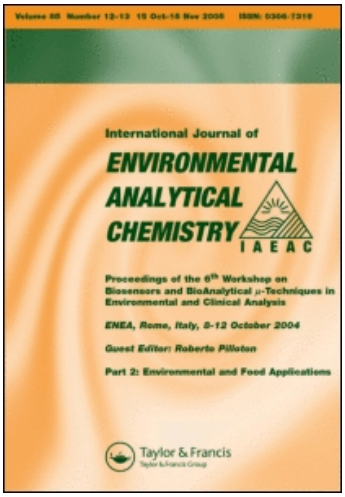

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

An Evaluation of High Performance Liquid Chromatography-UV for the Multi-Residue Analysis of Organophosphorous Pesticides in Environmental Water

Victorin N. Mallet[a]; Monique Duguay[a]; Marc Bernier[a]; Natalie Trottier[a]
[a] Chemistry and Biochemistry Department, Université de Moncton, Moncton, NB, Canada

To cite this Article Mallet, Victorin N. , Duguay, Monique , Bernier, Marc and Trottier, Natalie(1990) 'An Evaluation of High Performance Liquid Chromatography-UV for the Multi-Residue Analysis of Organophosphorous Pesticides in Environmental Water', International Journal of Environmental Analytical Chemistry, 39: 3, 271 — 279
To link to this Article: DOI: 10.1080/03067319008032070
URL: http://dx.doi.org/10.1080/03067319008032070

Intern. J. Environ. Anal. Chem., Vol. 39, pp. 271–279
Reprints available directly from the publisher
Photocopying permitted by license only

AN EVALUATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY—UV FOR THE MULTI-RESIDUE ANALYSIS OF ORGANOPHOSPHOROUS PESTICIDES IN ENVIRONMENTAL WATER

VICTORIN N. MALLET*, MONIQUE DUGUAY, MARC BERNIER and NATALIE TROTTIER

Chemistry and Biochemistry Department, Université de Moncton, Moncton, NB E1A 3E9, Canada

(Received 28 September 1989; in final form 15 December 1989)

In this study isocratic high performance liquid chromatography in the reversed-phase mode (RP-8 or RP-18 column) with UV-detection (254 nm), was evaluated for the analysis, directly or after extraction, of organophosphorous pesticides in environmental water.

Fifteen pesticides were studied and good resolution was obtained for eight by direct analysis of a multi-residue water sample. Reproducibility in terms of retention times was found to be very good. Linear calibration curves were obtained down to 0.5 ng/μL (3 ng injected) with a coefficient of variation near 10%. The limit of quantitation for direct analysis was found to be 0.5 mg/L (3 ng) in water. However, by extraction with methylene chloride or ethyl acetate and concentration of the extract some of the pesticides could be determined at 0.5 μg/L (ppb) in water.

INTRODUCTION

Gas liquid chromatography (GLC) has traditionally been the method of choice for the analysis of volatile organophosphorous pesticides and for good reasons. The method is selective, sensitive and reproducible, and it is readily amenable to automatic manipulations. Recent advances in macro open-tubular columns offer greater resolution without significant loss in sample capacity when compared with conventional packed-columns[1,2,3] and new stationary phases have enlarged the spectrum of chemicals that may be analysed.[4] One disadvantage with GLC is that some organophosphorous pesticides are difficult to analyse because of their lack of volatility. Also there is always the need for confirmation methods.

The technique of high performance liquid chromatography (HPLC) offers an alternative to the analysis of non-volatile and thermally unstable pesticides and may be used for confirmation purposes. One advantage of HPLC is that the analysis may be performed directly in aqueous medium without the extraction step. This aspect may be important in methods development but it is more so when monitoring of the parent compound in the presence of breakdown products is required for kinetic studies or other applications. One serious drawback with the

*Author to whom correspondence should be addressed.

