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CONVERSION OF A CONVENTIONAL PACKED-COLUMN GAS CHROMATOGRAPH TO ACCOMMODATE MEGABORE COLUMNS

I. EVALUATION OF THE SYSTEM FOR ORGANOPHOSPHORUS PESTICIDES

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SUMMARY

A conventional packed-column chromatograph was modified for use with a megabore column using a commercial conversion kit, with the intention of developing a multi-residue method for organophosphorus pesticides. The results indicate that the conversion does not affect the resolving power of the megabore column, since fourteen organophosphorus pesticides could be separated in a single injection. The megabore column in such a system proved to be much more efficient than a packed column in terms of resolution and qualitative reproducibility, and comparable with a similar column in a dedicated chromatograph.

However, the conversion did affect the quantitative reproducibility of the system to some degree, as indicated by coefficients of variation between 5 and 27%, although it was determined that the particular nitrogen–phosphorus detector system (filament bead) used in this study was partly responsible for the variation. Nevertheless, calibration curves were obtained down to 0.1 ng per component, and a limit of quantitation of 1.0 ng was established for each component in a sample containing fourteen organophosphorus compounds. The results indicate that the system is sufficiently reproducible to develop a multi-residue method for organophosphorus pesticides in environmental waters.

INTRODUCTION

The technique of gas chromatography has evolved tremendously since its inception in 1951[1]. Initially the instrument was crude and simple, and consisted essentially of a heated injection port, an oven and a thermal conductivity detector. The column was initially made of copper tubing filled with an absorbant, but this was later replaced by glass tubing.

The first capillary (open tubular) column was introduced in 1957 by Golay[2], but its use was somewhat curtailed owing to a lack of capacity and easy breakage. A breakthrough came in 1979 with the introduction of fused-silica columns[3] (0.23 or

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0.32 mm I.D.). They were characterized by inertness, flexibility and ease of handling, and could be prepared with very thin stationary phases. However, column bleed was still a problem[4]. Subsequent studies resulted in a new breed of capillary columns with chemically bonded stationary phases[5–10]. These columns have excellent chemical and thermal stability, although the problem of capacity still remains[11]. Megabore columns (0.53–1.0 mm I.D.) have recently been introduced[12]. They are characterized by speed, inertness, thermal and chemical stability and efficacy (better resolution), but they have much better capacity[11] than conventional capillary (microbore) columns.

Many laboratories are still equipped with conventional packed-column chromatographs since it is only recently that dedicated capillary instruments have been marketed. Thus several authors[13,14] have reported on the conversion of their chromatograph to accept a megabore column for specific applications. Several prototypes of conversion kits have recently been marketed.

This paper reports on the conversion of a packed-column chromatograph to accommodate a megabore column, and on the evaluation of the system for the multiresidue determination of organophosphorus pesticides.

EXPERIMENTAL

Chemicals and apparatus

Organophosphorus pesticide standards were obtained from agriculture Canada (Ottawa, Canada). Stock solutions of 1 mg/ml were prepared in ethyl acetate (Anachemia). A complete list of all the pesticides studied is presented in Table I.

A Tracor 560 gas chromatograph with a Tracor 702 nitrogen–phosphorus detector was used. The chromatograph was modified to accept a megabore column using the "direct injection kit. No. 2" and the "make-up gas adaptor kit No. 2" from Supelco (Bellefonte, CA, U.S.A.).

The borosilicate megabore column (Supelco) was 30 m × 0.75 mm I.D. and contained 1.0-μm SPB-5 (a mixture of 5% dimethylpolysiloxane, 1% vinylmethylsiloxane and 94% diphenylpolysiloxane). The column was inserted 2 cm into the conversion tube (inlet) and 13 cm (outlet) into the detector system, so that the tip of the column came to rest *ca.* 2 mm from the jet. Another megabore column (30 m × 0.75 mm I.D.) containing SPB-1 (1.0 μm) was used for comparison purposes.

