460 were obtained from our depository. For perchlorination technique antimony pentachloride from Alfa Products (Danvers, Mass., U.S.A.) and

tetrachloromethane, analytical grade quality from Canlab (Toronto, Ont.). All other chemicals and carbon dioxide (Alphagas, Oakville, Ont.) were of suitable purity for supercritical fluid chromatography.

## 2.2 Extraction and Cleanup Procedure

Unfortunately, there is no source available of a certified reference sample of sediments contaminated with PCTs. We prepared a sufficient quantity of the sediment sample by spiking 5 grams of blank sediment. The sediment was added to a round bottom flask filled with n-pentane containing 200 ug of PCTs. The flask was then sealed and allowed to equilibrate overnight. Afterwards, n-pentane was evaporated to dryness under a gentle stream of nitrogen. The sediment samples were spiked with 40 ug/g of individual polychlorinated terphenyls and PCBs. The samples were extracted using n-hexane by soxhleting them for 8 hours. The extracts were cleaned up by column chromatography on Florisil (12).

## 2.3 Instrumentation

Microbore column supercritical fluid chromatographic experiments were conducted with a Jones Chromatography oven, Model 7960 equipped with a Rheodyne 7520 injector having 0.5 uL internal volume modified to accommodate supercritical fluid mobile phases. The instrument was equipped with a variable wavelength UV detector (ISCO V<sup>4</sup> Absorbance detector, Lincoln, NE). The high pressure flow cell having an illuminated volume of 0.125 uL was employed. Spectra Physics Chromjet recorder/integrator was used for recording chromatograms (Figure 1).

## 2.4 Chromatographic Columns and Conditions

The columns used for this study were conventional liquid chromatography microbore columns that had been purged with helium of the manufacturer's mobile phase for 30 minutes before their chromatographic retention characteristics were recorded. Microsphere C-18 (Alltech, Deerfield, Ill.) silica 25 cm x 1.0 mm columns were used.

Open tubular column SFC separations were performed using a Model 501 supercritical fluid pump purchased from Lee Scientific Inc. (Salt Lake City, Utah, U.S.A.). Split injections (split ratio ca 1:30) were performed as previously described (14), using a Valco Model A 90 computer controlled pneumatically actuated valve fitted with a 200 nL volume. All separations were performed using CO<sub>2</sub> as the carrier fluid at a temperature of 140°C and a 10 m home-made SE-52 50 um i.d. 0.25 um film thickness column. Column pressure was maintained using an integral restrictor as described by Guthrie and Schwartz (13). The restrictor was constructed to yield a gas flow rate of approximately 2 mL/min at 200 atm CO<sub>2</sub>. The same UV-detection system was employed as described above. The UV-detector was set to monitor responses at 208 nm.

## 2.5 Perchlorination and Recoveries

Perchlorination is accomplished in a sealed ampoule at 140°C overnight. In agreement with previous work (11), we found that recoveries for the PCBs ranged at 95.0+ 4.7% and for PCTs between 89 to 98%. Comparison of the residue levels of PCBs and PCTs obtained by the direct injection of the cleaned up extract of individual Aroclors

and the total PCTs after conversion to a particular decachlorobiphenyl and tetradecachloroterphenyls is presented in Table 2. Each sample was run in triplicate to evaluate conversion efficiency. If traces of solvents are present in a sample a solid black residue is formed and it may affect the final recovery of expected perchlorination products.

### 3.0 RESULTS AND DISCUSSION

Previous studies in our laboratory have indicated that non-polar compounds such as PCB congeners can be eluted with supercritical carbon dioxide from conventional microbore and open tubular capillary columns (13). Different selectivity was observed for each column but the closest equivalent to the polarity was C-18 and SE-52 pair.

Supercritical fluid chromatography (SFC) has been applied to nine mixtures of various chlorinated biphenyls and terphenyls using both open tubular and microbore columns with supercritical carbon dioxide as the mobile phase. The selected Aroclors used in this study were those commonly used during their wide-spread industrial applications until their ban in the mid 1970's. The separation of the Aroclor mixtures was possible to achieve with  $\text{CO}_2$  as the mobile phase because their solubility in supercritical  $\text{CO}_2$  is very high and C-18 microbore column indicated very good partitioning for polychlorinated biphenyls (14).