Table 1 List of pesticides

Common name	*Chemical name*
Azinphosmethyl	O-O-Dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl]phosphorodithioate
Coumaphos	O-(3-Chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) O-O-diethylphosphorothioate
Crufomate	4-*tert*-Butyl-2-chlorophenyl methyl methylphosphoramidate
Diazinon	O-O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
Dicapthon	O-O-Dimethyl O-2-chloro-4-nitrophenyl phosphorodithioate
Fenchlorphos	O-O-Dimethyl O-2,4,5-trichrlorophenyl phosphorothioate
Fenitrothion	O-O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate
Fensulfothion	O,O-Diethyl O-[4-(methylsulfinyl)phenyl]phosphorothioate
Fenthion	O,O-Dimethyl O-(3-methyl-4-methylthiophenyl)phosphorothioate
Fonofos	O-Ethyl S-phenyl ethylphosphonodithioate
Gophacide	O,O-Bis (4-chlorophenyl) acetimidoyl phosphoramidothioate
Parathion-methyl	O,O-Dimethyl O-p-nitrophenyl phosphorothioate
Phosmet	O,O-Dimethyl-S-phthalimidomethyl phosphorodithioate
Temephos	O,O,O',O'-tetramethyl O,O'-thiodi-p-phenylene diphosphorothioate
Zytron	O-(2,4-dichlorophenyl O'-methyl N-isopropylphosphoroamidothioate

direct approach is the lack of sensitivity with conventional detectors such as the UV and as in GLC, environmental water samples have to be extracted and concentrated prior to analysis.

The analysis of pesticides and other organic contaminants by HPLC is not new. Lawrence and Turton[5] have collected data on 166 pesticides but most of the studies involved standards, not multi-residue analysis. Krause[6] studied 75 compounds by HPLC to determine those that could be analysed by fluorescence with improved detection limits. Price *et al.*[7] modified a multi-residue method for chlordane, toxaphene and PCBs in food by HPLC. Seymour *et al.*[8] also studied PCBs by HPLC.

One objective of this study was to evaluate the use of HPLC in the reversed-phase mode with UV detection in terms of resolution, reproducibility, sensitivity and detection limit, for the direct multi-residue analysis of some organophosphorous pesticides in water. Another objective was to determine the limit of quantification of these pesticides in environmental water following extraction and concentration.

EXPERIMENTAL

Chemicals

The organophosphorous pesticides were obtained as analytical standards from Agricultural Canada, Ottawa, Ontario. A list of the pesticides tested and their chemical names are presented in Table 1. Stock solutions were prepared 1 μg/μL in

ethyl acetate (pesticide grade). All other organic solvents were suitable for liquid chromatography (HPLC grade). Water for sample preparation was twice distilled, de-ionized and passed through a 0.45 μm filter. Water used as mobile phase was passed through a Millipore, Nylon 66, 0.22 μm filter. Mobile phases were de-gassed with helium prior to use. Local tap water that originates from a surface lake was used as a source of environmental water.

Method

Provided the concentrations of the pesticides are at least 0.1 mg/L the water sample may be injected directly into the chromatograph after passing through a 0.45 μm filter. Some pesticides may be analysed at 0.5 mg/L.

For less concentrated samples (0.1 μg/L) the following procedure may be used: extract 1-L with 3 × 50 mL of methylene chloride or 3 × 100 mL of ethyl acetate. Dry with anhydrous sodium sulfate. Evaporate almost to dryness, transfer to a 1.0 mL volumetric flask with acetonitrile and make-up to the mark. Pass an aliquot through a 0.45 μm filter and inject into the chromatograph.

Instrumentation

Some analyses were performed with a Perkin-Elmer Series 10 (LC-10), Liquid Chromatograph (without pre-column) equipped with a UV detector (LC-90), at 254 nm. The columns (25 cm × 4.6 mm i.d.) were reversed-phase (Altech C-8 and C-18), with 10 μm particles. The injection loop was 6 μL. Other experiments were conducted with a Spectra-Physics (SP-8000B) HPLC (with pre-column) using the same columns and a 10 μL injection loop.

RESULTS AND DISCUSSION

Because of its intermediate polarity a RP-8 column was initially selected to study individual retention times. Taking into consideration that the pesticides were soluble in methanol or acetonitrile, 75% aqueous solutions were chosen as initial mobile phases. Individual retention times for 14 OPs are presented in Table 2. Retention times are shorter in acetonitrile:water and the chromatograms show less band broadening which favors resolution and detection limits. Also Crufomate, Fenchlorfos and Zytron could not be detected in methanol:water, presumably because they were not eluted. Therefore, acetonitrile:water was favored as mobile phase for further studies.

