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Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water

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SUMMARY

An automated reversed-phase high-performance liquid chromatographic (HPLC) method has been developed for the determination of trace concentrations of propoxur, carbofuran, carbaryl, propham, captan, chloropropham, barban and butylate in drinking water. A 100-ml of sample water is passed through a 3-cm precolumn, packed with 5-μm ODS sorbent, at a flow-rate of 5 ml/min. The HPLC system is then switched to an acetonitrile–water gradient elution program. The analytes, which are concentrated on the precolumn, are eluted and separated on a 25-cm C_8 analytical column and determined by measuring the UV absorption at 220 nm. The resolution of analytes is excellent regardless of whether the elution from the precolumn is done unidirectionally or with backflushing. The precolumn can be used repeatedly for at least 30 samples without a significant decrease in efficiency. The total analytical time is 60 min. Tap, distilled, deionized, commercial spring and HPLC-grade waters were analyzed. The lowest detectable concentrations are in the range of $10 \cdot 10^{-12}$–$460 \cdot 10^{-12}$ g/ml for the eight pesticides with 100 ml of sample.

INTRODUCTION

In recent years, for the determination of trace amounts of organic pollutants in water, much attention has been focused on sample preconcentration techniques involving the use of a solid sorbent phase, as opposed to conventional liquid–liquid extraction techniques[1,2]. The use of a solid sorbent should, in theory, result in a more effecient recovery of analyte and better reproducibility between replicate sample ex-

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tractions. Both the amount of organic solvent and the period of time needed for the procedure are greatly reduced. The evaporation procedure required in liquid–liquid extractions is also eliminated. Several workers have described solid phase extraction (SPE) methods for determination of selected carbamate pesticides using Waters Assoc. Sep-Pak cartridges in conjunction with reserved-phase high-performance liquid chromatography[3,4]. EPA method 531[22] uses HPLC for the determination of carbamates. None of the above methods, however, is completely automated using SPE and HPLC methodologies for the determination of multiple residues of carbamate pesticides in water.

Unlike organochlorine pesticides, carbamates are difficult to determine by gas chromatography (GC) mainly owing to their thermal lability[5]. Methods involving GC have been described[6,7]. Spectrophotometric[8,9], enzymic[10,11], spectrofluorometric[12,13] and mass spectral techniques[14,15] for the determination of carbamates and their metabolic derivatives in various sample matrices have also been described. However, each of these methods has limitations making it inappropriate for the analysis of large volumes of aqueous solution containing pesticide residues at the 10^{-9} or 10^{-12} g/ml level. As a result, HPLC is generally regarded as the best technique for carbamate residue determination.

In an on-line pre-concentration method[16–19], the entire sample can be analysed quantitatively. An on-line technique also offers the possibility of constructing a totally automated HPLC system for the determination of trace amounts of organic pollutants in aqueous samples. In this paper, we report the development of an automated on-line preconcentration and determination method for eight pesticides in drinking water. The parameters investigated included (1) size of packing material used in the precolumn, (2) the rate of sample loading onto the precolumn, (3) properties of the solid sorbent phase, (4) precolumn longevity, (5) cost of operation, (6) whether backflushing of the precolumn is required, (7) type of analytical column and (8) minimum detectable concentrations. Seven of the eight pesticides were carbamate insecticides, herbicides or fungicides, chosen because they are of concern in Ontario environmental samples; the other pesticide was captan.

EXPERIMENTAL

Solvents

Acetonitrile was of HPLC grade from Fisher Scientific (Fairlawn, NJ, U.S.A.) and Caledon Labs. (Georgetown, Canada). Water used for the preparation of standards was distilled in glass in the laboratory.

Pesticides

Solid pesticide standards were obtained from the U.S. Environmental Protection Agency (EPA) (Research Triangle Park, NC, U.S.A.). Purities of the individual standards ranged from 97.5 to 100%. The pesticides, listed in the order in which they appear in the chromatograms, are (1), propoxur, (2) carbofuran, (3) carbaryl, (4) propham, (5) captan, (6) chloropropham, (7) barban and (8) butylate.

