*Journal of Chromatography*, 302 (1984) 205–219
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 405

# CAPILLARY FUSED-SILICA ON-COLUMN INJECTION OF CHLORINATED PESTICIDES WITH AN ULTRA-LOW VOLUME ROTARY VALVE

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## SUMMARY

Quantitative on-column injections into a fused-silica capillary column were carried out by using a ten-port ultra-low volume rotary switching valve to determine the utility and reproducibility of this rotary valve as an on-column injector. Good reproducibility for repeated injections of chlorinated pesticides was obtained by using an external sample loop and wash loop with the rotary valve. The problems commonly associated with a fine fused-silica or stainless-steel needle on-column capillary injection technique were minimized. The valve was used in the manual mode but can be completely automated with commercially available equipment.

## INTRODUCTION

The development of fused-silica bonded-phase capillary columns has given capillary chromatography a more durable tool for the qualitative and quantitative analysis of organic compounds. The fused-silica bonded-phase capillary column is ideal for certain techniques, such as on-column injections, because the liquid phase is chemically bound to the surface and the columns tend to straighten when uncoiled thus making installation easy.

On-column injections with long, fine stainless-steel needles[1,2] and fused-silica needles[3,4] are routinely used for the quantitative introduction of solutes directly into the fused-silica capillary column. This on-column technique is reproducible but cannot easily be automated. An automated on-column introduction system would be ideal for routine use, because injection errors can be minimized. The rotary valve on-column injector recently described by Steele and Vassilaros[5] is ideal for on-column injections because it is easy to use and does not require any needles or septums. They used a Rheodyne Model 7413 rotary injection valve for introducing solutes into a capillary column. They used a 1-$\mu$l internal sample loop in the active position of the valve for all injections. The valve had to be cooled before each injection so that the sample loop could be filled. The absolute reproducibility of this system was found to be $<2\%$ (R.S.D.) for four replicate injections of seven compounds in a commercially prepared test mixture. The valve was tested in the manual mode but has the capability of being automated with commercially available equipment.

We describe an on-column injector for fused-silica capillary gas chromato-

0021-9673/84/$03.00 

graphy, featuring external sample and wash loops with a ten-port rotary valve having less than 0.2 μl dead volume between any two connected ports. The reproducibility of the ultra-low volume ten-port rotary valve was checked using chlorinated pesticides in two different solvents and with a variety of system parameters.

## EXPERIMENTAL*

### *Equipment*

A Tracor 560 gas chromatograph, equipped with a flash-vaporization packed-column injector and a $^{63}$Ni electron-capture detector was used for determining the packed-column results. A Varian 3700 gas chromatograph, equipped with a capillary inlet/splitter, a flash-vaporization packed-column injector and $^{63}$Ni electron capture detector was used for determining the capillary results. A ten-port, ultra-low volume sampling and switching rotary valve, Model 4N10WT, manufactured by Valco Instruments (Houston, TX, U.S.A.) was used. Special ferrules (FSR.4, FSR.5, ZF.5, ZF1, and ZF1TF), nuts (ZN.5), unions (ZRU1.5T) and 1/32 in. stainless-steel tubing with inside diameters of 0.005 in. and 0.019 in. were used with the valve and were purchased from Valco Instruments. The ends of all of the 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing used with the rotary valve were machined flat and square. The valve required no prior conditioning. The fused-silica capillary column was a 30 m × 0.32 mm I.D. Durabond-5, equivalent to SE-54 (methyl, 5% phenyl, 1% vinyl silicone), 1.0 μm film thickness, purchased from J & W Scientific, (Rancho Cordova, CA, U.S.A.).

The sample introduction system and valve are diagrammed in Figs. 1 and 2, respectively. The septum nut and insert hardware were removed from the column injector before the valve was installed. A 27 mm long injector extension shown in Fig. 2, made from a piece of round aluminum rod (12 mm O.D.), was used in checking the reproducibility of the system. The injector will be called an extended injector when the extension is installed in the injection port and a regular injector when the

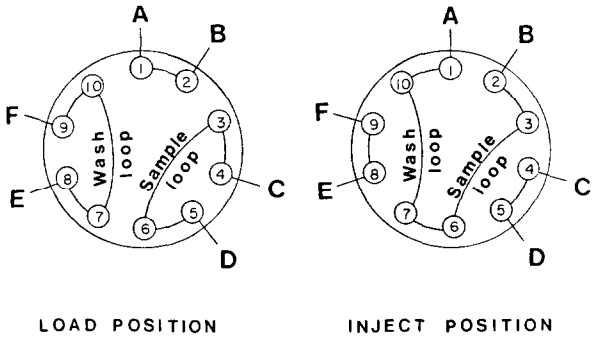


Fig. 1. Diagram of the rotary valve in the Load and Inject position. A: Carrier gas; B: capillary column; C: sample inlet; D: sample waste; E: wash waste; F: wash inlet.