The addition of methanol to the carbon dioxide mobile phase substantially reduced the retention times of the Aroclors. The reduced retention is attributed to the intermolecular attraction between the methanol and solute molecules and the increased solvating power due to the presence of methanol (15). Identification of the individual peaks, however, is only partially possible using microbore and OTCs. These peaks in individual Aroclor mixture are not homogenous and concentration of individual solutes vary from Aroclor mixture to another. For their characterization and identification, a more specific detector such as mass spectrometer is required. Retention data of PCBs and PCTs on the microbore C-18 packed column are given in Table 1 and Table 2. An increase in chlorine content generally increases the retention times of the PCB and PCT congeners.

Sensitivity of PCBs and PCT detection is in range of 1 ug/mL for PCBs and 5 to 10 ug/mL for PCTs.

As shown in Figure 2, with the microbore C-18 column separation of lower chlorinated biphenyls (Aroclor 1221) showed 9 peaks but the SE-52 OTC separated only 8 peaks. A simple visual comparison indicated only slight differences in resolution between the microbore and capillary columns. Each separation was carried out at 50°C with different pressure programming on both columns.

The perchlorination procedure of Hutzinger et al (11) was evaluated to determine quantitation of PCBs and PCTs by means of these two methods. Results of spiked PCBs and PCTs are illustrated in Table 3. It is very important to evaporate cleaned up extracts to dryness before reaction with antimony pentachloride + iodine.

The absolute retention times and UV-detector chromatographic traces of different PCBs and PCTs monitored at 208 nm are presented in Tables 1 and 2 and Figures 2 and 3. In general, increasing the chlorine content of PCBs and PCTs is a major factor in increasing the retention time in SFC. Congeners with the same chlorine content tend to be eluted at similar times. The UV-detector responses were in agreement with previously reported spectral data (17, 18). Thus with congener series of PCBs and PCTs, the UV-detector absorbance responses decrease with increasing retention times.

The microbore C-18 column used in these studies separates individual congeners in Aroclor mixtures on the basis of the relative extents of hydrophobic affinities. Thus, o-chlorine substituted congeners which cause the two phenyl rings of biphenyl or p-terphenyl out of coplanarity, tend to decrease the hydrophobicity of PCBs and PCTs relative to those with the same number of chlorine substituents but in different positions and they elute earlier on non-polar phases.

The detector responses of the PCBs and PCTs are based on the absorbance band at 208 nm, which has been attributed to the  $\pi - \pi^*$  transition as shown in Figure 4 for tetradecachloroterphenyl with  $E_{\text{max}} = 130,000 \text{ l/mol.cm}$  confirming previously published values (16). Identification of the individual congeners, however, is impossible to estimate. For their characterization a more specific detector such as mass spectrometer is required.

Although the analysis of PCBs and PCTs by SFC using a UV-detector at 208 nm has been shown to be a viable method, producing separation of some congeners that are equal to those of packed column gas chromatography, the relative lack of sensitivity compared to the ECD presents a serious problem. In the present study, sensitivity of PCBs is in range of 1 ug/mL when 0.125 uL flow-cell is used. The sensitivity could be increased almost on order of magnitude by increasing the cell volume without markedly reducing the separation efficiency. The microbore SFC system permits a much greater volume of sample to be loaded per analysis than is possible with open tubular column SFC without splitting the sample. Sample splitting also may contribute to errors in quantitation. Together with the use of higher concentrations of sample solutions would to a large extent overcome the relatively diminishing sensitivity of the SFC method.

As far as the analysis of PCTs by SFC is concerned using a UV-detector may be very advantageous. The detector does not require high temperature operation. Analysis can be performed at any convenient column temperature below 100°C. Again, the lack of sensitivity as compared to the ECD may be a problem. It can be partially improved in the same way as for the PCBs.