The detector source (filament) was operated at 7.5 V with the polarizing voltage at low. The flow-rates of air and hydrogen were set at 125 and 3.0 ml/min, respectively. The helium (carrier gas) flow-rate was set at 5.0 ml/min. A gas purifier operated at 600°C (Demery Lindberg) was installed to remove oxygen and water. Helium was also used as make-up gas when necessary.

Under normal conditions, the chromatograph was programmed as follows: 150°C for 3 min, up to 250°C at 5°C/min, hold for 8 min.

RESULTS AND DISCUSSION

The conversion of a packed-column chromatograph to accept a megabore column is straightfoward. Fig. 1 shows the location of the column in the injection port and the detector. There is some dead volume created by the glass tubing in the injection port and also in the second port, which is used as an inlet for make-up gas.

TABLE I

ADJUSTED RETENTION TIMES OF ORGANOPHOSPHORUS COMPOUNDS STUDIED

N.D. = Not detected.

Common name (trade name)	*Chemical name*	t'_R *(min)*
Acephate (Orthene)	O,S-Dimethyl acetylphosphoroamidothioate	9.3
Azinphos-methyl (Guthion)	O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazine-3(4*H*)-yl)methyl] phosphorodithioate	N.D.
Chlorpyrifos (Dursban)	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	22.2
Diazinon (Basudin)	O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate	18.0
Dichlorvos (Nogos)	2,2-Dichlorovinyl dimethyl phosphate	4.5
Dimethoate (Cygon)	O,O-Dimethyl S-(methylcarbamoylmethyl) phosphorodithioate	16.5
Disulfoton (Disyston)	O,O-Diethyl S-2-(ethylthio)-ethyl phosphorodithioate	18.3
Fenitrothion (Folithion)	O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate	21.3
Bis-fenitrothion	O-methyl-O,O-[di-(3-methyl-4-nitrophenyl)]phosphorothioate	N.D.
S-Methyl-bis-fenitrothion	S-methyl-O,O-di-3-methyl-4-nitrophenyl phosphorothiolate	N.D.
Fensulfothion (Dasanit)	O,O-Diethyl O-[4-(methylsulphinyl) phenyl]phosphorothioate	27.3
Fenthion (Baycid)	O,O-Dimethyl O-[3-methyl-4-(methylthio)phenyl]phosphorothioate	21.9
Fonofos (Dyfonate)	O-Ethyl S-phenyl ethylphosphonodithioate	17.5
Malathion (Mercaptothion)	O,O-Dimethyl S-1,2-di-(ethoxycarbonyl) ethyl phosphorodithioate	21.6
Methamidophos (Monitor)	O,S-Dimethyl phosphoramidate	4.4
Oxydemeton-methyl (Metosystox-R)	S-[2-(Ethylsulfinyl)ethyl] O,O-dimethyl phosphorodithioate	N.D.
Parathion (Thiophos)	O,O-Diethyl O-*p*-nitrophenyl phosphorothioate	22.2
Phorate (Thimet)	O,O-Diethyl S-(ethylthiomethyl) phosphorodithioate	15.9
Phosalone (Zolone)	S-6-Chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl O,O-diethyl phosphorodithioate	N.D.
Phosmet (Imidan)	O,O-Dimethyl-S-phthalimidomethyl phosphorodithioate	32.7
Temephos (Abate)	O,O,O',O'-Tetramethyl O,O'-thiodi-*p*-phenylene diphosphorothioate	N.D.
Tetrachlorvinphos (Gardonna)	2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethylphosphate	24.6
Trichlorfon (Dipterex)	Dimethyl-(2,2,2-trichloro-1-hydroxyethyl phosphonate	9.8

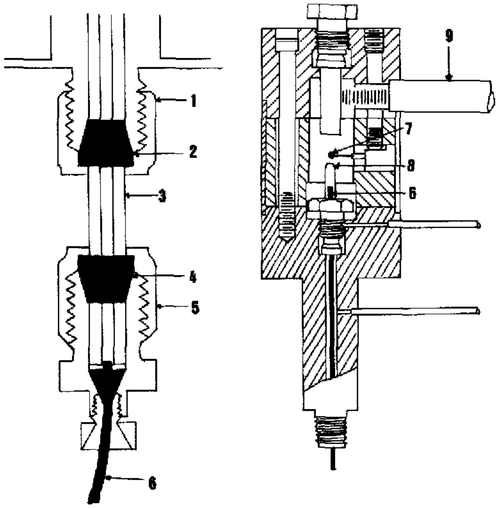

Fig. 1. Location of the column in the injection port (left) and in the Tracor detector (right): 1 = nut; 2 = ferrule; 3 = glass tubing; 4 = ferrule; 5 = nut; 6 = megabore column; 7 = filament and bead; 8 = jet; 9 = collecting electrode.