* Reference to any commercial materials, equipment or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

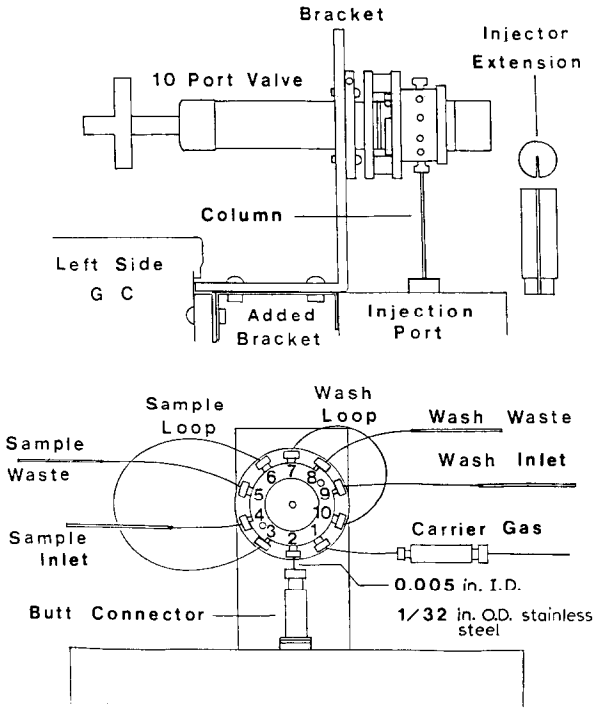


Fig. 2. Side and front view of the rotary valve mounted on the gas chromatograph.

extension is not installed. A 5 mm length of one end of the rod was cut down to a diameter of approximately 10 mm so that it would fit snug inside the injector and extend 22 mm above the injector. A groove, 1 mm wide and 6 mm deep, was cut along the length and to the center of the rod. The machined piece of aluminum rod, when used, was placed around the column and mounted inside the column injector. The top of the injector block was insulated so that the valve would be shielded from any excess heat. A bracket was added between the frame of the gas chromatograph and the left injection port as shown in Fig. 2 so the valve bracket could be mounted. This bracket was constructed of flat aluminum material (*ca.* 2 mm thick) bent in a channel configuration with one side longer than the other. The bracket was 75 mm long × 37 mm wide with channel sides 30 mm and 10 mm long. The bracket was mounted with existing holes and bolts in the instrument.

A Valco LC valve bracket was used to mount the rotary valve body on the added bracket in the gas chromatograph. Valve port 2 used for the capillary column was mounted with the bracket so it was 30 mm above and directly over the center of the open injector body. The end of the fused-silica capillary column was threaded through the column injector body and attached to port 2 with a FSR.5 ferrule (see Fig. 2). The column was connected directly to the valve for the first part of the evaluation. A butt connector and ferrule (Supelco, Bellefonte, PA, U.S.A.) was used to connect the column to a 33-mm piece of 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing, which in turn was connected to the valve with a ZF.5 ferrule and ZN.5 nut. The butt connector ferrule was made of 10% PTFE, 15% graphite and 75%

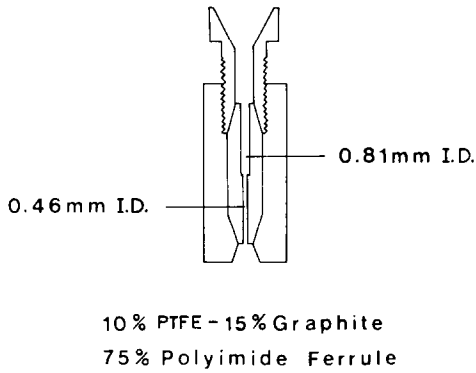


Fig. 3. Diagram of butt connector used to connect 0.32 mm fused-silica capillary column to 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing.

polyimide. A 0.81-mm hole was drilled through half of the ferrule and a 0.46 mm hole through the other half, as shown in Fig. 3. The end of the 0.81-mm hole inside of the ferrule was conformed to the square end of a piece of 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing by pushing both together hard and twisting back and forth. This was done until the inside of the ferrule was flat, as determined by viewing it through a microscope.

The helium carrier gas (pressure-controlled) was supplied to port 1 of the valve through a 1/16 in. to 1/32 in. zero dead volume reducing union (ZRU1.5T) and a piece of 0.019 in. I.D. × 1/32 in. O.D. stainless-steel tubing. Stainless-steel ferrules (ZF.5, and ZF1) were used to attach the carrier gas line.

External loops used with this rotary valve were constructed of deactivated fused-silica needle material (0.12 mm I.D.) purchased from J & W Scientific, and 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing. The wash loop was installed between valve ports 7 and 10. The sample loop was installed between valve ports 3 and 6. The fused-silica needle-material (0.12 mm I.D.) loops were installed with FSR.4 ferrules. The ferrules had to be tightened more than recommended by the manufacture in order to stop them from leaking. The 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing was installed with ZF.5 ferrules. Loops with volumes of 1 $\mu$l (80 mm long) and 2 $\mu$l (160 mm long) were constructed from the fused-silica needle-material and the stainless-steel tubing. The same loop lengths were used for both materials, because the internal diameters were equal. The smallest length allowed for the wash and sample loops is 80 mm, because of the location of the ports on the valve. The ends of the stainless steel tubing were machined so a zero dead volume fit could be obtained.

