ELSEVIER

Journal of Chromatography A, 725 (1996) 129–136

JOURNAL OF CHROMATOGRAPHY A

Solid-phase microextraction of the herbicide metolachlor in runoff and tile-drainage water samples

K.N. Graham[a], L.P. Sarna[a], G.R.B. Webster[a,*], J.D. Gaynor[b], H.Y.F. Ng[c]

[a]*Department of Soil Science, University of Manitoba, Winnipeg, MB, R3T 2N2 Canada*

[b]*Harrow Research Centre, Agriculture and Agri-Food Canada, Harrow, ON, N0R 1G0 Canada*

[c]*Aquatic Ecosystem Protection Branch, National Water Research Institute, 867 Lakeshore Road, Burlington, ON, L7R 4A6 Canada*

Abstract

The new rapid solid-phase microextraction (SPME) technique developed in Canada by Pawliszyn and co-workers has been used in the analysis of water for pesticide residues in laboratory studies. SPME used with gas chromatography and electron-capture detection (GC–ECD) of metolachlor in runoff water showed linear response over a wide range. The lowest concentration analyzed was 0.002 μg/l (2 ppt), and the highest was 20 000 μg/l (20 ppm). Over this span of seven orders of magnitude, the standard curve had an R^2 of 0.9954 for ten data points, each of which was averaged over three or more trials. The curve below 200 μg/l, or 0.20 ppm had a slightly different slope (R^2 0.9996). Earlier analyses by automated SPME–GC and flame ionization detection (FID) in distilled water showed linear response over the range 180 to 180 000 μg/l. A 100-μm polydimethylsiloxane-coated fibre was used; metolachlor residues in the runoff water were 0.17 to 50.7 μg/l. This is the first time, to our knowledge, that SPME has been used in the analysis of herbicide residues in runoff water.

Keywords: Solid-phase microextraction; Water analysis; Environmental analysis; Pesticides; Metolachlor

1. Introduction

Pesticides are used extensively in Canadian agriculture, and their residues are of concern in the contamination of ground- and surface water. Many studies have reported that pesticide use has led to the presence of residues in groundwater, surface water, and tile drainage water [1–5]. Monitoring of pesticide residues in water must be performed to ensure that levels do not exceed those judged to be harmful to the environment or exceed drinking water standards.

Metolachlor (2-chloro-6′-ethyl-N-(2-methoxy-1-methylethyl)acet-*o*-toluidide) is a chloracetanilide herbicide used to control grassy weeds in southwestern Ontario and in soybean (*Glycine max.*), field corn (*Zea mays*), and field bean (*Phaseolus* spp.), and in corn (maize) and potatoes in Manitoba. Much of the agricultural land in southwestern Ontario is underlain with a network of tile drains to facilitate the control of the water levels in these soils and to minimize loss of soil nutrients by leaching.

Residues of metolachlor have been found with increasing frequency in surface waters in this region as a result of runoff from agricultural land during and following periods of excessive rainfall [1,2,4]. Monitoring for metolachlor requires specialized instrumentation and techniques. Conventional techniques have made use of solvent extraction [6].

The U.S. EPA method 507 [7] is a standard method of analysis for metolachlor in water. This method was adopted following the U.S. National

*Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)00956-6

Pesticide Survey of 1990 which identified the need to amalgamate a variety of methods for uniform pesticide techniques in water [8]. The method involves the solvent extraction of one litre water sample using methylene chloride. The extract is dried over anhydrous magnesium sulfate and concentrated to a volume of 5.00 ml after solvent substitution with methyl *tert.*-butyl ether (MTBE). Analysis is performed by GC with nitrogen phosphorus detection (NPD) and has a minimum quantification limit for metolachlor of 1.5 μg/l [9].

The U.S. EPA method 'Atrazine and Metolachlor EPA - 1' [10] similarly uses 3.0 g dipropyl phthalate dissolved in 1000 ml acetone to extract the analyte from a one-litre sample. This is performed by shaking for 30 min after which the extractant is analyzed by GC with flame ionization detection (FID).

The Alberta Environmental Centre method No. A109.0 [11] is listed for analysis of organochlorine pesticides in water. This method extracts one litre of sample with 140 ml methylene chloride, which is exchanged for hexane and concentrated to a volume of 2.00 ml. The concentrate is analyzed by GC with electron-capture detection (ECD).