These two aspects are obvious drawbacks to this particular conversion kit as opposed to those kits that allow on-column injection.

The carrier gas was optimized using fenitrothion as a good representative of the organophosphorus compounds under study. It was found that a flow-rate of 5 ml/min offered good peak symmetry and reasonable retention time (21.3 min.) for fenitrothion. This is in agreement with the results of Lubkowitz *et al.*[15] who showed that the response of the nitrogen–phosphorus detector is at a maximum with a helium flow-rate of 5.0 ml/min. The response of the detector to fenitrothion was best with the air and hydrogen flow-rates set at 120 and 3.0 ml/min, respectively. It was also found that the response was better without make-up gas.

All the organophosphorus compounds available were tested individually under the above conditions. The results given in Table I show that 17 of the 23 compounds gave positive responses, with retention times varying from 4.4 to 32.7 min. However, some were not detected because of lack of volatility, and also several had similar retention times.

Attempts to separate all seventeen organophosphorus compounds by temperature programming failed. For this reason dichlorvos, fenthion and chlorpyrifos were excluded from further studies. Very good resolution was then achieved by temperature programming (140°C for 5 min up to 250°C at 3°C/min) for the remaining fourteen organophosphorus compounds, but the analysis time was unacceptably long at 115 min. Thus the programme time was shortened to 31 min with some loss in resolution ($R = 0.78$ between peaks 6 and 7, which means 30% overlap), as illustrated in Fig. 2.

Linearity study

Standard mix solutions of the fourteen organophosphorus compounds were

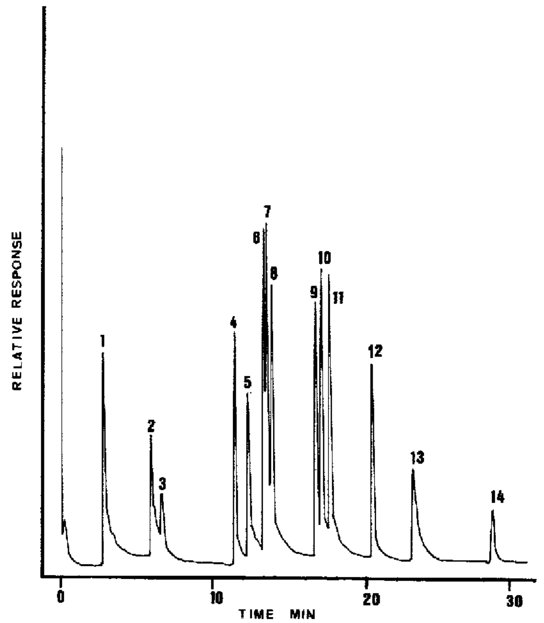

Fig. 2. Chromatogram showing separation of fourteen organophosphorus compounds on Megabore SPB-5. Amount injected, 1 ng of each. Peaks: 1 = methamidophos; 2 = acephate; 3 = trichlorfon; 4 = phorate; 5 = dimethoate; 6 = fonofos; 7 = diazinon; 8 = disulfoton; 9 = fenitrothion; 10 = malathion; 11 = ethyl-parathion; 12 = tetrachlorvinphos; 13 = fensulfothion; 14 = phosmet.

prepared such that 100.0, 10.0, 5.0, 1.0, 0.1 and 0.01 ng/μl of each pesticide were injected. Only a few pesticides, such as diazinon and ethyl-parathion, could be detected at 0.01 ng. Thus further studies with all fourteen organophosphorus were limited to 0.1 ng/μl solutions. A typical cailbration curve for metamidophos is shown in Fig. 3. The slope of the curve is 0.92, which indicates good sensitivity. However, below 1.0 ng, the deviation from linearity becomes excessive. All the other organophosphorus compounds showed similar response curves, with slopes (sensitivity) varying between 0.90 and 1.0; exceptions were phorate and disulfoton, with values of 0.60 and 0.77, respectively.