The inlet for the wash loop at valve port 9 and the inlet for the sample loop at valve port 4 were installed with a short piece of machined 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing 60 mm long and 30 mm of PTFE tubing 0.5 mm I.D. from Rainin (Woburn, MA, U.S.A.) was slipped over the end of the stainless-steel tubing. The exit for the wash loop at valve port 8 and the exit for the sample loop at valve port 5 were installed with a piece of 0.019 in. I.D. × 1/32 in. O.D. stainless-steel tubing and waste lines made from PTFE tubing 0.5 mm I.D. (Rainin) were slipped over the end of the stainless-steel tubing.

*Standards*

A standard mixture of chlorinated pesticides, prepared in hexane and isooctane, was used to evaluate the ultra-low volume rotary valve as an on-column injection device for capillary chromatography (see Table I).

*Chromatographic conditions*

*Capillary chromatography.* Temperature programming with rates of 5°C/min and 15°C/min for the column oven and 80°C and 120°C column oven starting temperatures, was used in determining the operating parameters of the system. Temperature programming with a rate of 15°C/min for the column oven and 120°C column oven starting temperature were used in determining the reproducibility of the valve with a hexane solution of standard mixture, an isoocatane solution of standard mixture and sample extracts in hexane. A 1-min hold at the start and a 26-min hold at the final temperature of 220°C were used with all temperature programs. The column injector block was maintained at 240°C and the electron-capture detector block was maintained at 300°C for all tests. The attenuation of the electron-capture detector electrometer was set at $64 \times 10^{-11}$ A f.s. for all of the tests and the make-up gas was 10% methane in argon, flow-rate 35 ml/min. A 30 m × 0.32 mm I.D. Durabond-5 fused-silica column was used with helium as the carrier gas at a flow-rate of

TABLE I

STANDARDS

| *Compound* | *Peak Number* | *Concentration (μg/ml)* |
|---|---|---|
| *Standard mixture 1* | | |
| α-BHC | 1 | 0.003 |
| γ-BHC | 2 | 0.005 |
| Heptachlor | 3 | 0.005 |
| Chlorpyrifos | 4 | 0.015 |
| Heptachlor epoxide | 5 | 0.010 |
| *cis*-Chlordane | 6 | 0.010 |
| Dieldrin | 7 | 0.015 |
| Endrin | 8 | 0.015 |
| *Standard mixture 2* | | |
| Hexachlorobenzene | 1 | 0.003 |
| *trans*-Chlordane | 2 | 0.010 |
| *trans*-Nonachlor | 3 | 0.010 |
| *p,p*-DDT | 4 | 0.030 |
| *p,p*-Methoxychlor | 5 | 0.100 |
| *Standard mixture 3* | | |
| Pentachlorobenzene | 1 | 0.002 |
| Pentachlorophenyl methyl ether | 2 | 0.002 |
| Quintozene | 3 | 0.004 |
| Pentachloroaniline | 4 | 0.005 |
| Pentachlorphenyl methyl sulfide | 5 | 0.004 |
| Octachlor epoxide | 6 | 0.010 |
| *p,p*-DDE | 7 | 0.015 |
| *p,p*-TDE | 8 | 0.020 |

54 cm/sec. A Spectra-Physics SP4270 electronic integrator was used to determine the areas of the peaks.

*Packed-column chromatography.* A 4 mm I.D. × 1/4 in. O.D. × 6 ft. glass column, packed with 5% OV-101 on Chromosorb WHP (80–100 mesh) was used with 5% methane in argon as the carrier gas at a flow-rate of 72 ml/min. The column oven temperature was maintained at 200°C. The column oven injector block was maintained at 230°C, and the electron-capture detector block was maintained at 350°C for all tests. The electron-capture detector electrometer attenuation was set at $1.8 \times 10^{-10}$ A f.s. Peak heights were used in quantitating the packed column results.

*Procedure*

The wash loop was filled with pure solvent while the sample loop was filled with a standard mixture. A 50-$\mu$l and a 25-$\mu$l syringe were used to fill the wash and sample loops, respectively. The loops were filled until all air bubbles were expelled. The valve was then quickly rotated to the inject position. The data system and temperature program were started. The valve was left in the inject position until the analysis was finished. No baseline disturbance was noticed when the valve was rotated to the inject position.

## RESULTS AND DISCUSSION

The injector extension was used so as to provide a heated zone 3 mm from the body of the valve which closely duplicates the conditions used by Steele and Vassilaros[5]. This valve is different from the one used by them in that external loops of different size can be mounted in series and do not have to be cooled before each injection. The ten-port valve was initially installed with a 1-$\mu$l wash loop and a 2-$\mu$l sample loop. Both were made from 0.12 mm I.D. fused-silica needle material and installed on the valve as shown in Fig. 2. This arrangement of the loops allows one loop to wash and flush the contents of the other loop through the heated injector zone into the column. There is less than 0.2 $\mu$l dead volume between the wash and sample loops.