A liquid–solid extraction [12] method followed by reversed-phase high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection has been described for pesticide analysis in water. The liquid–solid extraction is performed using cartridges filled with Carbopack B (graphitized carbon black) followed by elution with the solvents methylene chloride and methanol. This permits a stepwise elution of base–neutral and acidic pesticides. The eluent is analyzed by HPLC with a UV detector set at 220 nm. The reported limit of detection for this method is 0.009 μg/l [12].

The above methods are typical of conventional analytical techniques and have the common requirement for the use of organic solvents which are costly to purchase, hazardous to work with, and problematic to dispose of. These methods also often require a time consuming preparative clean-up step which is costly in time and labour and which may reduce recovery of the analyte.

Enzyme linked immunoassay, or ELISA, is a recent technology which has promise as a screening technique for metolachlor in water. This method is based on the use of antibodies or β lymphocytes which specifically bind to a foreign molecule, or antigen [13]. In this type of analysis, the analyte and a similar enzyme conjugate compete for binding sites on the antibody which is either coated in microwells of an analytical plate, or is attached to paramagnetic particles. Any unreacted antibody is complexed with a chromogenic agent allowing determination of analyte concentration on the basis of colour by comparison with standards. Colour intensity is inversely related to the amount of analyte present [14]. The limit of detection for metolachlor using this method has been reported to be 0.1 μg/l [13].

A new technology has recently been designed by Pawliszyn and co-workers at the University of Waterloo, Waterloo, Ontario, Canada [15–18]: solid-phase microextraction (SPME) has been shown to be able to extract residues of organic analytes from water for direct injection into a gas chromatograph by thermal desorption. This new method preserves the quality associated with conventional analytical techniques, but is essentially solventless and avoids

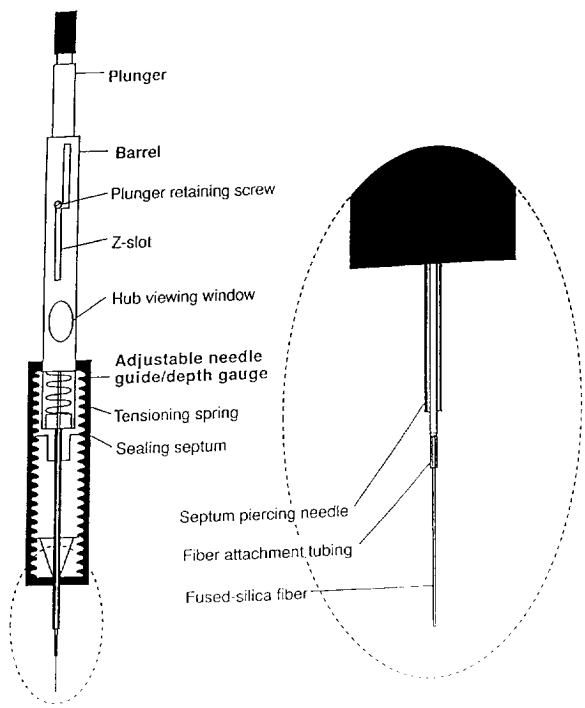

Fig. 1. SPME apparatus for manual sorption and injection [15].

intermediate steps such as cleanup and solvent evaporation; it also avoids the expense and inconvenience of the use and disposal of solvent at the end of analyses.

The SPME method involves a simple syringe-like apparatus (Fig. 1) [15] which houses a fused-silica fibre that is coated with a known volume of a polymeric stationary phase. Extraction occurs within the sample vial when the fibre is extended and exposed directly to the aqueous sample (Fig. 2). The analyte partitions between the aqueous phase in which it is dissolved and the polymeric coating of the fibre.

This partitioning is dependent on the two constants: K, the distribution constant of the analyte, and V_s, the volume of the fibre coating, plus C_{aq}, the concentration of analyte in the sample solution. The amount of analyte which may be sorbed to the fibre coating, n is determined by these three factors, as expressed in the following equation:

$$n = KV_sC_{aq}$$

This method does not involve an exhaustive extraction, but rather establishment of an equilibrium. A linear relationship exists between the number of moles, n, of analyte sorbed by the coating, and the original concentration of the analyte in the sample, C_{aq} [19].

Initial success in the analysis of metolachlor in distilled water by SPME–GC–FID and SPME–GC–ECD was reported in 1994 [20]. The current paper reports for the first time the use of SPME–GC–ECD for the analysis of residues of a herbicide in environmental water samples: ground water and tile drainage water.