Apart from the potential to analyze environmental samples for PCBs and PCTs, the SFC method provides a simple means of the supercritical fluid extraction - SFC as a non-destructive technique for purifying individual PCBs and PCTs, which can then be used for toxicological investigations, where trace impurities could significantly alter their toxicological properties. The limitation of the UV-detector could also be used to advantage by effectively

eliminating the interference problem of other non-absorbing impurities in environmental samples during PCB and PCT analysis.

#### 4.0 CONCLUSIONS

It has been demonstrated that the determination of PCB and PCTs by SFC using microbore columns and UV detection can be used at ppm level. Our results indicate that perchlorination and pattern comparison provide the same quantitative data.

#### ACKNOWLEDGEMENT

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**Table 1. Retention Times for all Peaks in Aroclor 1200 Series  
on the C-18 Microbore Column**

PEAK NO.	RT (min)	PEAK NO.	RT (min)
1	3.58	13	12.11
2	4.81	14	13.17
3	4.91	15	14.54
4	5.10	16	15.60
5	5.94	17	17.16
6	6.55	18	17.78
7	7.40	19	20.36
8	8.33	20	20.78
9	8.94	21	23.33
10	10.88	22	24.25
11	11.00	23	26.78
12	11.40	24	26.69

**Table 2. Retention Times for Aroclor Peaks of 5400 Series**

PEAK NO.	RT (min)	PEAK NO.	RT (min)	PEAK NO.	RT (min)
1	6.22	11	12.86	21	22.56
2	7.16	12	13.46	22	24.35
3	8.26	13	14.58	23	25.82
4	8.68	14	15.14	24	26.43
5	9.25	15	15.87	25	27.04
6	9.57	16	16.32	26	27.52
7	10.37	17	17.52	27	28.03
8	10.86	18	18.21	28	30.46
9	11.49	19	20.04	29	32.06
10	11.89	20	21.68	30	35.54

**TABLE 3.      Spiked Sediment Samples with PCBs and PCTs:  
Comparison of Data for Spiked Sediments with Aroclor  
1254 and Aroclor 5460 by Two Methods. (n = 30)**

SAMPLE	SPIKE (ug/g)	SFC-PATTERN (ug/g)	PERCHLORINATION (ug/g)
Aroclor 1254	1.0	0.89	0.90
Aroclor 1254	10.0	9.25	9.30
Aroclor 1254	100.0	94.05	92.55
Aroclor 5460	10.0	10.06	9.10
Aroclor 5460	50.0	51.10	46.55
Aroclor 5460	100.0	100.70	91.95