Preparation of stock standard solutions

Solid standards were dissolved in acetonitrile and diluted in acetonitrile. These individual stock standard solutions were combined at different concentrations be-

cause of varying sensitivities to UV detection. The combined standard solution thus prepared was diluted with water to give standard water samples as below.

Water samples

Standard water samples were prepared by diluting 1 ml of the combined standard solution (prepared as above) to 1000 ml with distilled water from the laboratory unless indicated otherwise. The following types of water samples were investigated: two municipal tap waters, two distilled waters, three commercial HPLC-grade waters, two commercial spring drinking waters, one reverse osmosis water and one ion-exchange water.

Apparatus

The HPLC system consisted of a Model 510 pump, a Model 501 pump, a WISP Model 710B sample processor and a Model 484 tunable absorbance UV detector (all from Waters Assoc., Milford, MA, U.S.A.), a Fisher Recordall Series 5000 strip-chart recorder and a Digital Professional 350 computer system (Digital Equipment, Maynard, MA, U.S.A.) incorporating Waters Assoc. 840 chromatography software. A Waters Assoc. Model 600 Powerline solvent-delivery system was used in additional sample loading rate experiments.

Precolumns were 5-μm Spherisorb C_{18} and C_8 3 cm $\times$ 4.6 mm I.D. cartridges from Brownlee Labs. (Santa Clara, CA, U.S.A.) and 3 cm $\times$ 4.6 mm I.D. laboratory-packed with 10-μm Vydac Reverse Phase TP-201 (Separations Group, Hesperia, CA, U.S.A.), 10-μm Ultrasil ODS (Altex Scientific, Berkeley, CA, U.S.A.) and 40-μm Co-Pell ODS (Whatman, Clifton, NJ, U.S.A.). The analytical columns were a 5-μm Supelcosil LC-8 (25 cm $\times$ 4.6 mm I.D.) (Supelco, Bellefonte, PA, U.S.A.) and a 5-μm Spherisorb C_{18} (15 cm $\times$ 4.6 mm I.D.) (Phenomenex, Torrance, CA, U.S.A.).

The on-line preconcentration apparatus (Fig. 1) incorporated two high-pressure in-line filters with 0.5-μm frits from Mandel Scientific (Guelph, Canada) and three Rheodyne (Cotati, CA, U.S.A.) Model 7000 two-position six-port switching valves, one of which was equipped with a Model 5701 air actuator controlled by a Model 7163 solenoid valve kit.

Operating conditions

The following conditions were used: wavelength, 220 nm; flow-rate, 1.5 ml/min; chart speed, 0.5 cm/min; detector sensitivity, 0.075 a.u.f.s. (1 mV = $1 \cdot 10^{-3}$ absorbance); recorder range, 10 mV full-scale and column temperature, ambient.

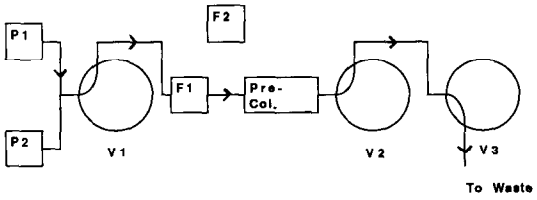
On-line preconcentration

A 100-ml volume of water sample was passed through the precolumn while the apparatus was in the 'load' position.

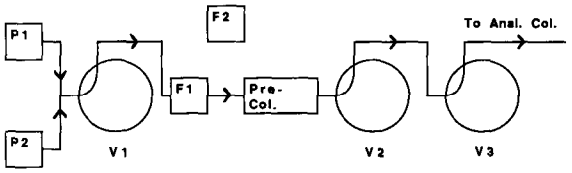
Elution

The following gradient program was run after switching the valves to the 'elute' position from the 'load' position, with elapsed time and composition of the acetonitrile–water mobile phase: initial, 30:70; 5 min, 30:70; 15 min, 60:40; 25 min, 60:40; 30 min, 30:70 and 35 min, 30:70. Changes in the percentage of acetonitrile throughout the gradient program occurred linearly. The final 10 min of the gradient program serve to return the system to the initial conditions to permit another analysis run.