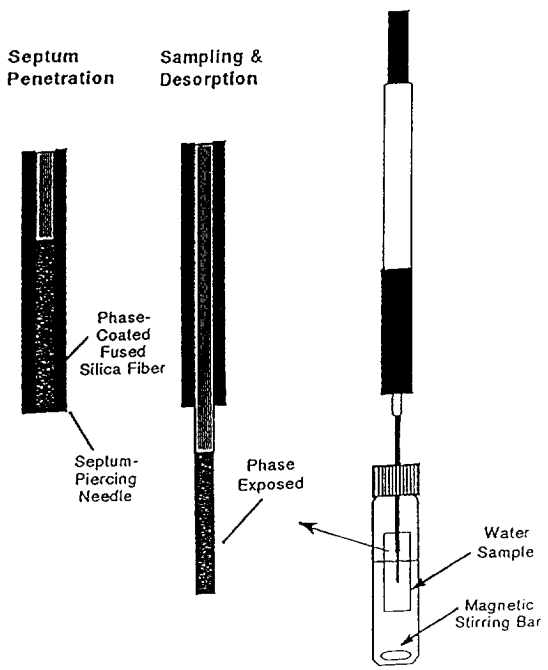

Fig. 2. SPME apparatus showing withdrawn and exposed fibre.

2. Experimental

2.1. Sample collection

Runoff water and tile drainage samples from agricultural plots treated with metolachlor were obtained from test plots at the Harrow Research Centre, Agriculture and Agri-Food Canada in Harrow, Ontario. The herbicide was applied preemergence at 1.68 kg/ha with a Chelsea sprayer using 8004 EVS flat fan or VS nozzles (TeeJet) on May 13, 1994. Samples were collected in glass bottles August 13–14, 1994, during the first rainfall producing runoff after herbicide application. The water samples were stored at 4°C and shipped to the laboratory where they were received on September 9 and 20, 1994 in 200- and 1000-ml glass bottles. No filtration or other pretreatment occurred.

2.2. SPME extraction

From these bottles 30-ml aliquots were transferred into 40 ml screw-cap glass vials with Teflon coated septa. Vials were also fitted with 13×8 mm magnetic stirbars. For extraction, a Supelco manual SPME fibre holder assembly was used equipped with a 100-μm polydimethylsiloxane coated fibre. Extraction occurred within the sample vial, at room temperature, as the SPME septum piercing needle was inserted through the septa and the fibre was immersed in the liquid sample. Sorption time was 15 min during which the sample was stirred with the magnetic stirplate set at 60% maximum speed.

Once sorption was complete, the fibre was retracted into the septum piercing needle and the apparatus was removed from the vial septum. The fibre was then directly inserted into the GC for desorption and analysis. Desorption of analyte from the SPME fibre occurred in the injection port of the GC at 200°C for 2 min after which the purge gas was turned on and the desorbed sample extract was analyzed by GC.

2.3. GC analysis

Automated SPME

Initial analyses were attempted on a Varian 3400 gas chromatography equipped with an 8200 autosampler modified for SPME and an flame ionization detector. The gas chromatograph was fitted with a 30 m×0.25 mm J and W Scientific fused-silica DB-5 capillary column with a film thickness of 0.1 μm. The helium carrier gas flow-rate was 1 ml/min with a total flow at the detector of 30 ml/min. GC conditions (59 min total run time): 100°C (5 min), 5°C/min, 250°C (3 min), 2°C/min, 280°C (6 min). Data was collected and analyzed using a Varian Star Chromatography Workstation.

Manual SPME

Analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with an electron-capture detector and a 30 m×0.25 mm J and W Scientific fused-silica DB-5 capillary column with a film thickness of 0.1 μm. The helium carrier gas flow-rate was 1.1 ml/min, with a total flow at the detector of 60 ml/min. GC conditions (19 min total run time): 100°C (2 min), 10°C/min to 250°C (2 min).

Data was collected and analyzed using HP Chemstation software. Each runoff water subsample was analyzed in triplicate using the above method. A standard curve was created using metolachlor spiked HPLC grade water at concentrations ranging from 0.002 μg/l to 20 000 μg/l (or 2 ppt to 20 ppm).

Confirmation of analyte identity was performed using the SPME–GC parameters described above followed by mass spectral analysis on a Finnigan MAT model 801 ion trap detector. The ion trap detector was run in full scan acquisition mode with a scan range of 50–400 amu. The multiplier voltage was 1700 eV. The detector temperatures were as follows: transfer line 280°C, exit nozzle 260°C and manifold 225°C.

