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Miniaturisation of size-exclusion chromatography as a powerful clean-up tool in residue analysis

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ABSTRACT

A miniaturised size-exclusion chromatography (SEC) column with a 2 mm I.D. was developed and compared to a routinely used SEC column with a 10 mm I.D. for the determination of organochlorine pesticides. The flow-rate and sample size were decreased in proportion with the decrease in diameter and the column was tested for recovery of pesticides and for clean-up of animal fat and vegetable samples. Analysis was performed with capillary gas chromatography–electron-capture detection. The miniaturised column proved to be better than the standard SEC column with regard to the removal of the matrix in vegetable samples, while comparable results were obtained with regard to recoveries obtained and results of analysis. Sample size and solvent consumption for the miniaturised SEC column as well as the volume of the collected fraction are dramatically decreased. This facilitates evaporation of solvent which may be necessary to achieve the needed limit of detection.

INTRODUCTION

Since the introduction by Stalling *et al.* [1] of size-exclusion chromatography (SEC) as a versatile and easy to use clean-up method in the area of analysis of organic contaminants, the method has become wide spread. SEC is used to separate the analytes from co-extracted compounds with a higher molecular weight, such as fats and dyes.

Until now a large number of articles have been published dealing with the applicability of SEC in the area of trace analysis of organic contaminants. Several experimental conditions have been used including a number of different column sizes, gel materials, solvents and solvent mixtures [1–4]. Specht and Tillkes [2,3] investigated the elution volume of a large number of organic compounds on a SEC column. Their results indicate very clearly the versatility of SEC as a clean-up method since basically all organic contaminants elute in the same fraction, separated from higher-molecular-weight material. In our laboratory a SEC column (10 mm I.D.) is used on a routine basis for the clean-up of different agricultural products and animal fats in the analysis of pesticides [4]. Other authors use SEC in the determination of animal drugs [5] or polycyclic aromatic hydrocarbons [6].

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However, even when using a 10 mm I.D. SEC there are drawbacks, *e.g.* the large solvent consumption and the relatively large volume of the fraction containing the organic contaminants, causing a high degree of dilution of the sample. Dilution of the sample is especially inconvenient when analysis at trace level is to be performed. Evaporation of the solvent, when necessary, is possible but time-consuming and may cause losses of volatile compounds when heating and/or vacuum is used. The results reported by Fernandez *et al.* [6] show clearly the losses due to evaporation; recovery percentages of as low as 52% are reported for some polychlorinated biphenyls, (PCBs) in the analysis of PCBs when using SEC as a clean-up.

In this paper the development of a SEC column of small internal diameter is described and the results are compared to the results obtained with a routinely used SEC system. In general the elution volume of the organic compounds, as well as the flow-rate and the injected sample volume, changes linearly with the square of the column diameter so the same limit of detection (LOD) may be obtained with much lower solvent consumption. Furthermore, concentration of the collected fraction, if necessary, is rapid without the need for heating or applying a vacuum. Evaporation losses are thus reduced to a minimum.

The aim of this work is to miniaturise SEC to such an extent that the resulting small fraction may in future be introduced on-line into a capillary gas chromatographs. In this way a highly automated and very sensitive system for the analysis of pesticides may be obtained.

EXPERIMENTAL

Instrumentation

The 10 mm I.D. SEC column and its operating conditions have already been described by Roos *et al.* [4]. The miniaturised SEC column consisted of a 60 cm × 2 mm I.D. glass-lined stainless-steel tubing (Techmation, Utrecht, The Netherlands) equipped with column end-fittings and 2-μm frits. The column was packed with Bio-Beads SX3 gel and eluted with a mixture of ethyl acetate–cyclohexane (1:1) at a flow-rate of 40 μl/min. All tubing connections were made of 0.18 mm I.D. stainless-steel tubing or 0.3 mm I.D. PTFE tubing and were kept as short as possible to minimise peak broadening. A Gilson 305 high-performance liquid chromatography (HPLC) pump (Meyvis, Bergen op Zoom, The Netherlands) was used together with a Gilson 202 fraction collector. A WISP autosampler (Millipore-Waters, Etten-Leur, The Netherlands) was used to inject 20-μl aliquots onto the SEC column. SEC chromatograms were recorded using a Merck Hitachi L 4000 UV detector (Merck, Amsterdam, The Netherlands) operated at 280 nm.