CAPTIONS

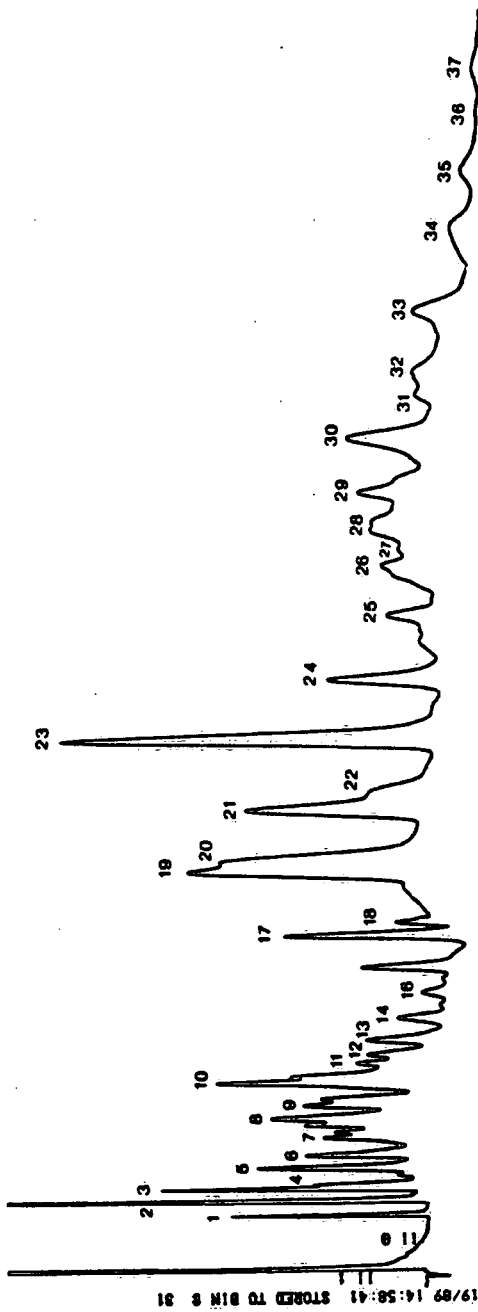
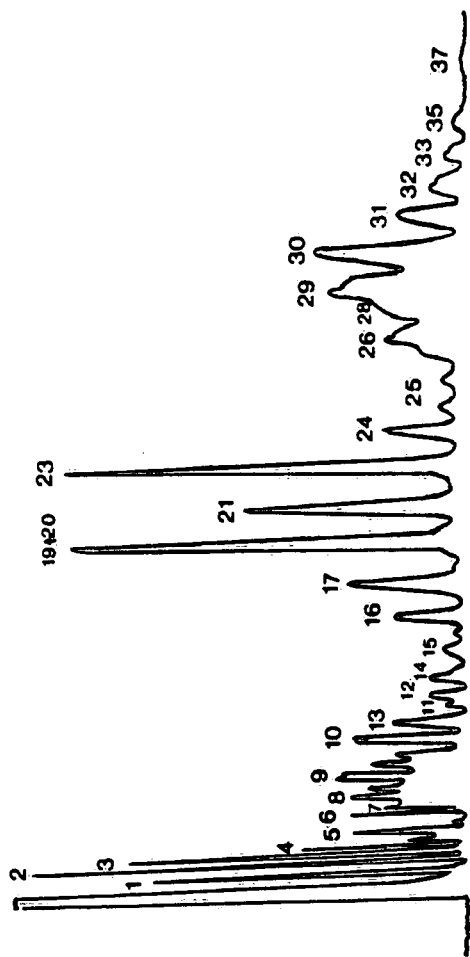
Figure 1. Separation of PCB and PCT Mixture on the Microbore (A) and Capillary (B) Columns.

Conditions: (A) 1 mm I.D. C-18 microbore column 25 cm in length; dp = 5  $\mu$ m; oven 45 C; restrictor 10 cm x 25  $\mu$ m I.D.; injector volume 500 nL; UV-detector @ 208 nm; sensitivity 0.05 absorbance units; 0.125  $\mu$ L cell volume; CO<sub>2</sub> as a supercritical fluid; atten. 8 x; pressure programming from 2000 psi for 5 minutes and 13.3 psi/min. to 2400 psi, then hold for 20 minutes.

Figure 2. Separation of Aroclor 1200 Series on the Microbore C-18 Column. Conditions as in Fig. 1.

Figure 3. Separation of Aroclor 5400 Series on the Microbore C-18 Column. Conditions as in Fig. 1.

Figure 4. The UV-Spectrum of Tetradecachloro-p-Terphenyl.  $c=10^{-5}$ M in acetonitrile; 1 cm cuvette.