3. Results

Automated SPME

Originally the possibility of performing these analysis on an automated SPME system attached to a GC–FID was investigated since work had shown that metolachlor at concentrations as low as 180 μg/l

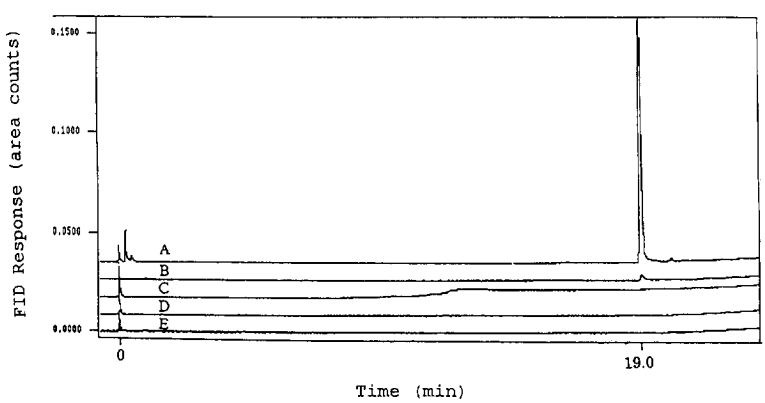

Fig. 3. Autosampler–SPME–GC–FID chromatograms for metolachlor. A=spiked standard at 18 000 μg/l (18 ppm), B=blank desorption run following standard run (A), C=sample number 31, D=sample number 27, E=sample number 32.

could be analyzed this way (Fig. 3). Unfortunately the detection limits of FID were not compatible with the low concentrations of metolachlor in the runoff water samples. As demonstrated in Fig. 3, the spiked standard (chromatogram A) at 18 000 μg/l showed a clear metolachlor peak at 19 min. Chromatogram B is a blank run following the standard run, showing some carry-over on the fibre at this concentration. Chromatograms C, D and E are analyses of samples numbered 31, 27 and 23, respectively. There was no metolachlor peak detectable for the water samples using this method.

Manual SPME

The standard curve was created using HPLC-grade water (Anachemia, Rouses Point, NY, USA) spiked with analytical grade metolachlor (Riedel-de Haen, Seelze, Germany) at ten concentrations ranging from 0.002 μg/l to 20 000 μg/l (2 ppt to 20 ppm). Under these conditions metolachlor had a retention time of 14.2 min (Fig. 4). The average of three replicate analyses per sample showed that metolachlor was present in the runoff and tile drainage water samples at concentrations ranging from 0.17 μg/l to 50.70 μg/l (or 170 ppt to 50 ppb). This was well within the range of linearity for the method.

3.2. SPME fibre robustness

The effects of organic and particulate matter on the SPME fibre is unknown, but they appear to reduce the GC response after several extractions. The fibre performance was routinely checked using standard solutions. In total, eight fibres were used to perform over 200 analysis, averaging 27 analysis per fibre before its performance became questionable, showing decreased reproducibility. As this number of samples is considerably lower than the reported 100+ analysis possible per SPME fibre [21] for distilled water samples, it appears that organic and particulate matter, possibly in combination with stirring, decrease fibre life.

3.3. Analyte carryover

Carryover of analyte on the fibre after thermal desorption from the previous analysis was regularly monitored. The fibre was re-desorbed in the injector