The analysis of pesticide-containing fractions was performed by splitless injection of 2-μl aliquots on a Perkin Elmer 8700 gas chromatograph (Perkin Elmer, Gouda, The Netherlands) equipped with a Perkin Elmer AS 2000 B autosampler, a 25 m × 0.25 mm I.D. CP SIL 8 CB column with 0.41 μm film thickness (Chrompack, Middelburg, The Netherlands) and a ^{63}Ni electron-capture detector. Helium was used as carrier gas at a linear velocity of 30 cm/s. After injection, the oven was kept at 90°C for 2 min then the split vent was opened and the oven was heated at a rate of 10°C/min to 250°C. This temperature was maintained for 20 min.

All solvents were purchased from Merck and were distilled in glass prior to use.

For testing purposes a mixture of pesticides was used containing 0.1 μg/ml HCB (hexachlorobenzene), 0.2 μg/ml α-HCH (hexachlorocyclohexane), γ-HCH and β-heptachloroepoxide, 0.4 μg/ml β-HCH, heptachlor, α-chlordane, γ-chlordane, dieldrin, endrin, *p,p'*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], *p,p'*-TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] and *o,p'*-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane] and 0.8 μg/ml *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-

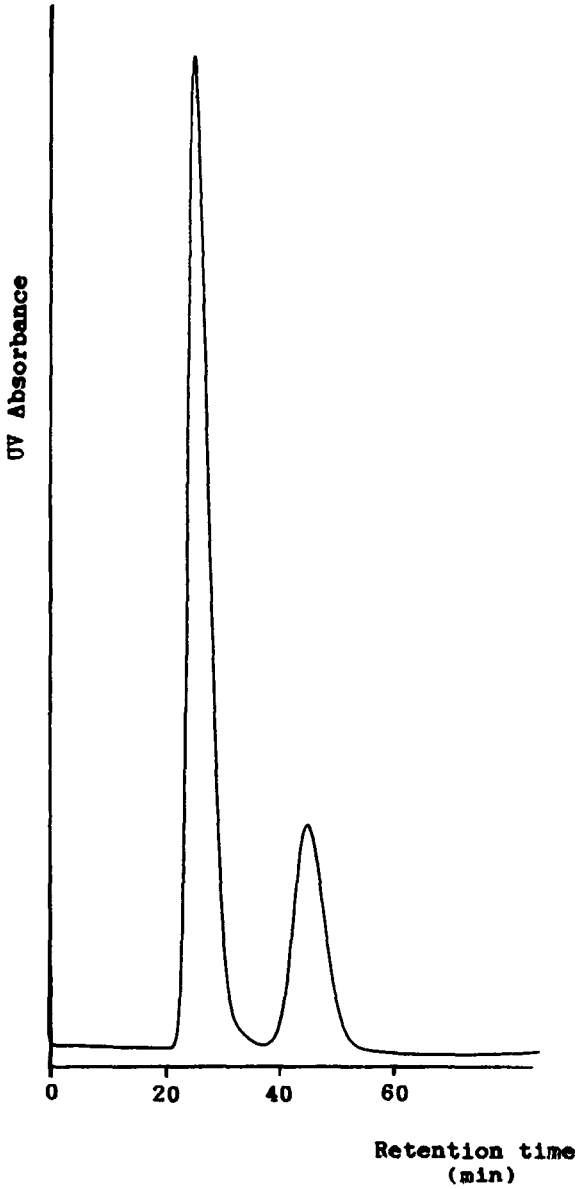

Fig. 1. Typical separation of animal fat and pesticides on a 2 mm I.D. SEC column.

chlorophenyl)ethane]. For the gas chromatographic (GC) determination, this solution was diluted 100-fold to obtain a solution containing 0.001, 0.002, 0.004 and 0.008 μg/ml, respectively, of the pesticides mentioned. PCB 138 was added as an internal standard (I.S.) at a final concentration of 0.02 μg/ml in the GC standard solution. For SEC exeriments monitored by UV detection, a mixture of the same pesticides was used at concentrations of 10, 20, 40 and 80 μg/ml, respectively.