Unfortunately, it was not possible to separate all 14 OPs in a multi-residue standard because of the closeness in retention times for a few pesticides. In the end eight pesticides were selected for further studies but in principle those that were excluded could be analysed by HPLC in the absence of interfering pesticides. Thus, best resolution was observed with a 65% aqueous solution of acetonitrile for a mixture of eight pesticides as shown in Figure 1. All the OPs were separated with an acceptable resolution (>1.0) in less than 20 minutes and with excellent

Table 2 Individual retention times (min) of various OPs on a RP-8 column

Common name	*Methanol:water (75:25)*	*Acetonitrile:water (75:25)*
Azinphosmethyl	7.1	6.5
Coumaphos	12.3	7.7
Crufomate	N.D.	6.2
Diazinon	13.0	9.2
Dicapthon	9.4	6.7
Fenchlorphos	N.D.	10.9
Fenitrothion	8.5	6.4
Fensulfothion	6.6	5.1
Fonofos	12.0	8.9
Gophacide	12.6	7.1
Parathion-methyl	6.6	5.9
Phosmet	7.0	5.6
Temephos	23.0	10.4
Zytron	N.D.	10.2

Conditions: 1.0 mL/min; concentration: 100 ng/μL in ethyl acetate.
Instrument: PE LC-10.
N.D.: Not detected.

reproducibility in terms of retention times. Attempts to obtain better resolution with RP-18 or RP-2 columns failed. In principle, however, better resolution should be achieved with a 5 μm particle size PR-8 column instead of a 10 μm.

Reproducibility in terms of peak heights at various concentrations (in ethyl acetate, acetonitrile and water) was then studied using the above conditions. The data for 100 ng/μL solutions in acetonitrile are presented in Table 3. They indicate that the coefficients of variation vary from 1.6 to 3.2% at that concentration. At 1 ng/μL the coefficients of variation varied from 10.6% (fensulfothion) to 1.5% for Azinphosmethyl which is quite acceptable. Five of the pesticides (see Table 3) could also be detected comfortably at 0.1 ng/μL. Calibration curves between 1000 and 0.1 ng/μL for these pesticides were perfectly linear. Thus, for some pesticides (indicated in Table 3) the limit of detection may be considered to be 0.1 ng/μL (0.6 ng) but for the others 0.5 ng/μL (3 ng) is more appropriate.

Solutions of the eight pesticides were prepared 1 ppm in distilled and tap water (originally surface water). Aliquots were injected within the hour to reduce degradation. As compared with 1 ng/μL solution of the pesticides in ethyl acetate there is an important decrease in peak heights for most of the pesticides in tap water (Table 4), presumably caused by the increase in polarity of the solvent. Coumaphos is barely detectable and Temephos is not detected. The other pesticides (see Table 4) may be analysed in water at 0.5 mg/L.

It is clear that the direct determination of OPs in water at a limit of quantitation 0.5 mg/L would not be applicable to environmental samples which usually contain less than 10 μg/L. Therefore an extraction and concentration step was introduced to improve the limit of quantitation. Extraction of environmental water with organic solvents such as chloroform or ethyl acetate introduces co-extractives which interfere with the resolution. However, it was found that use of a