The series wash and sample loops used in this rotary valve on-column injection technique resemble the solvent plug injection technique used for packed column chromatography. This technique uses a solvent plug to wash and flush the sample plug out of the syringe and into the heated injector zone of the packed column. Chromatography with this on-column injection system was optimized to allow a wide range of chlorinated pesticides, prepared in solvents such as hexane and isooctane, to be injected so that good peak separation and peak symmetry and reasonable elution times could be obtained.

Solvents such as hexane and isooctane were chosen because these are the primary solvents used in our laboratory for the samples and standards respectively in the analysis for pesticide residues. Light petroleum (boiling range 30–60°C) and diethyl ether are also used in our laboratory but could not be evaluated with this particular system because the ambient temperature of the valve body was 36°C. This is above the boiling point of the solvents and volatilization of these solvents in the loops would produce bubbles, thus preventing quantitative transfer from the loops.

The following chromatographic conditions for this system were found to be

optimal. The injection port temperature had to be above 200°C because peaks broadened as the temperature dropped below 200°C. An injector temperature of 240°C gave good results while temperatures as high as 280°C did not appear to improve the chromatograms. The temperature of the injector extension was found to be 140°C when the injector block temperature was maintained at 240°C. The initial column oven temperature of programmed gas chromatography had to be above the boiling point of the highest-boiling solvent injected, otherwise, the leading edge of the chlorinated pesticides investigated was distorted. Such an effect has been previously reported[6]. An initial column oven temperature of 120°C gave good results for our tests. These parameters would need to be validated for other compounds and solvents.

Tables II and III show reproducibility results obtained by using fused-silica loops and a column attached directly to the valve. Each standard was injected with a 2-μl sample loop and a 1-μl wash loop filled with the solvent used for the standard.

Quantitative transfer of all the sample from the loop to the column could not be achieved until the wash loop was employed. The percent of each pesticide left in the loop ranged from 4.2% for α-1,2,3,4,5,6-hexachlorocyclohexane (α-BHC) to 7.2% for dieldrin when the wash loop was not employed.

The results in Table II show that good reproducibility was achieved with six consecutive injections of standards in either isooctane or hexane with an extension on the injector at 240°C. The relative standard deviation (R.S.D.) between each solvent for each compound was also good which indicated that solvents such as hexane and isooctane could be used interchangeably.

Six consecutive injections of a standard mixture in hexane were made with the extended injector and the regular injector. The R.S.D. of the compounds for each injector at 240°C are shown in Table III. The R.S.D. was good for each injector type. This indicates that the extended injector is not needed for good reproducibility. Later

TABLE II

REPRODUCIBILITY OF SIX CONSECUTIVE INJECTIONS OF STANDARDS IN HEXANE AND ISOOCTANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Fused-silica loops. Isooctane = standard (2 μl) and wash (1 μl) in isooctane. Hexane = standard (2 μl) and wash (1 μl) in hexane. Average peak area calculated for six consecutive injections.

| *Compound* | *Isooctane* | | | *Hexane* | | |
|---|---|---|---|---|---|---|
| | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* |
| α-BHC | 160444 | 1428 | 0.89 | 151906 | 862 | 0.56 |
| γ-BHC | 231724 | 1544 | 0.67 | 222579 | 4318 | 1.94 |
| Heptachlor | 214463 | 2686 | 1.25 | 202205 | 1041 | 0.52 |
| Chlorpyrifos | 221682 | 2214 | 0.99 | 223529 | 2176 | 0.97 |
| Heptachlor epoxide | 405612 | 4763 | 1.17 | 388709 | 1454 | 0.37 |
| *cis*-Chlordane | 408908 | 4480 | 1.09 | 394177 | 2087 | 0.52 |
| Dieldrin | 619823 | 6992 | 1.12 | 595240 | 2380 | 0.40 |
| Endrin | 430007 | 5545 | 1.28 | 406876 | 1242 | 0.30 |

TABLE III

REPRODUCIBILITY OF SIX CONSECUTIVE INJECTIONS WITH STANDARDS IN HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Fused-silica loops. Standard (2 μl) and wash (1 μl) in hexane. Average peak area calculated for six consecutive injections.

| *Compound* | *Extended injector* | | | *Regular injector* | | |
|---|---|---|---|---|---|---|
| | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* |
| α-BHC | 258161 | 2620 | 1.01 | 186033 | 3562 | 1.91 |
| γ-BHC | 376518 | 2958 | 0.78 | 262978 | 2641 | 1.00 |
| Heptachlor | 340406 | 5153 | 1.51 | 241259 | 4166 | 1.70 |
| Chlorpyrifos | 317301 | 6353 | 2.00 | 260295 | 1991 | 0.76 |
| Heptachlor epoxide | 667038 | 7239 | 1.08 | 458010 | 6239 | 1.36 |
| *cis*-Chlordane | 661219 | 7177 | 1.08 | 462052 | 4788 | 1.04 |
| Dieldrin | 1030891 | 10593 | 1.02 | 716312 | 8171 | 1.14 |
| Endrin | 702334 | 7185 | 1.02 | 508762 | 6067 | 1.19 |

results show that isooctane can also be injected without the injector extension and comparable results can also be obtained for isooctane. The results in Tables II and III have shown that reproducible injections of standards in hexane and isooctane can be made which would allow good quantitation of chlorinated pesticides. Cross-contamination between injections was observed when a cleaned-up sample extract of jelly beans containing chlorinated pesticide residues was injected into the system, along with a standard, for quantitation. An unidentified incurred residue in the samples showed up in the standard injected after the sample. The unidentified incurred residue also showed up in several chromatograms following successive solvent injections, where each one was less than the first. This problem had not been encountered in the initial tests.