Reproducibility studies

The reproducibility of a chromatogram in terms of individual retention times in multi-residue analysis is very important for identification purposes. Table II gives the coefficients of variation (C.V., n = 16) of the retention times for the fourteen organophosphorus compounds studied. The variation is slightly higher at low retention values but the average C.V. is only 2%, which is quite acceptable considering that variations between 1 and 10% are common in gas chromatography[16]. Such little variation enables the analyst to detect a particular organophosphorus compound with reasonable accuracy and is a clear indication that the conversion does not adversely affect column performance.

Reproducibility as a function of peak height is more of a problem. C.V. for 1.0

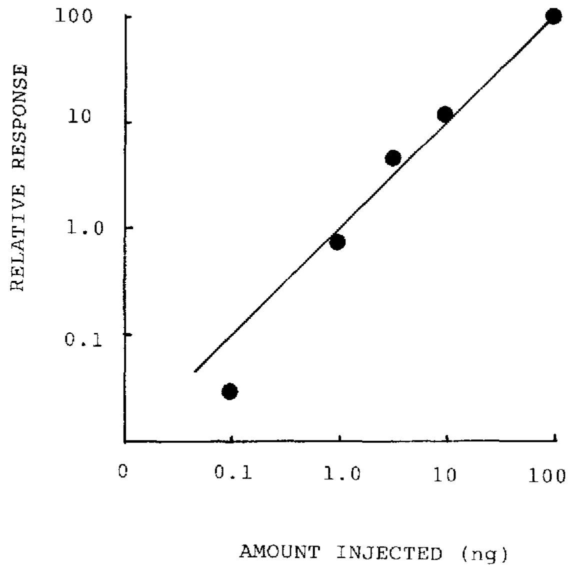

Fig. 3. Calibration curve for methamidophos.

ng of each component in the multi- residue standard are given in Table III. They show a minimum of 5% for ethyl-parathion and a maximum of 27% for phosmet. This variation may be attributed to several reasons. The main one is the greater surface area (due to extra plumbing) that the injected sample comes in contact with before

TABLE II

REPRODUCIBILITY STUDY OF RETENTION TIMES OF A MULTI-RESIDUE STANDARD (1.0 ng)

Organophosphorus compound	*Average (n=16) retention time (min)*	*S.D.*	*C.V. (%)*
Methamidophos	2.9	0.1	4
Acephate	5.9	0.2	3
Trichlorfon	6.4	0.2	3
Phorate	11.1	0.3	2
Dimethoate	12.0	0.3	2
Fonofos	13.1	0.3	2
Diazinon	13.2	0.3	2
Disulfoton	13.5	0.3	2
Fenitrothion	16.3	0.3	2
Malathion	16.7	0.2	1
Ethyl-parathion	17.1	0.2	1
Tetrachlorvinphos	19.9	0.3	1
Fensulfothion	22.6	0.4	2
Phosmet	27.3	0.5	2
		Average	2

TABLE III

REPRODUCIBILITY STUDY OF DETECTOR RESPONSE TO A MULTI-RESIDUE STANDARD (1.0 ng)

Organophosphorus compound	*Average (n=16) peak height*	*S.D.*	*C.V. (%)*
Methamidophos	12.1	1.8	15
Acephate	2.3	0.4	15
Trichlorfon	6.3	0.6	9
Phorate	21.6	1.6	7
Dimethoate	14.7	2.3	16
Fonofos	34.0	3.8	11
Diazinon	52.1	6.1	12
Disulfoton	38.8	3.8	10
Fenitrothion	42.7	3.0	7
Malathion	36.4	3.4	9
Ethyl-parathion	48.4	2.6	5
Tetrachlorvinphos	30.0	3.3	11
Fensulfothion	6.4	1.6	25
Phosmet	3.9	1.1	27
		Average	12

entering and at the exit of the column, which contributes to band broadening and inevitably affects reproducibility. Another important reason is believed to be detector variability. In the past, while working with packed columns, this particular nitrogen–phosphorus detector (Tracor 702) showed occasional variability, which was related to the particular filament bead in the detector. A third cause of variation is related to the particular organophosphorus compound (for example, tetrachlorovinphos and phosmet), which sometimes lack volatility. This results in broad and unsymmetrical peaks, which have a strong influence on reproducibility.