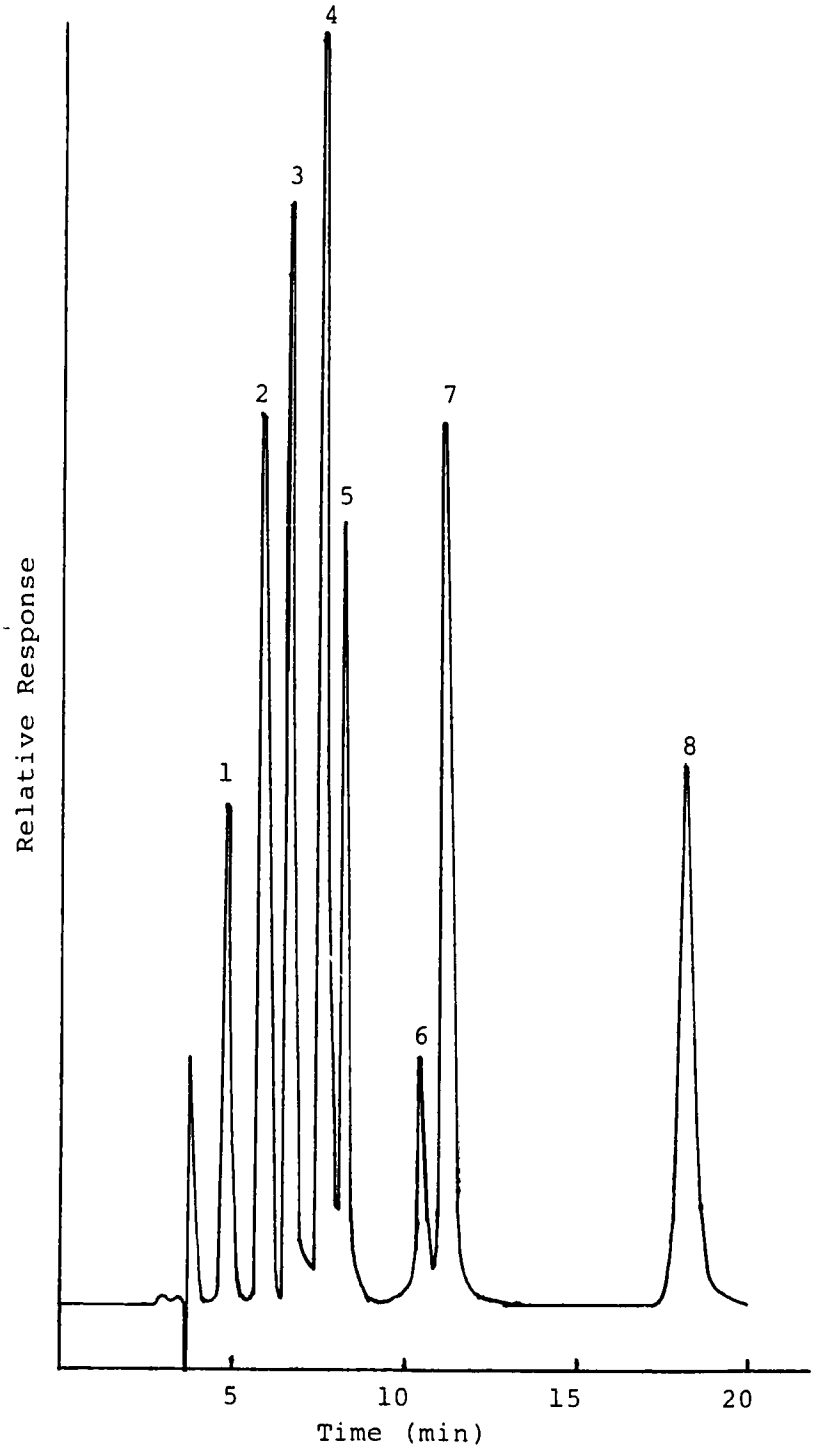

Figure 1 HPLC chromatogram (LC-10) showing separation on RP-8 column of eight organophosphorous pesticides in water (100 mg/L). 1. Fensulfothion; 2. Azinphosmethyl; 3. Parathion-methyl; 4. Fenitrothion; 5. Dicapthon; 6. Coumaphos; 7. Diazinon; 8. Temephos. Mobile phase: acetonitrile:water (65:35) at 1.3 mL/min.

Table 3 Reproducibility study of OPs at 100 ng/μL in acetonitrile

Pesticide	*Average height (cm) (n=6)*	*Standard deviation*	*Coefficient of variation*
Azinphosmethyl[a]	13.0	0.38	2.9
Fensulfothion	7.9	0.23	2.9
Parathion-methyl[a]	16.0	0.46	2.9
Fenitrothion[a]	18.3	0.52	2.9
Dicapton[a]	11.4	0.37	3.3
Coumaphos	3.5	0.09	2.5
Diazinon[a]	13.0	0.21	1.6
Temephos	7.9	0.25	3.2
Average coefficient of variation	2.8		

Conditions: Mobile phase, acetonitrile:water (65:35), 1.0 mL/min.
[a]May be detected at 0.1 ng/μL.

Table 4 Peak heights (cm) ($n=2$) of OPs (1.0 mg/L) in various media

Pesticide	*Media*		
	Ethyl acetate	*Purified water*	*Tap water*
Fensulfothion[a]	2.0	2.1	1.9
Azinphosmethyl	2.9	2.7	2.5
Parathion-methyl[a]	3.7	3.0	2.8
Fenitrothion[a]	4.1	2.7	2.4
Dicapthon[a]	2.5	1.4	1.1
Coumaphos	0.8	0.2	0.2
Diazinon[a]	2.7	1.6	1.2
Temephos	1.5	—	—

Conditions: flow rate, 1.0 mL/min; 65:35 (acetonitrile:water).
[a]May be analysed directly at 0.5 ng/μL.