The cross-contamination between injections was obviated by using 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing in place of the fused-silica needle material used for the external loops and using a butt connector (Fig. 3) to join the 0.31-mm I.D. fused-silica column to a piece of 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing attached to the valve (Fig. 2). The end of the stainless-steel tubing is machined square so that a zero dead-volume fit is created between the valve and the fused-silica column. The butt connector was placed between the valve body and the top of the injector. This did not allow the extension of the injector to be used, but, based on data taken early, it probably was not needed. It is very important that the inside of the butt connector ferrule be made square with the end of the machined stainless-steel tubing so there is no dead volume in the connection. The dead volume in the ferrule is created by the bullet shaped end of the drill bit but can be removed by pushing the ferrule and a machined piece of stainless-steel tubing together hard and twisting back and forth. A 2-μl wash loop in conjunction with a 1-μl sample loop was found to give no cross-contamination between injections when a standard twenty times more concentrated than the standard normally used was injected. No peaks

TABLE IV

REPRODUCIBILITY OF FIVE CONSECUTIVE INJECTIONS OF STANDARDS IN ISOOCTANE AND HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Stainless-steel loops. Standards (1 μl) in hexane and isooctane, wash (2 μl) in hexane. Average peak area calculated for five consecutive injections.

| *Compound* | *Isooctane* | | | *Hexane* | | |
|---|---|---|---|---|---|---|
| | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* |
| α-BHC | 172902 | 2130 | 1.23 | 180460 | 1517 | 0.84 |
| γ-BHC | 242434 | 3625 | 1.49 | 246300 | 2782 | 1.12 |
| Heptachlor | 220718 | 1281 | 0.58 | 225449 | 3039 | 1.34 |
| Chlorpyrifos | 261171 | 1901 | 0.72 | 270130 | 853 | 0.32 |
| Heptachlor epoxide | 406609 | 4890 | 1.20 | 443283 | 5773 | 1.30 |
| *cis*-Chlordane | 417932 | 4239 | 1.01 | 444158 | 4698 | 1.06 |
| Dieldrin | 656511 | 6466 | 0.98 | 700404 | 8149 | 1.16 |
| Endrin | 465617 | 3527 | 0.76 | 489314 | 5840 | 1.19 |

were found in the subsequent solvent chromatogram. The connection between the column and valve body was found to be the greatest contributor to the cross-contamination problem. The same cross-contamination problem was observed if the inside of the butt connector ferrule was not made square with the end of a piece of machined stainless-steel tubing and if the holes drilled in the ferrule were too big and allowed the stainless-steel tubing and column to fit too loosely.

Tables IV, V, and VI show reproducibility results obtained by using 0.005 in. I.D. × 1/32 in. O.D. stainless-steel loops and a butt connector which was used to

TABLE V

REPRODUCIBILITY OF EIGHT CONSECUTIVE ALTERNATING INJECTIONS OF FOUR EACH FOR STANDARDS IN ISOOCTANE AND HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Stainless-steel loops. Standards (1 μl) in hexane (4) and isooctane (4), wash (2 μl) in hexane. Average peak area calculated for eight consecutive injections.

| *Compound* | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* |
|---|---|---|---|
| α-BHC | 189178 | 1983 | 1.04 |
| γ-BHC | 270079 | 6221 | 2.30 |
| Heptachlor | 167501 | 3088 | 1.84 |
| Chlorpyrifos | 200123 | 6013 | 3.00 |
| Heptachlor epoxide | 458661 | 6214 | 1.35 |
| *cis*-Chlordane | 458924 | 5471 | 1.19 |
| Dieldrin | 715330 | 12038 | 1.68 |
| Endrin | 504535 | 5788 | 1.14 |

TABLE VI

REPRODUCIBILITY OF FIVE CONSECUTIVE INJECTIONS WITH STANDARDS IN HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Stainless-steel loops. Standards (1 μl) and wash (2 μl) in hexane. Average peak area calculated for five consecutive injections.