For packing the miniaturised SEC column, the column end-fittings were removed and a reservoir consisting of a piece of 6.4 mm O.D. stainless-steel tubing, length 10 cm, was mounted on top of the column. The reservoir was filled with 10 ml of a slurry of Bio-Beads SX3 in ethyl acetate–cyclohexane (1:1), pre-swollen for 24 h. Trapping of air bubbles in the column packing was avoided by allowing a large part of the thick slurry to flow through the column by gravity. When trapping of air bubbles nog longer seemed likely to occur, the column end fitting on the bottom end of the column was replaced and the HPLC pump was connected to the gel reservoir.

The column was than packed over a period of 24 h at a flow-rate of 100 μl/min. When packing was completed, the gel reservoir and the surplus of gel slurry were removed and a column end-fitting was mounted. The column was then ready for use.

Procedure

The miniaturised SEC column was connected to a Merck-Hitachi UV detector operated at 280 nm. The flow-rate was set at 40 μl/min. A 20-μl aliquot of a solution was injected containing the mentioned fourteen pesticides at concentrations ranging from 10 to 80 μl/ml together with 0.2 g/ml animal fat. The resulting UV chromatogram is shown in Fig. 1. From this chromatogram the elution volume of the pesti-

TABLE I

COMPARISON OF RECOVERY OF PESTICIDES WITH EITHER A 2 MM I.D. SEC COLUMN OR A 10 MM I.D. SEC COLUMN (n = 10)

C.V. = Coefficient of variation.

Pesticide	2 mm I.D.		10 mm I.D.	
	Recovery (%)	C.V. (%)	Recovery (%)	C.V. (%)
α-HCH	103	4.2	101	4.2
HCB	89	5.3	102	5.1
β-HCH	85	4.5	101	5.1
γ-HCH	101	4.8	102	4.8
Heptachlor	99	4.7	102	4.1
Heptachloroepoxide	100	3.9	103	3.8
γ-Chlordane	97	2.8	109	3.7
α-Chlordane	98	3.2	106	2.8
p,p'-DDE	94	4.0	110	3.0
p,p'-TDE	92	3.3	107	2.8
o,p'-DDT	96	2.3	108	2.0
p,p'-DDT	99	2.3	109	3.0
Dieldrin	96	3.7	109	3.0
Endrin	96	2.7	105	3.4

TABLE II

RESULTS OF ANALYSIS FOR AN ARTIFICIALLY CONTAMINATED ANIMAL FAT USING EITHER A 2 MM I.D. SEC COLUMN OR A 10 MM I.D. SEC COLUMN (n = 10)

Pesticide	Spiking level (μg/g)	2 mm I.D. SEC		10 mm I.D. SEC	
		Content (μg/g)	C.V. (%)	Content (μg/g)	C.V. (%)
α-HCH	0.50	0.50	7.1	0.56	3.8
HCB	0.25	0.24	6.3	0.28	5.6
β-HCH	1.00	0.73	7.7	1.08	3.1
γ-HCH	0.50	0.49	7.3	0.56	3.8
Heptachlor	1.00	1.01	5.4	1.07	3.2
Heptachloroepoxide	0.50	0.49	7.8	0.53	4.3
γ-Chlordane	1.00	0.94	6.7	1.04	4.4
α-Chlordane	1.00	0.97	6.5	1.05	3.7
p,p'-DDE	1.00	1.05	6.7	1.08	4.2
p,p'-TDE	1.00	0.97	7.4	1.04	4.6
o,p-DDT	1.00	1.00	6.2	1.04	3.6
p,p'-DDT	2.00	2.14	8.5	2.10	5.2
Dieldrin	1.00	1.01	5.9	1.08	3.5
Endrin	1.00	0.92	5.7	1.09	3.9