RP-18 column instead of a RP-8 forced many of the co-extractives to elute early. Thus a RP-18 column was preferred with environmental samples.

To illustrate the applicability of HPLC–UV to environmental water following a pre-concentration step five typical pesticides were selected for further studies. These were fensulfothion, fenthion and temephos, and azinphosmethyl which is difficult to analyse by GLC.[3,4] Fenitrothion was introduced as a control to compare with a GLC method.[3,4] A typical calibration curve for a standard of azinphosmethyl in acetonitrile between 1000 ng/μL and 0.1 ng/μL gave a slope of 1.2 with a correlation coefficient of 0.9999. A coefficient of variation of less than 10% may be expected at 0.1 ng/μL which is considered to be the limit of detection. This performance compares favorably with that observed by GLC for the same pesticides with the exception of azinphosmethyl for which the HPLC approach is much better.

Application to environmental water samples was then evaluated. Results for the extraction of multi-residue samples with methylene chloride and ethyl acetate at 0.5 μg/L are given in Table 5. By this method a concentration of 0.5 μg/L may be

Table 5 Multi-residue analysis of organophosphorous pesticides in environmental water

Pesticide	*Methylene chloride*		*Ethyl acetate*	
	x (%)	*C.V.* (%)	*x* (%)	*C.V.* (%)
Azinphosmethyl	73	8	95	10
Fenitrothion	70	14	92	10
Fenthion	65	13	99	12
Temephos	64	11	87	11

[a] Recovery ($n = 6$) (0.5 μg/L).

considered to be the limit of quantitation as limited by the coefficients of variation which increase over 15% at lower levels. A typical chromatogram (RP-18) of a water sample extract is presented in Figure 2. It shows some co-extractives which did not interfere in this case. However, in several extractions, fensulfothion could not be detected. As compared with methylene chloride, extraction with ethyl acetate yields better recoveries (see Table 5). However, extraction with methylene chloride is easier and only 3×50 mL of solvent is needed. Recoveries at higher concentrations (1.0 μg/L or 10 μg/L) are also quantitative and coefficients of variation are smaller as expected. One drawback with the RP-18 column is the longer retention times presumably caused by greater compatibility of the pesticides with the stationary phase.

CONCLUSION

This study shows that HPLC using a RP-8 column and a UV detector at 254 nm may be used to monitor organophosphorous pesticides directly in water provided the concentration is near 1 mg/L. The resolution is excellent and reproducibility both in terms of qualitative and quantitative analysis is acceptable. Although the direct approach would be quite useful for formulations or for kinetic studies to monitor the parent compound in presence of degradation products, it would be limited for environmental samples where the concentration is usually in the parts per billion range. However, the method is compatible with methylene chloride or ethyl acetate extraction at the low parts per billion level which is within the range expected with environmental samples. Typically the method could be used to analyse azinphosmethyl in water at 0.5 μg/L. This compares favorably with GLC since azinphosmethyl is very difficult to analyse by that method. For other pesticides, such as fenitrothion, which is readily analysed by GLC, the HPLC method could be used for confirmation purposes.