SAMPLE LOAD



UNIDIRECTIONAL ELUTION



BACKFLUSH ELUTION

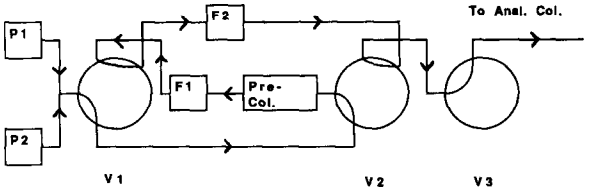


Fig. 1. Schematic diagram of the valve-switching system and the directions of liquid flow. V, P and F denote valves, pumps and filters, respectively; Anal. Col. = analytical column; Pre-Col. = precolumn. During the sample loading step, P1 dispenses sample. During the elution steps, P1 dispenses water and P2 dispenses acetonitrile as part of the mobile phase.

RESULTS AND DISCUSSION

The valving system employed in the on-line pre-concentration apparatus (Fig. 1) allows the application of the mobile phase to the precolumn in the same direction in which the sample was loaded (unindirectional elution), or in a direction opposite to that in which the sample was loaded (backflush elution). This makes the system more versatile than those employing only one high-pressure valve. Our studies revealed close similarities between unidirectional and backflush elutions. This is in sharp contrast to results obtained by straight injection of a concentrated stock solution (1000 times the concentration of the standard water samples used for this study) into the HPLC system (Fig. 2). The peak heights and shapes of the earlier eluting analytes are

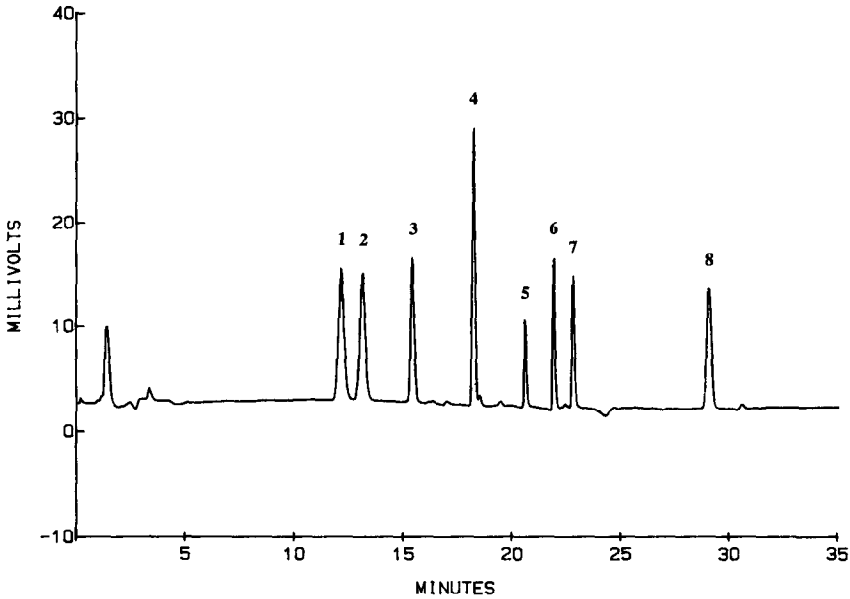


Fig. 2. Chromatogram resulting from a direct 150-μl injection without the preconcentration procedure of a concentrated stock solution prepared in acetonitrile–water (30:70). Peak numbers as in Table I.

very different to those from the preconcentration sample runs. However, no difference was found in the average peak area counts by the two methods. Adsorption on and elution from the precolumn increases the peak sharpness for propoxur and carbofuran although the relative retention times for the two compounds are shifted closer together. A C_8 sorbent packing in the precolumn was also investigated as an alternative to the C_{18} packing. In contrast to the results obtained with the C_{18} packing, backflush elution improved the resolution between propoxur and carbofuran (Fig. 3). No difference was found in the average peak area counts when using the C_{18} or C_8 sorbent. In the developed method, a C_{18} sorbent was used and unidirectional elution was employed, as backflush elution offered no advantage when using a C_{18} sorbent. A C_{18} analytical column was also investigated but no improvement was found in the separation of the eight pesticides.