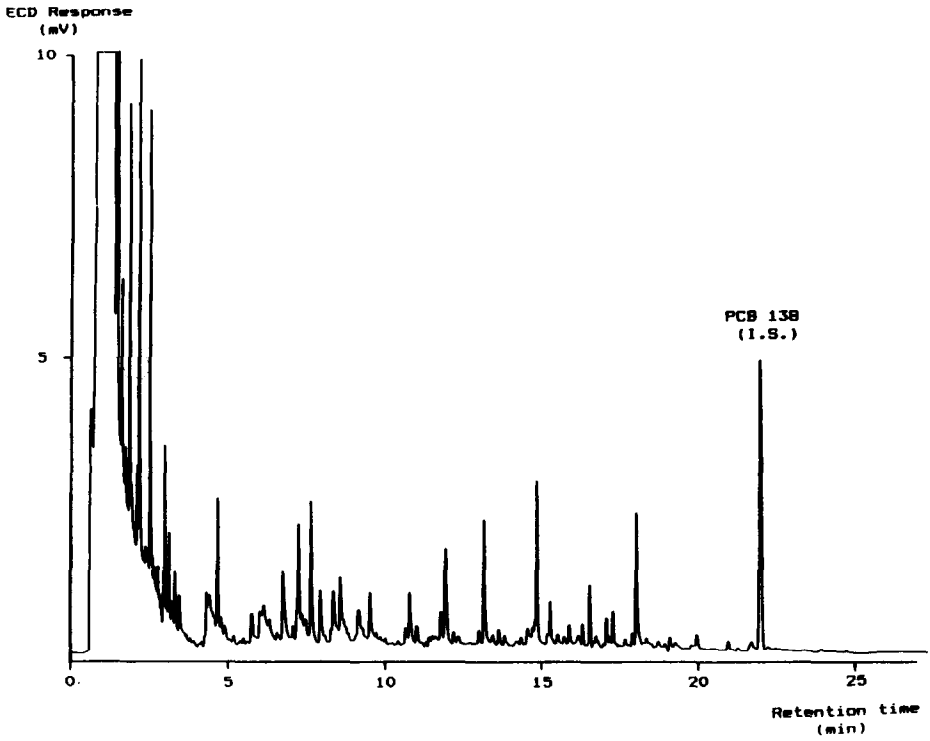

Fig. 2. Matrix blank of pepper obtained with the 10 mm I.D. SEC column. The injected volume corresponds to 0.5 mg of sample.

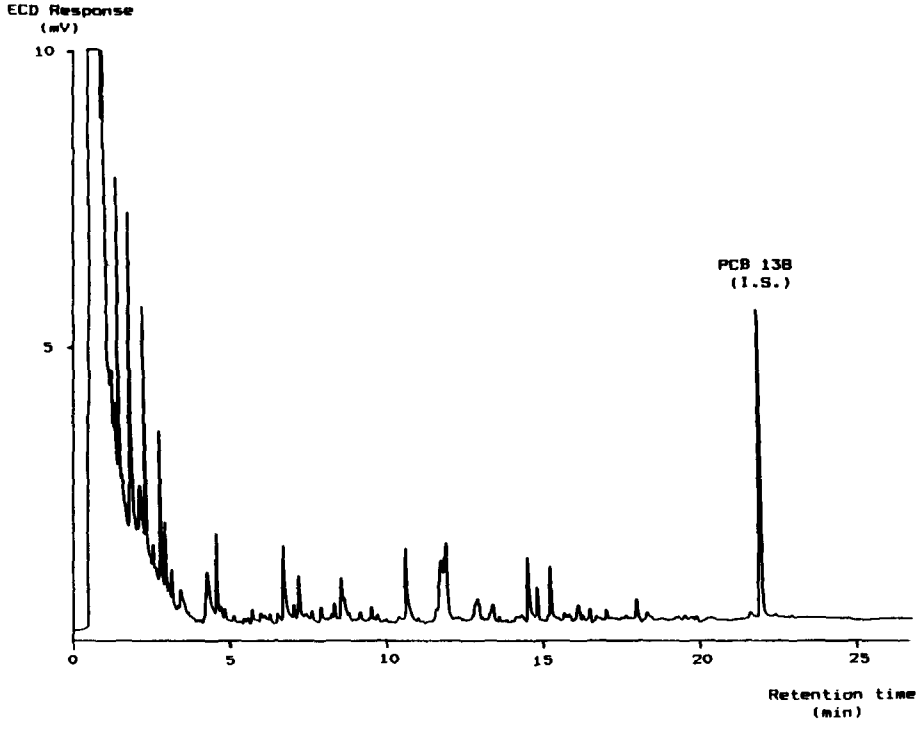

Fig. 3. Matrix blank of pepper obtained with the 2 mm I.D. SEC column. The injected volume corresponds to 0.5 mg of sample.

cides was estimated and the collect and dump times of a fraction collector were adjusted accordingly. Further optimisation was done by injection of a solution of the pesticides over the concentration range 0.1–0.8 μg/ml and replacing the UV detector by the fraction collector. The pesticides in the fraction collected using the pre-determined collect and dump time were analysed by GC–electron-capture detection (ECD). The collect and dump times were adjusted to obtain a recovery of β-HCH of at least 85% while the recovery of the other pesticides should exceed 90%. These quantitative recoveries of the pesticides could be obtained collecting the eluate between 38.5 and 55 min after injection.