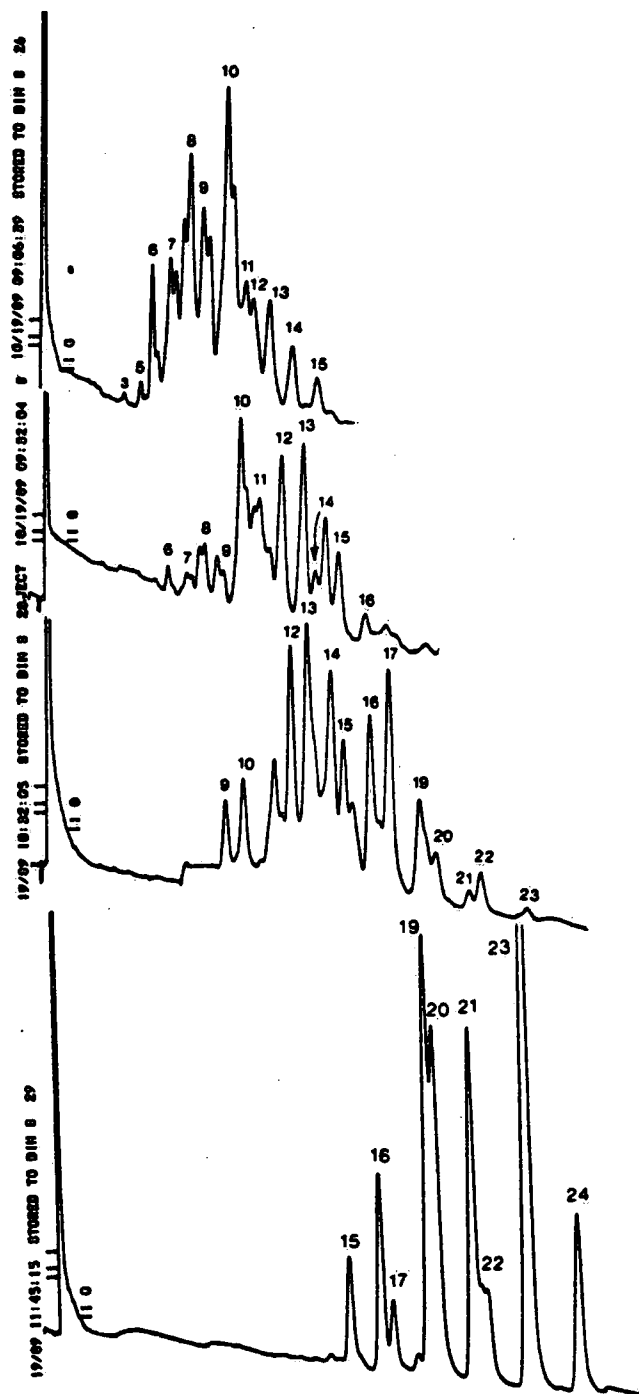
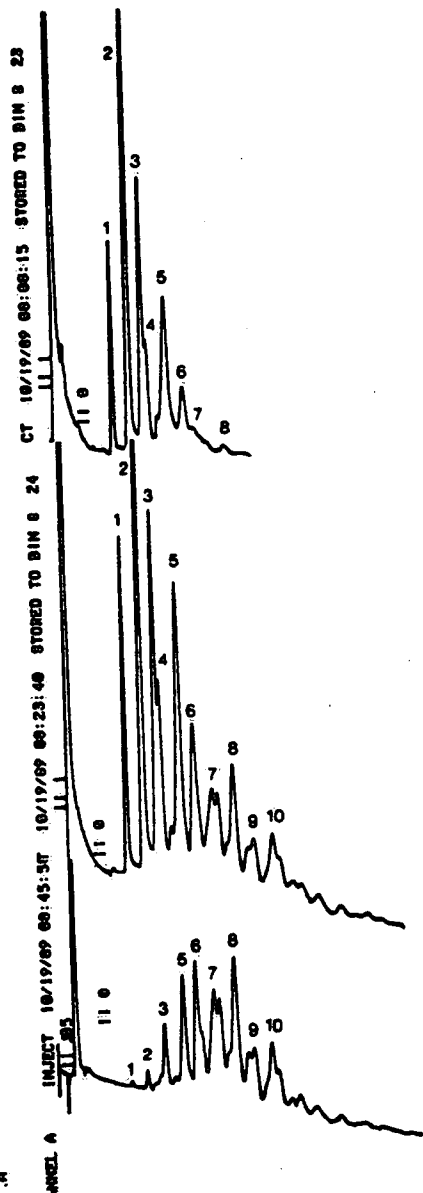


1 10/19/99 14:58:41 STORED TO BIN 8 31

ARO 1232 11UC/UL 45C 2000 SHIN-2400 30MIN HOLD 20MIN

1 1242 .95UC/UL 2000 SHIN -2400 30MIN

.A





2.0 mg/ml

ARO 5469 2000 5MIN -2400 30MIN HOLD 20MIN 40C  
DS1.A FAULT 10

MIN -2400 30MIN HOLD 20 MIN 40C  
ULT 10

N -2400 30MIN HOLD 20MIN  
T 10

CHANNEL A INJECT 10/16/89 10:26:37 STORED TO BIN 8 15  
INJECT 10/16/89 09:34:18 STORED TO BIN 8 14ECT 10/16/89 09:09:24 STORED TO BIN 8 13