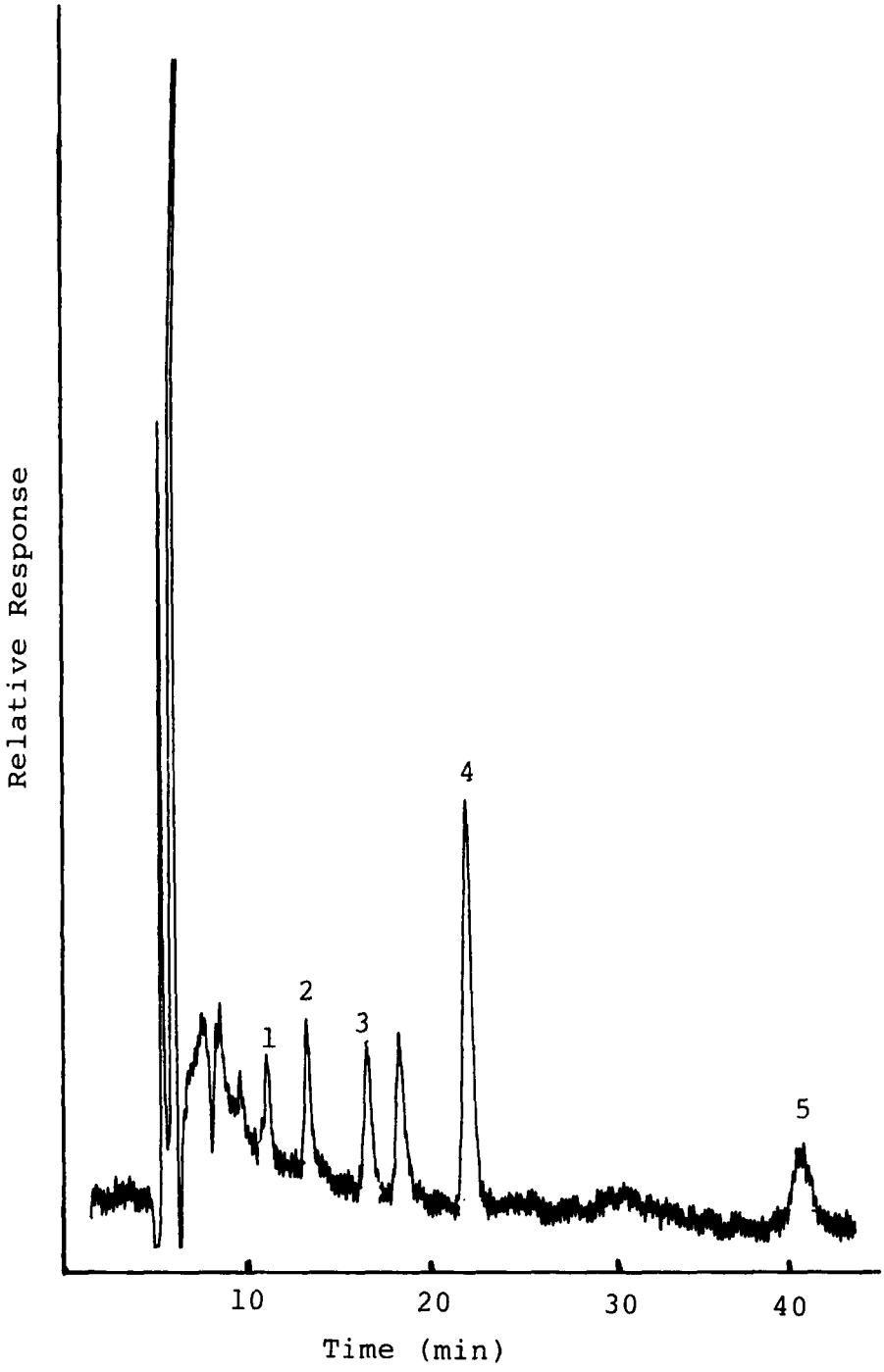

Figure 2 HPLC chromatogram (SP-8000B) using RP-18 column of methylene chloride extract from water (0.5 μg/L). 1. Fensulfothion; 2. Azinphosmethyl; 3. Fenitrothion; 4. Fenthion; 5. Temephos. Mobile phase: acetonitrile:water (70:30) at 25 °C and flow of 1.0 mL/min.

Acknowledgement

This work was financially supported by the National Science and Enginering Research Council of Canada and by Université de Moncton.

References

1. N. V. Fehringer and S. M. Walters, *J. Assoc. Offic. Anal. Chem.* **69,** 90 (1986).
2. C. Mallet and V. N. Mallet, *J. Chromatogr.* **481,** 27–35 (1989).
3. C. Mallet and V. N. Mallet, *J. Chromatogr.* **481,** 37–44 (1989).
4. S. M. Staroverov, A. A. Serdan and G. V. Lisichkin, *J. Chromatogr.* **364,** 377 (1986).
5. J. F. Lawrence and D. Turton, *J. Chromatogr.* **159,** 207 (1978).
6. R. T. Krause, *J. Chromatogr.* **255,** 497 (1983).
7. H. A. Price, R. L. Welch, R. H. Scheel and L. A. Warren, *Bull. Environ. Contam. Toxicol.* **37,** 1 (1986).
8. M. P. Seymour, I. W. Duncan, T. M. Jefferies and L. J. Notoarianni, *J. Chromatogr.* **368,** 174 (1986).