| *Compound* | *Absolute average peak area* | *S.D.* | *R.S.D. (%)* |
|---|---|---|---|
| Pentachlorobenzene | 80235 | 1271 | 1.58 |
| Pentachlorophenyl methyl ether | 162421 | 4147 | 2.55 |
| Quintozene | 185593 | 3973 | 2.14 |
| Pentachloroaniline | 161304 | 1965 | 1.21 |
| Pentachlorophenyl methyl sulfide | 229321 | 6836 | 2.98 |
| Octachlor epoxide | 349553 | 5889 | 1.68 |
| *p,p*-DDE | 637155 | 9424 | 1.47 |
| *p,p*-TDE | 612232 | 11530 | 1.88 |
| Hexachlorobenzene | 187337 | 2090 | 1.11 |
| *trans*-Chlordane | 528171 | 8396 | 1.58 |
| *trans*-Nonachlor | 483736 | 7743 | 1.60 |
| *p,p*-DDT | 1119599 | 6105 | 0.54 |
| *p,p*-Methoxychlor | 1812932 | 15914 | 0.87 |

attach the column to a piece of 0.005 in. × 1/32 in. stainless-steel tubing attached to the valve. Each standard was injected with a 1-μl sample loop and 2-μl wash loop filled with hexane. Results in Table IV show that good reproducibility between five consecutive injections of standards in isooctane and hexane was achieved. The R.S.D. between each solvent for each compound were good. Results in Table V show that good reproducibility was achieved with eight consecutive injections of standards in hexane and isooctane. The injections were alternated so every other standard was in

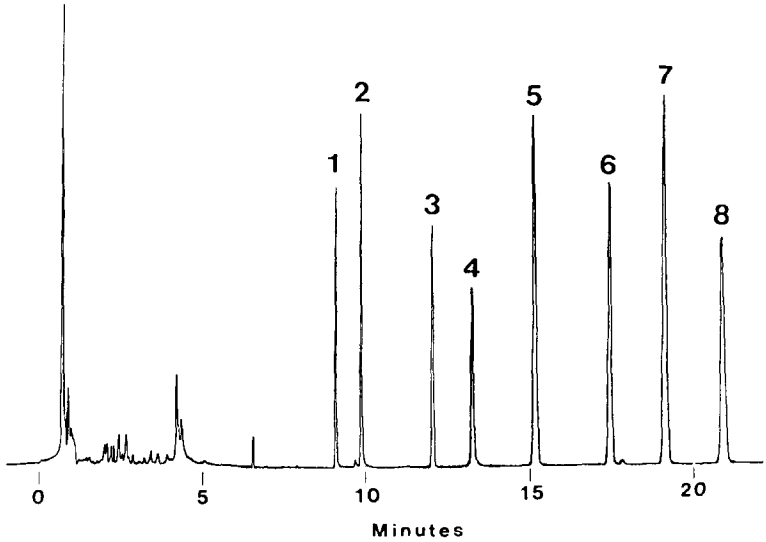


Fig. 4. Chromatogram of standard mixture 1 with peak numbers and concentrations as in Table I.

a different solvent. This shows that samples in hexane and standards in isooctane could be injected without change in response. This is important because the standards in our laboratory are diluted with isooctane. Further dilutions of any standard with hexane will still contain a small percentage of isooctane.

Five consecutive injections of thirteen other chlorinated pesticides gave good reproducibility, as shown in Table VI. The results shown in Tables IV, V, and VI demonstrate that a wide range of chlorinated pesticides in hexane or isooctane can be injected with good reproducibility using a rotary valve on-column injection technique. Typical chromatograms of twenty one chlorinated pesticides injected with the rotary valve on-column injection technique are shown in Figs. 4–6. Chromatographic conditions given above were used to chromatograph 1 $\mu$l of standard mixtures 1–3 (see Table I).

*Analytical applications*

Samples of creamy peanut butter and candied sweet potatoes were analyzed for pesticides in our laboratory by using packed column chromatography and the capillary rotary valve on-column injection technique described above. The creamy peanut butter and candied sweet potatoes were cleaned up by the methods described in the *Pesticides Analytical Manual* (PAM)[6] and by gel permeation chromatography (GPC)[7].

The peanut butter fat was extracted according to Section 211.13c, of PAM *i.e.* triple extraction with 100 ml of 50% ethyl ether in light petroleum. SX-3 Gel beads were used as column material in GPC and 50% (v/v) methylene chloride in hexane as the solvent to separate the pesticide residues form the extracted peanut butter fat. Eluate A described in Section 252.13 (Alternate Florisil Elution System) consisted of 20% (v/v) methylene chloride in hexane.

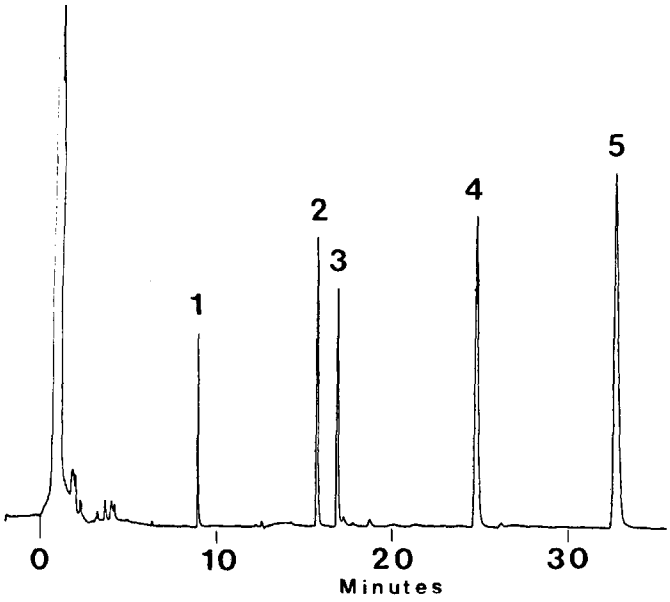


Fig. 5. Chromatogram of standard mixture 2 with peak numbers and concentrations as in Table I.