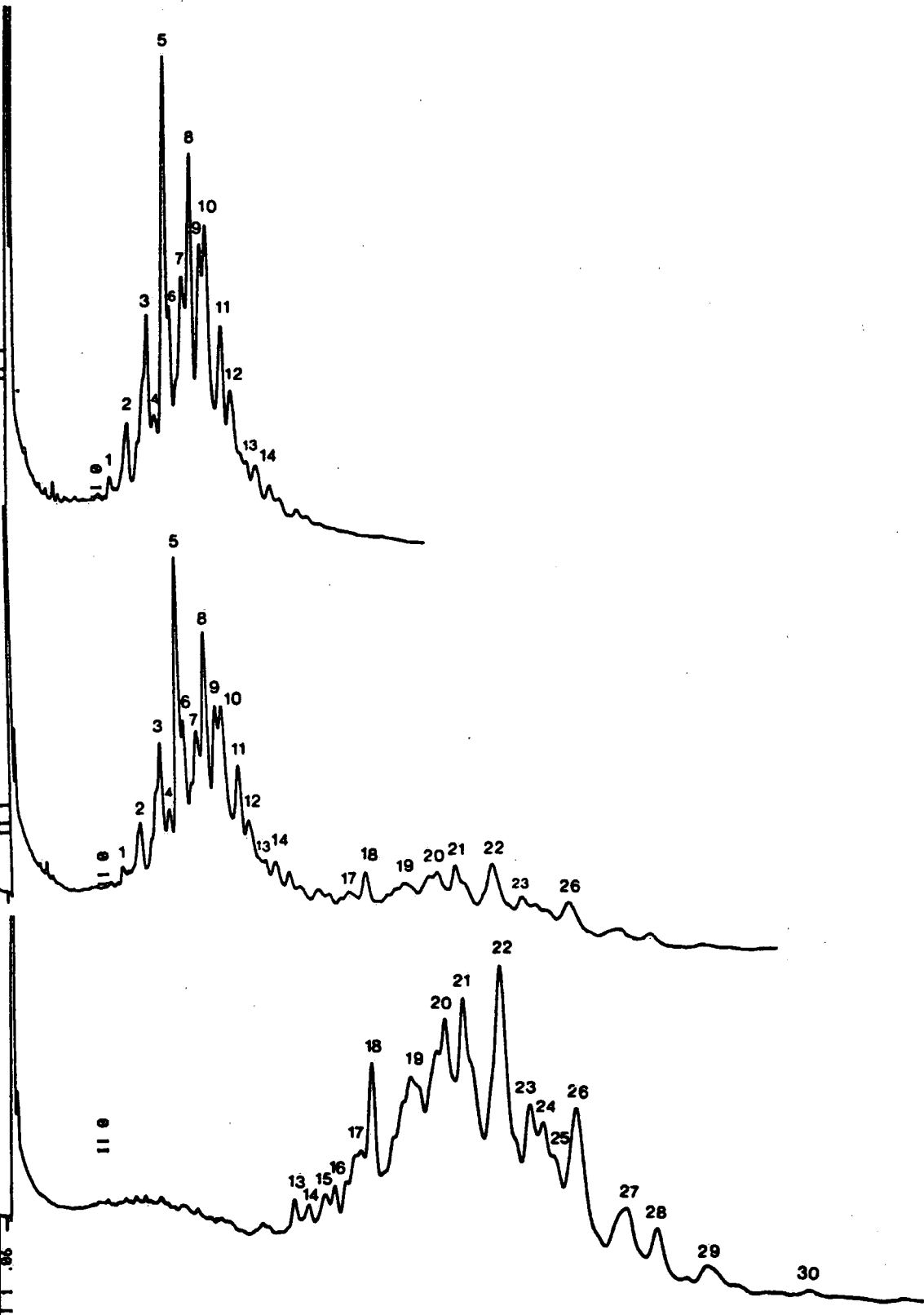


Fig 3