Table I gives the retention times of the eight pesticides, peak-area counts with their relative standard deviations (R.S.D.) from five replicate measurements at the concentrations listed and the minimum detectable concentrations. The minimum detectable concentrations were calculated based on a 100-ml sample using a 3:1 ratio of signal to baseline noise. The detection limit can be influenced by the number and concentration of co-eluting impurities in the sample matrix. The minimum detectable amounts can vary depending on the sample volume and concentration.

Fig. 4A shows a chromatogram for a distilled water sample with low concentration (one tenth of the concentrations listed in the sample concentration column in Table I) of the eight pesticides. At these concentrations, the peaks from the impurities in the sample matrix are roughly equal in height and area to those of the sample peaks. The reproducibility of the method is excellent, as evidenced by an average R.S.D. of *ca.* 2% for all of the compounds of interest. Background subtraction (as

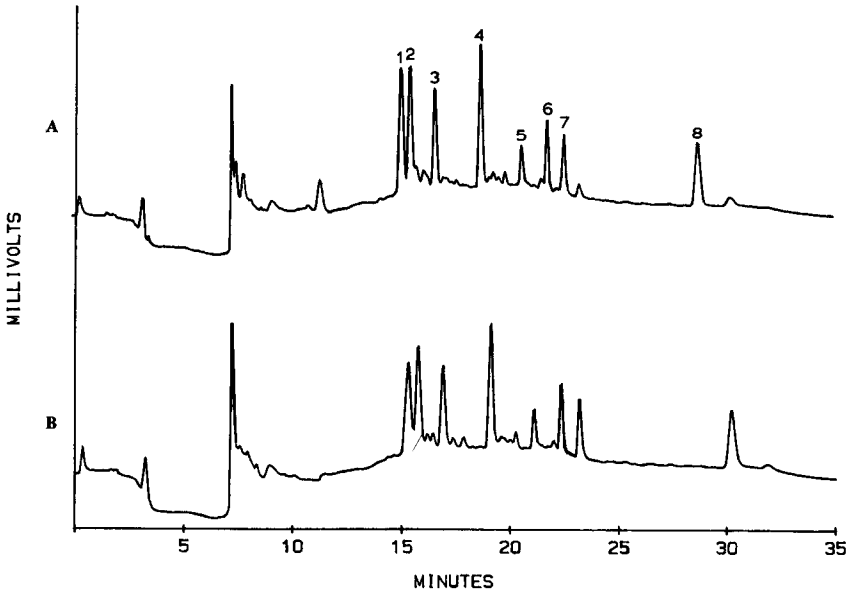


Fig. 3. Chromatograms showing the effect of (A) backflush elution and (B) unidirectional elution for a 5-μm C_8 precolumn. Each chromatogram is plotted at 90 mV. Peak numbers as in Table I.

shown in Fig. 4) would prove to be a valuable asset in the determination of analytes, but the absence of a blank for field samples makes this impossible. Baseline correction (gradient subtraction) could be used to improve the chromatogram profile but a solvent blank does not accurately duplicate the conditions to which the precolumn has been subjected.

Separation of the analytes when using a 40-μm sorbent is good but the peak areas for the first three eluting compounds (propoxur, carbofuran and carbaryl) are

TABLE I

SELECTED PESTICIDES, THEIR RETENTION TIMES, AVERAGE PEAK-AREA COUNTS ± R.S.D. FROM FIVE REPLICATE MEASUREMENTS USING A 5-μm PRECOLUMN, SAMPLE CONCENTRATIONS AND MINIMUM DETECTABLE CONCENTRATIONS FOR A 100-ml SAMPLE

No.[a]	*Compound*	*Retention time (min)*	*Peak area ($\times 10^3$)*	*Sample (10^{-9} g/ml)*	*Minimum detectable (10^{-12} g/ml)*
1	Propuxur	16.00	598 ± 3	3.84	65
2	Carbofuran	16.40	597 ± 24	4.35	70
3	Carbaryl	17.35	377 ± 7	0.42	10
4	Propham	19.25	580 ± 12	3.17	50
5	Captan	21.10	164 ± 6	9.70	460
6	Chloropropham	22.25	242 ± 6	0.98	30
7	Barban	23.00	252 ± 5	1.08	40
8	Butylate	29.40	428 ± 10	4.07	150

[a] The pesticides are numbered to coincide with those in the figures.