Comparison of the miniaturised SEC column with the 10 mm I.D. SEC column was peformed by determining the recovery of fourteen pesticides on each column (Table I). Therefore a 20-μl aliquot of the pesticide solution was injected ten times on each column and the collected fractions were, after addition of PCB 138 as an internal standard, made up to 2 ml, thus yielding the same concentration of pesticides and internal standard in the extract as in the GC standard solution when 100% recovery is obtained.

Artificially contaminated animal fat was also analysed ten times to compare the results of analysis on each column. Pork fat obtained by pentane extraction was used.

The spike levels for the fourteen pesticides and the results of these experiments

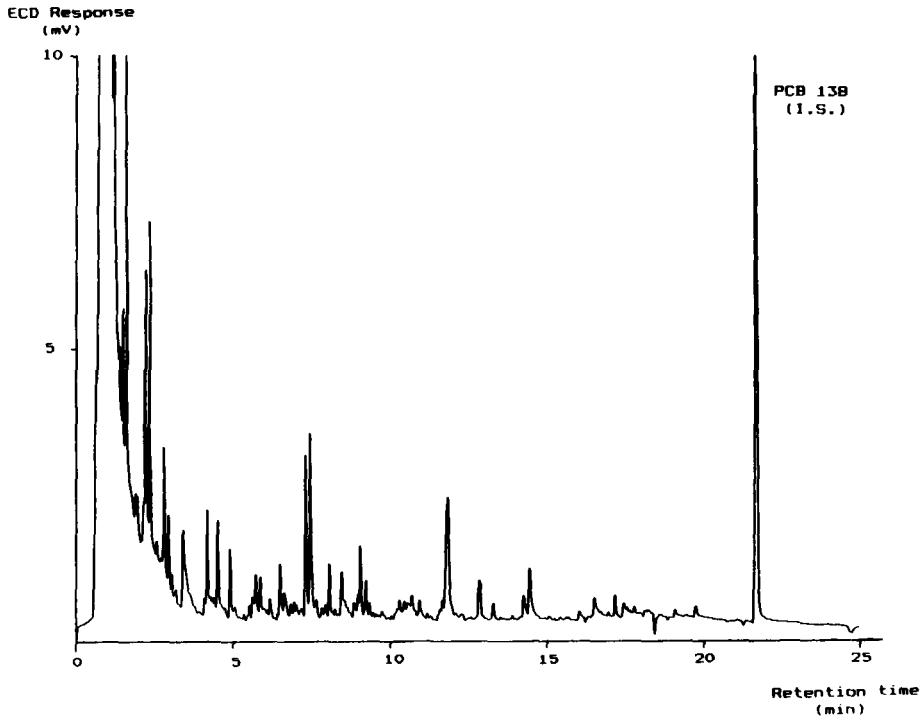

Fig. 4. Matrix blank of pork fat obtained with the 10 mm I.D. SEC column. The injected volume corresponds to 4 mg of sample.