Limits of detection and quantitation

The limit of detection (LOD) (peak height double the noise) varies with each chemical. In order to detect each compound in the multi-residue standard, a solution of at least 0.1 ng/μl has to be injected. However, considering the variability expressed above for each pesticide, a limit of quantitation (LOQ) of 1.0 ng per analyte (ten times LOD) was chosen (average C.V. 12%) to ensure that all pesticides could be analysed with precision.

Comparison with SPB-1 megabore

The SPB-1 is considered to be less polar than SPB-5. A chromatogram of fourteen organophosphorus compounds at 1.0 ng/μl is shown in Fig. 4. Resolution is better for peaks 8, 9, 10 and 11 than with the SPB-5 column. However, peaks 6 and 7 co-elute and peaks 1 and 2 are not as sharp. Overall, the SPB-5 stationary phase gives better results than the SPB-1.

Sasaki *et al.*[17] have evaluated three megabore columns installed in a dedicated capillary gas chromatograph with a flame photometric detector in a multi-residue study of OPs in agricultural products. All the columns were 0.53 mm I.D., with

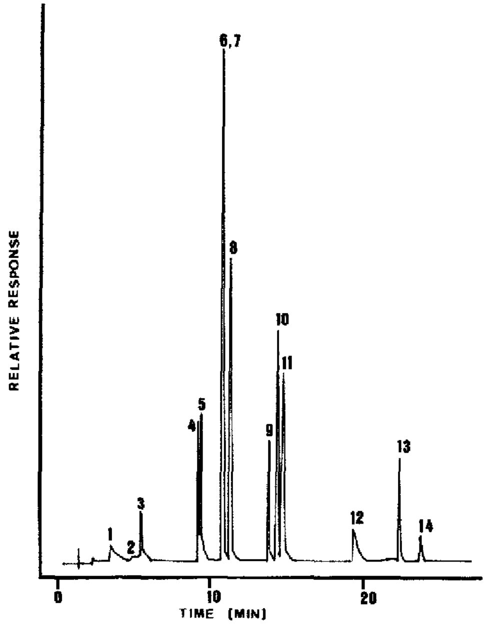

Fig. 4. Chromatogram of fourteen organophosphorus pesticides on SPB-1; amount injected, 1.0 ng of each. Peak numbers as in Fig. 2.

lengths between 10 and 12 m. These authors studied individually 23 organophosphorus compounds on CBP-10, CBP-1 and 5% phenylmethylsilicone. For comparison purposes, only the results with 5% phenylsilicone will be mentioned since this stationary phase compares favourably with SPB-5. The retention times varied from 0.9 min (dichlorvos) to 24.3 min for phosmet. The LOD varied between 0.01 and 0.08, which compares favourably with our data.

CONCLUSION

This study shows the separation of fourteen organophosphorus compounds on a megabore column installed in a conventional gas chromatograph. The results compare well with those obtained with a dedicated capillary gas chromatograph[17]. In comparison, a packed column (1.8 m × 4 mm I.D., OV-101:OV-210) in the same chromatograph can separate only five of the fourteen chemicals. Others co-elute or are simply not volatile under these conditions.

The quantitative data, on the other hand, show more variability, some of which may be caused by the conversion (more dead volume and surface area) of the gas chromatograph to accept megabore columns. However, it is suspected that much of the variability may be due to detector instability in our particular case.

It was felt that the results were sufficiently reproducible to warrant further studies. Thus, the system was used to evaluate several methods for the recovery of organophosphorus compounds from water. The results of this study appear in the following paper.

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