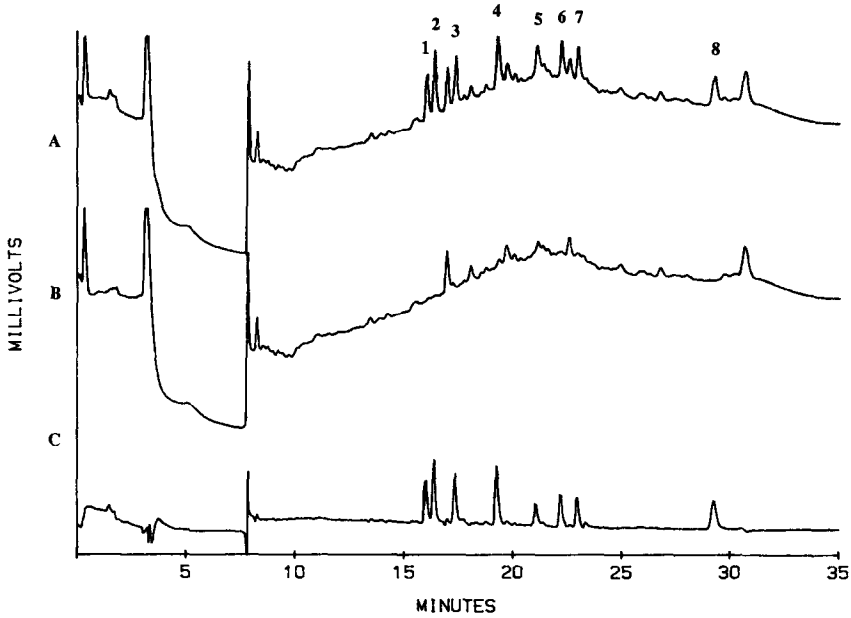


Fig. 4. Chromatograms corresponding to (A) a standard sample, (B) a distilled water blank and (C) chromatogram of A after subtracting B as a background. The samples were preconcentrated on a 10-μm Ultrasil ODS precolumn. The concentrations of the pesticides are one tenth those listed in Table I. Each chromatogram is plotted at 15 mV. Peak numbers as in Table I.

not as large as those of the 5-μm material. The peak areas with the 10-μm Ultrasil ODS sorbent were as large as those with the 5-μm sorbent except for propoxur and propham, whose peak areas were 65% and 75%, respectively, of those obtained with the 5-μm sorbent. The 5-μm material shows excellent retention of all eight analytes. From these results, it was concluded that the use of the 5-μm sorbent was most appropriate as no breakthrough of the early eluting pesticides was observed.

The results of the investigation of sample loading rate indicated no variation of retention of analytes under practical conditions. The flow-rates through the precolumns were increased in 1 ml/min increments from 3 to 6 ml/min for the 5-μm packing and from 3 to 7 ml/min for the 10-μm packing. In both instances the flow-rate did not significantly affect the retention of analytes by the precolumn. Investigation of higher flow-rates using the Model 501 single-head pump to determine the point of sample breakthrough was not possible owing to restrictions imposed by the high column back-pressures at flow-rates exceeding 6 ml/min for the 5-μm packing and 7 ml/min for the 10-μm packing. However, experiments with a dual-head pump (Model 600 Powerline) and a 5-μm precolumn showed that sample loading rates of 10 ml/min can be attained without breakthrough of any of the analytes. This result is in good agreement with those obtained by Goewie *et al.*[20]. With the Model 501 pump, we judged that a sample loading rate of 5 ml/min with the 5-μm packing was most appropriate. Under these conditions, the total sample loading time for 100 ml is 20 min, which is adequate considering the subsequent 35-min chromatographic step. A completely automated procedure (including sample loading and analysis) takes approximately 60

min with the developed method. If the concentrations of carbamate residues are at least ten times those investigated in our study, or the detection limits achieved by conventional liquid–liquid extraction techniques are acceptable, then a sample size of only 5–10 ml is needed and the analysis time becomes much shorter. It is important to note that the sensitivity of the developed method when using 100 ml of sample is at least ten times greater than that of the method currently in use[21].