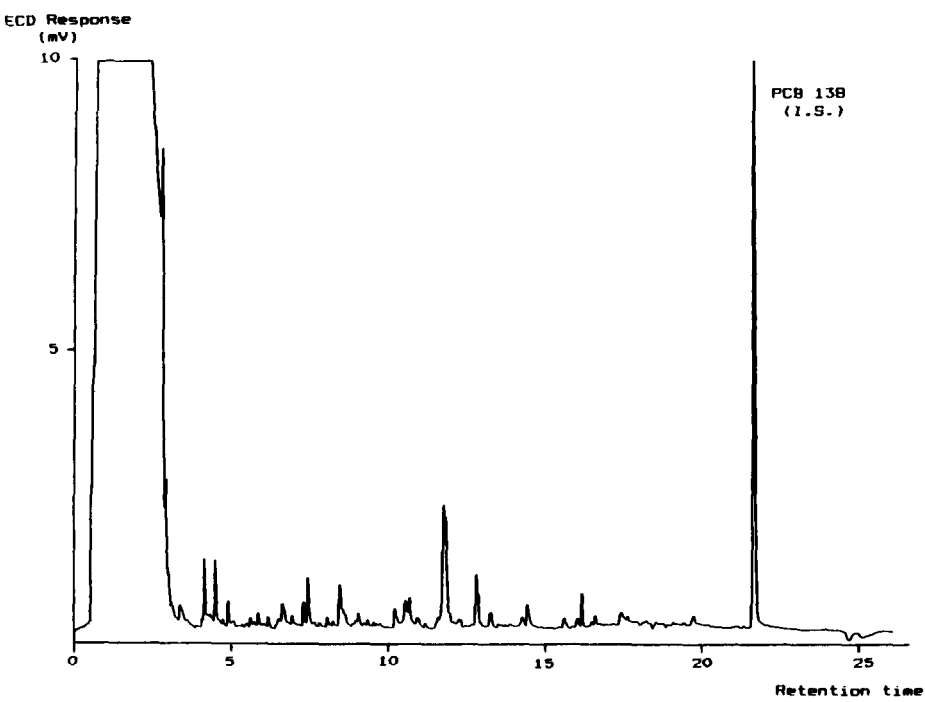

Fig. 5. Matrix blank of pork fat obtained with the 2 mm I.D. SEC column. The injected volume corresponds to 4 mg of sample.

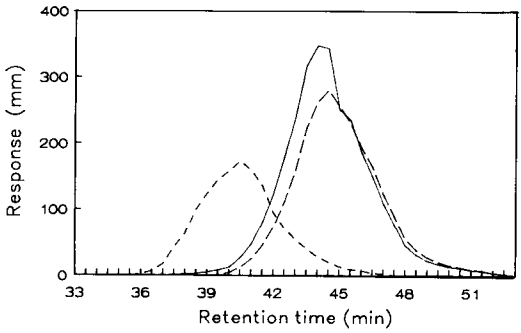

Fig. 6. Elution curves of α-HCH (———), β-HCH (---) and γ-HCH (– – –) on the 2 mm I.D. SEC column (see text).

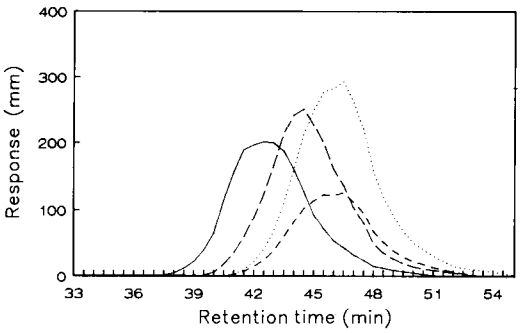

Fig. 7. Elution curves of *p,p'*-DDE (···), *p,p'*-TDE (———), *o,p'*-DDT (---) and *p,p'*-DDT (– – –) on the 2 mm I.D. SEC column (see text).

are shown in Table II. The injected aliquot (20 μl) corresponded to an amount of 4 mg of fat. After addition of PCB 138 as an internal standard, the collected fraction was made up to 1 ml, yielding the same concentration of pesticides and internal standard in the extract as in the GC standard solution when 100% recovery is obtained.

Vegetable extracts were also cleaned on both SEC columns and the chromatograms compared. In Figs. 2, 3, 4 and 5 matrix blanks of a pepper (capsicum) extract and blanks of a pork fat extract are shown as obtained with the 10 mm I.D. columns and the 2 mm I.D. column, respectively.

DISCUSSION

The parameters of the 10 mm I.D. SEC column were decreased in proportion with the square of the column diameter to obtain parameters for the 2 mm I.D. SEC column. Nevertheless, the pesticide-containing fraction on the 2 mm I.D. SEC column appeared larger as compared to the 10 mm I.D. SEC column than expected by calculation. Since the injection volume was reduced in proportion to the decrease of dimensions, this resulted in an even more dilute extract causing an LOD which is,

without evaporation, approximately 20% higher than the LOD obtained with the 10 mm I.D. SEC column.

From the results in Table I the recovery of pesticides appear to be comparable. For β-HCH in general a slightly lower recovery is observed on the miniaturised SEC column (Fig. 6). Increase in the recovery for β-HCH can be obtained by starting the collection of the pesticide fraction earlier, but at the expense of a less thorough clean-up. Since the mean recovery of β-HCH is still 85%, this slightly lower recovery is acceptable. For the other compounds, recoveries on both columns are reasonably good.