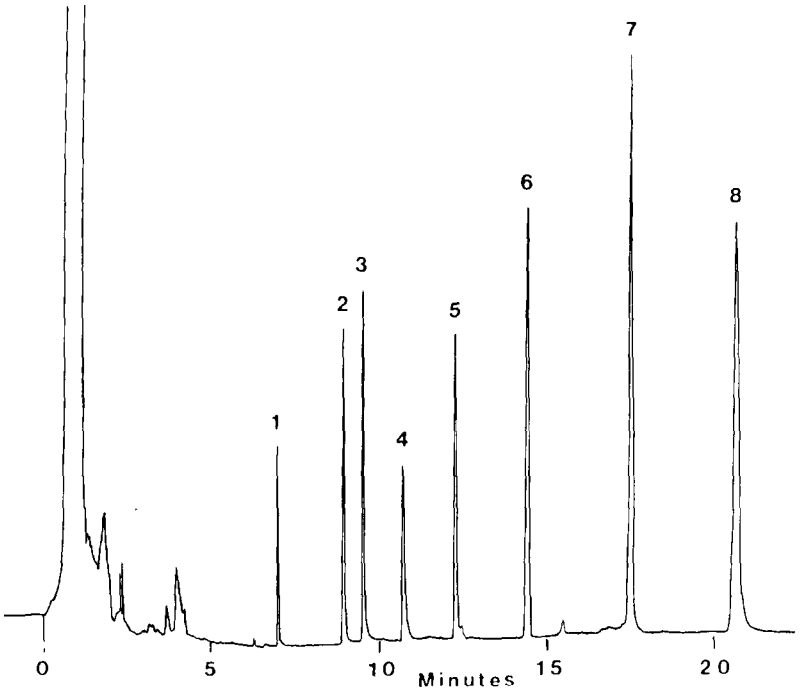


Fig. 6. Chromatogram of standard mixture 3 with peak numbers and concentrations as in Table I.

The candied sweet potatoes were extracted according to section 212.13b of the PAM, *i.e.* water–acetonitrile extraction. The "6%" eluate" of section 211.14d (Florisil Column) consisted of 6% (v/v) diethyl ether in light petroleum. Residues in eluate A, which represents 10 g/ml of the creamy peanut butter and in the "6% eluate", which represents 17.65 g/ml of the candied sweet potatoes were quantitated by packed-column chromatography with single 5-$\mu$l injections of each sample and standard. Peak heights were used to quantitate sample residues as usual in our laboratory.

Three 1-$\mu$l injections of eluate A, of the creamy peanut butter diluted to 1 g/ml and four 1-$\mu$l injections of the "6% eluate", of the candied sweet potatoes diluted to 8.82 g/ml, were injected into the capillary system along with standards for quantitation. The parameters for the packed and capillary systems are shown above (*Chromatographic conditions*). The reproducibility of six alternating injections (three each) of residues in the creamy peanut butter extract and standards was good, as shown in Table VII.

Table VIII shows that the results from the packed column and capillary system for three residues of chlorinated pesticides in the creamy peanut butter at the 0.002 ppm, 0.004 ppm, and 0.008 ppm level were comparable. A 1-mg equivalent of sample matrix was introduced into the system per injection and the elapsed time for the test was approximately 5 h.

Results in Table IX show that the reproducibility of alternating injections (four each) of residues in the candied sweet potatoes and standard was good. The reproducibility between the four sample injections was somewhat higher than the standard

TABLE VII

SIX CONSECUTIVE ALTERNATING INJECTIONS OF THREE EACH FOR SAMPLE AND STANDARD IN HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold 1 min, program rate = 15°C/min. Stainless-steel loops. Standard and sample (1 μl) in hexane; wash (2 μl) in hexane. Creamy peanut butter, 1 g/ml.

| *Compound* | *Reproducibility based on* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Sample residues (μg/g)** | | | *Absolute areas*** | | | | | |
| | *Average* | *S.D.* | *R.S.D. (%)* | *Sample* | | | *Standard* | | |
| | | | | *Average* | *S.D.* | *R.S.D. (%)* | *Average* | *S.D.* | *R.S.D. (%)* |
| Pentachlorophenyl methyl ether | 0.0024 | 0.000026 | 1.10 | 217592 | 4117 | 1.89 | 181620 | 1396 | 0.77 |
| Quintozene | 0.0037 | 0.000127 | 3.40 | 182811 | 5814 | 3.18 | 196663 | 4915 | 2.50 |
| Pentachloro-aniline | 0.0066 | 0.000256 | 3.80 | 261384 | 8664 | 3.32 | 195963 | 3832 | 1.95 |

* Three calculations.
** Three injections.

and is believed to be the result of the noisier baseline associated with each sample injection. An 8.8-mg equivalent of sample matrix per injection was introduced into the system and the elapsed time of the test was approximately 7 h.