The lifetime of the precolumn is an important economic consideration when making a choice between an SPE or an on-line preconcentration technique. Commercially available SPE cartridges are substantially cheaper (Canadian $1.50–2.50) than 5-$\mu$m precolumns (Canadian $60). However, in our study, one 5-μm precolumn stood up well to the analysis of at least thirty 100-ml water samples without showing any noticeable deterioration. The extent of deterioration was assessed by monitoring the resolution between propoxur and carbofuran and by monitoring the tailing of all analyte peaks. We judged that on a cost per analysis basis, the technique can compete with a method employing commercial SPE cartridges. The precolumn could be used substantially longer if only 10 ml or less of sample is used. The cost of operation of the method then becomes much lower.

The method was applied to the analysis of several kinds of water samples. Chromatograms of these water samples are shown in Fig. 5. Each water sample showed several peaks but they are all essentially unique for individual samples. The tap water samples are prone to huge peaks early in the chromatogram from early eluting impurities but the baseline is sufficiently stable after 15 min to allow for accurate determination of analytes.

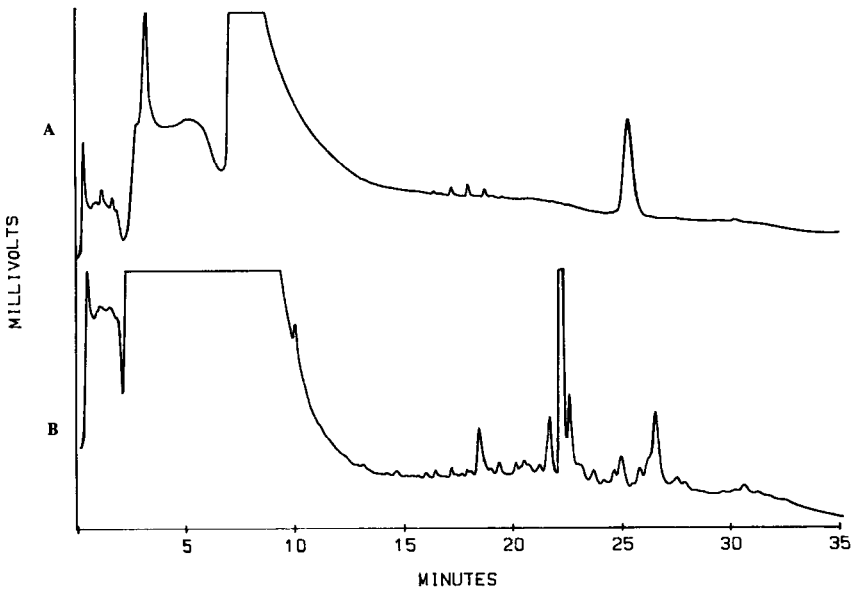


Fig. 5. Chromatograms of two commercial bottled spring waters, (A) a Canadian product and (B) a European product. Chromatograms are plotted at 40 mV. None of the above peaks corresponds to pesticides of interest in this study.

TABLE II

AVERAGE PEAK-AREA COUNTS OVER THREE ORDERS OF CONCENTRATION

Pesticide	*0.1 ppb*[a] *(× 10²)*	*1 ppb (× 10³)*	*10 ppb (× 10⁴)*
Propuxur	600	598	618
Carbofuran	620	597	580
Carbaryl	400	377	397
Propham	520	580	553
Captan	175	164	162
Chloropropham	300	242	263
Barban	270	252	230
Butylate	400	428	459

[a] The American billion (10^9) is meant.