The results of analysis of pesticides in an animal fat are comparable on both the miniaturised and the normal SEC column. Again β-HCH gives a lower result on the miniaturised column due to the lower recovery (Table II).

No significant differences in the precision of analysis are observed. From Figs. 2 and 3 a difference in the efficiency of the clean-up becomes clear. The matrix blanks of the pepper extract show some interferences. For the 2 mm I.D. SEC column some interferences are seen but these are significantly smaller than the interferences seen for the 10 mm I.D. SEC column. The greater length of the 2 mm I.D. column probably causes this difference. Also, the difference in residual color was very clear. The same, but to a lesser extent, applies to the clean-up efficiency of samples of animal fat (Figs. 4 and 5). It should be mentioned that when SEC is the sole clean-up tecnique, only large molecules are removed from the sample extract. Therefore some interferences in the gas chromatogram must be expected.

In Figs. 6 and 7 the elution profile for some pesticides are given. These elution profiles were obtained by fractionating the eluate from the SEC column at 30-s intervals (20 μl) and determining the pesticide concentration in each collected fraction by GC–ECD. The shown elution profiles were reconstructed from these data. From the difference in elution volume for β-HCH compared to α-HCH and γ-HCH, it is obvious that size exclusion is not the only separating mechanism. These compounds are isomers which differ only in equatorial or axial substitution of chlorine to the cyclohexane ring. When only size exclusion contributes to the separation, all three isomers should have approximately the same elution volume. The same applies to the elution volumes of the DDT-related compounds. In size exclusion the elution volume is influenced by molecular weight as well as molecular shape. The substitution of chlorine on either equatorial or axial positions on the cyclohexane ring of the HCHs may influence molecular shape. However, the observed difference in elution volume is large considering the fact that a low resolution gel is used. Therefore it does not seem likely that size exclusion, including both molecular weight and molecular shape effects, is the only separating mechanism. The difference in elution volume may be caused by adsorption effects. In that case adsorption for all the pesticides differs only slightly, with a sudden adsorption minimum for β-HCH. On the other hand some partitioning may occur, resulting in differences in elution volume. Partitioning may be due to small differences in eluent composition between the stationary and the mobile eluent.

From the data on elution profiles it is clear that β-HCH is the first eluting compound while endrin is the last eluting compound of the pesticides investigated. Using the recovery of these two compounds, the collect and dump times for the collection of a pesticide-containing fraction can be readily optimised.

In the near future, the authors will start experiments to interface a miniaturised SEC column with 2 mm or even smaller I.D., with capillary GC. This interfacing of clean-up method with the method of analysis will make complete automation of clean-up and determination possible.

CONCLUSIONS

Miniaturised SEC is comparable to normal-sized SEC, offers a means of lowering sample and solvent consumption, and facilitates concentration of the collected fraction by evaporation. The LOD is slightly higher for miniaturised SEC due to a less than proportionally larger pesticide fraction.

Both types of columns were compared with respect to the recovery of pesticides, the results of analysis and the precision of analysis. The data presented show that the columns are comparable with respect to these items. The 2 mm I.D. SEC column tested appeared to give better clean-up, probably due to the increased column length. This became most apparent when analyzing vegetable samples containing large amounts of dyes.

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REFERENCES

1 D. L. Stalling, R. C. Tindle and J. L. Johnson, *J. Assoc. Off. Anal. Chem.*, 55 (1972) 32.
2 W. Specht and M. Tillkes, *Fresenius Z. Anal. Chem.*, 301 (1980) 300–307.
3 W. Specht and M. Tillkes, *Fresenius Z. Anal. Chem.*, 322 (1985) 443–455.
4 A. H. Roos, A. J. van Munsteren, F. M. Nab and L. G. M. Th. Tuinstra, *Anal. Chim. Acta*, 196 (1987) 95–102.
5 M. Petz and U. Meetschen, *Z. Lebensm. Unters. Forsch.*, 184 (1987) 85–90.
6 P. Fernandez, C. Porte, D. Barcelo, J. M. Bayona and J. Albaiges, *J. Chromatogr.*, 456 (1988) 155–164.