Table VIII shows that the results from the packed column and capillary system for two incurred residues of chlorinated pesticides in the candied sweet potatoes at the 0.0005 μg/g and 0.0002 μg/g level were not very close. This is probably due to the fact that the lower limit of quantitation using this packed column system had been reached thus introducing a greater degree of variability in the results. The re-

TABLE VIII

RESIDUE VALUES DETERMINED BY PACKED AND CAPILLARY COLUMN CHROMATOGRAPHY

| *Compound* | *Packed column* | | *Capillary column* | |
|---|---|---|---|---|
| | *Creamy peanut butter** | *Candied sweet potatoes** | *Creamy peanut butter*** | *Candied sweet potatoes**** |
| Pentachlorophenyl methyl ether | 0.002 | – | 0.0024 | – |
| Quintozene | 0.004 | – | 0.0037 | – |
| Pentachloroaniline | 0.008 | – | 0.0066 | – |
| Octachlor epoxide | – | 0.0005 | – | 0.00072 |
| *p,p*-DDE | – | 0.0002 | – | 0.00046 |

* One determination.
** Average of 3 determinations.
*** Average of 4 determinations.

TABLE IX

EIGHT CONSECUTIVE ALTERNATING INJECTIONS OF FOUR EACH FOR SAMPLE AND STANDARD IN HEXANE

Initial column temperature: 120°C, programmed to 220°C after initial hold of 1 min, program rate = 15°C/min. Stainless-steel loops. Standard and sample (1 μl) in hexane; wash (2 μl) in hexane. Candied sweet potatoes, 8.825 g/ml.

| *Compound* | *Reproducibility based on* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Sample residues (μg/g)** | | | *Absolute areas*** | | | | | |
| | *Average* | *S.D.* | *R.S.D. (%)* | *Sample* | | | *Standard* | | |
| | | | | *Average* | *S.D.* | *R.S.D. (%)* | *Average* | *S.D.* | *R.S.D. (%)* |
| Octachlor epoxide | 0.00072 | 0.000043 | 5.98 | 245561 | 16061 | 6.50 | 385813 | 6324 | 1.63 |
| *p,p*-DDE | 0.00046 | 0.000020 | 4.32 | 184080 | 9715 | 5.20 | 673324 | 14371 | 2.13 |

* Four calculations.
** Four injections.

producibility between the standard injections in both sample runs is good and facilitates the use of a computing integrator and data system.

A final test for the percentage of pesticides left in the sample loop and 0.005 in. I.D. × 1/32 in. O.D. stainless-steel tubing between valve body and column after a normal analysis was made. Leaving the wash loop empty and the sample loop filled with 1 μl of a standard mixture an injection was made into the capillary column. The standard was twenty times more concentrated than standard mixture 1. The valve was left in the Run position and fresh solvent was flushed through the sample inlet line. The valve was switched to Load position after the programmed analysis was finished and the 2-μl wash loop was filled with hexane and injected. The percentage of pesticides left in the sample loop and tubing between valve and fused-silica column ranged from 7.16% for α-BHC to 16.11% for endrin. The above procedure was repeated except this time the sample loop was rinsed with fresh solvent before a solvent injection was made. The percentage of pesticides left in the stainless-steel tubing from the valve to the column ranged from 3.9% for Heptachlor epoxide to 8% for endrin. This further indicates that a solvent wash is needed to insure complete transfer of sample to the column when this on-column capillary injection system is used.

This technique will allow on-column injection without the use of long fine needles or septums. The tests were performed manually, but they can be automated with commercially available equipment. The R.S.D. between injections with the ultra-low volume rotary valve for the chlorinated pesticides in hexane are comparable to results obtainable when the same compounds are injected into a packed column. The amount injected on this capillary system using the rotary valve is 50 times less than what is normally injected for quantitation on packed column instruments in our laboratory.

## CONCLUSION

Our sample introduction technique has demonstrated that on-column capillary injections can be achieved with good reproducibility and without the problems commonly associated with a fine fused-silica or stainless steel needle on-column capillary injection techniques. Further validation and automation of this system is currently being investigated.

## REFERENCES

1 F.-S. Wang, H. Shangfield and A. Zlatkis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 562.
2 K. Grob and K. Grob, Jr., *J. Chromatogr.*, 151 (1978) 311.
3 E. Geeraert, D. de Schepper and P. Samder, *J. High Resolut. Chromatogr. Chromatogr. Commun.*,6 (1983) 386.
4 J. V. Hinshaw, Jr. and F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 555.
5 D. H. Steele and D. L. Vassilaros, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 561.
6 B. M. McMahon and L. D. Sawyer (Editors), *Pesticides Analytical Manual, Vol. 1*, Food and Drug Administration, Washington, DC, 1978.
7 M. L. Hopper, *J. Agr. Food Chem.*, 30 (1982) 1038.