The method of determination of analytes that was originally followed was to calibrate the instrument by four injections of increasing volume of a concentrated stock solution without employing the preconcentration procedure, and subsequently comparing the peak areas. This approach, however, was found to be unacceptable. As demonstrated in Figs. 2 and 3, the peak shapes are substantially different although by area integration all compounds showed quantitative relationships. Because of this difference, three concentrations of standard solutions were prepared (for most of the analytes at 0.1, 1.0 and 10 ppb) and the resulting peak areas were calculated by using the developed method. Because the response of the compounds was linear (Table II), it was concluded that the peak-area counts from the preconcentration of the 1 ppb standard were adequate for daily calibration.

If a solvent delivery system with a three-solvent capability (*e.g.*, the Waters 600E Multisolvent Delivery System) is incorporated in the system that we used, the method lends itself easily to complete automation for a single analysis. This is achieved by computer software control which permits switching of the valving system from the sample load position to the elute positon without manual manipulation. By the addition of a simple rotary switching device, the method could be automated for the analysis of many samples.

The lowest detectable concentrations given in the text could be improved by increasing the sample size, but this would also increase the time of analysis. Analysis time, sample loading rate and detection limit are all dependent on each other and must be selected according to the purpose of the analysis. The technique developed in this study is applicable to all sample matrices investigated.

CONCLUSION

The proposed method is simple, rapid, accurate, economical and reproducible. All of the sample can be injected into the HPLC system for analysis. The method also offers the possibility of complete automation for the analysis of many samples.

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REFERENCES

1 G. A. Junk and J. J. Richard, *Anal. Chem.*, 60 (1988) 451.
2 E. Chladek and R. S. Maranaro, *J. Chromatogr. Sci.*, 22 (1984) 313.
3 A. W. Wolkoff and C. Creed, *J. Liq. Chromatogr.*, 4(8) (1981) 1459.
4 R. J. Bushway, *J. Chromatogr.*, 211 (1981) 135.
5 R. J. Kuhr and H. W. Dorough, *Carbamate Insecticides: Chemistry, Biochemistry and Toxicology*, CRC Press, Cleveland, OH, 1976.
6 J. N. Seiber, *J. Agric. Food Chem.*, 20 (1972) 443.
7 S. Lau and R. L. Marxmiller, *J. Agric. Food Chem.*, 18 (1970) 413.
8 J. E. Fahey, M. C. Wilson and E. J. Armbrust, *J. Econ. Entomol.*, 63 (1970) 589.
9 M. Chiba, *J. Agric. Food Chem.*, 29 (1981) 118-131.
10 W. Thornburg, *Anal. Chem.*, 43 (1971) 145R.
11 J. H. Ruzicka, *Pestic. Sci*, 4 (1973) 417.
12 R. J. Argauer, H. Shimanuki and C. C. Aivarez, *J. Agric. Food Chem.*, 18 (1970) 688.
13 R. J. Argauer and W. Bontoyan, *J. Assoc. Off. Anal. Chem.*, 53 (1970) 1166.
14 R. O. Mumma and S. Khalifa, *J. Agric. Food Chem.*, 20 (1972) 1090.
15 J. A. Durden and W. J. Bartley, *J. Agric. Food Chem.*, 19 (1971) 441.
16 M. C. Harvey and S. D. Stearns, in J. F. Lawrence (Editor), *Liquid Chromatography in Environmental Analysis*, Humana Press, Clifton, NJ, 1984, Ch. 7 pp. 332–338.
17 J. J. Kirkland, *Analyst (London)*, 99 (1974) 1859.
18 J. N. Little and G. J. Fallick, *J. Chromatogr.*, 112 (1975) 389.
19 C. F. Poole and S. A. Schuette, *Contemporary Practice of Chromatography*, Elsevier, New York, 1984, pp. 461–462.
20 C. E. Goewie, W. F. Nielen, R. W. Frei and U. A. Th. Brinkman, *J. Chromatogr.*, 301 (1984) 325.
21 *Method PWACAR-W04A10, Determination of Carbamates in Water by HPLC*, Ontario Ministry of the Environment, Rexdale, 1988.
22 *EPA/600/4-85/054*, USEPA, EMSL, Cincinnati, OH.