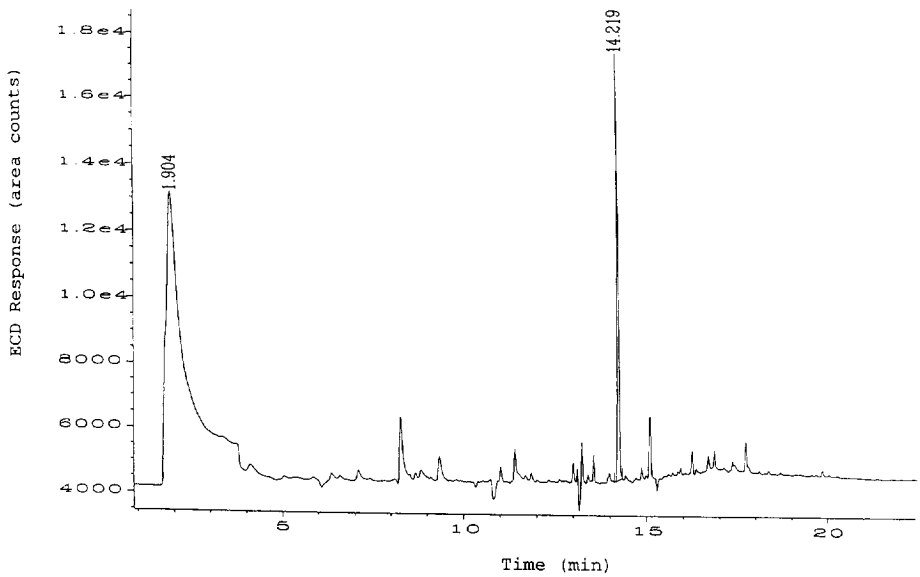

Fig. 4. SPME–GC–ECD chromatogram of sample number 11 showing selectivity of the 100-μm fibre (surface runoff water sample containing 11.8 μm/l metolachlor).

port following an analytical run. In this manner it was determined that carryover from one run to the next did not occur at real water sample concentration levels experienced in this study, but the effect was detected at 200 μg/l (0.2 ppm) or higher levels for standard runs. This effect was mitigated by performing prolonged (and multiple desorptions if necessary) of a fibre following exposure to high concentrations of analyte. Normally any carryover was eliminated under these circumstances by the second desorption run.

4. Discussion

4.1. Reproducibility

The relative standard deviation was 8% or lower over three replicates, with the exception of two samples (numbered 17 and 25) (Table 1). A high degree of reproducibility for this SPME method has thus been demonstrated. The two cases showing lower reproducibility appeared to be attributed to poor peak shape which interfered with the establishment of the baseline.

4.2. Sample stirring

Stirring decreases the amount of time needed to reach equilibrium between the analyte in the sample solution and the fibre coating. Stirring results in shorter sampling time, speeding diffusion of the analyte to the fibre. It also disrupts the layer of depleted water which otherwise tends to remain next to the fibre during sorption of the analyte.

4.3. Sample volume

Sample volume can also affect analytical sensitivity; for a given concentration, a larger volume of aqueous sample will give a greater response [22]. Thus, the 40-ml vials used in this study allowed greater sensitivity than the 2-ml vials initially used on the autosampler.

Table 1
Residues of metolachlor in runoff and tile drainage water

Sample No.	R.S.D. (%)	Metolachlor (μg/l)
1	7	9.33
2	1	13.17
3	8	0.18
4	8	7.95
5	6	0.81
6	5	5.01
7	1	0.17
8	6	0.81
9	4	7.49
10	4	8.21
11	6	11.83
12	7	1.05
13	7	0.51
14	1	0.42
15	6	0.33
16	7	0.33
17	10	0.33
18	2	0.67
19	2	7.43
20	6	4.20
21	3	6.00
22	4	2.62
23	3	11.34
24	2	0.27
25	9	1.03
26	5	50.70
27	5	4.78
28	8	0.57
29	7	12.18
30	2	18.61
31	4	5.14
32	3	6.22
33	5	1.40
34	2	4.20
35	4	5.78
36	7	5.16
37	2	3.32
38	3	0.27
39	3	5.22
40	1	5.20

4.4. Limits of detection and standard curve characteristics

The lowest reproducible concentration using this method was 0.002 μg/l (2 ppt), and the upper limit tested was 20 000 μg/l (20 ppm). Over this span of seven orders of magnitude, the standard curve had an R^2 of 0.9954 for ten data points, each of which was

averaged over three or more trials. While this is excellent linearity overall, upon further investigation it was noticed that the lower portion of the curve (below 200 μg/l, or 0.20 ppm) had a slightly different slope than the upper portion of the curve. When the lower seven concentrations were used to plot the standard curve, the R^2 value obtained improved to 0.9996. As the runoff water sample concentrations had a maximum value of 50 μg/l (sample number 26), the standard curve spanning five orders of magnitude from 0.002 μg/l to 180 μg/l was used (Table 2). Fibre selectivity for metolachlor was sufficient that samples did not need cleanup; Fig. 4 shows the chromatogram for sample 11 (runoff) which contained 11.8 μg/l.

4.5. Fibre degradation

The necessity to replace the SPME fibre after approximately 27 analyses is well below the expected lifespan of a fibre under normal use [20]. The possibility of fibre degradation due to the presence of organic and particulate matter was investigated. The fibre was observed under a low magnification microscope before and after use. The presence of particulate and fibrous contaminants was revealed, making the fibre appear discoloured, and dirty. This contamination raised questions about the possibility of a decrease in sorptive capability of the fibre coating due to mechanical interference. The longevity of the SPME fibre used for these analyses of runoff water samples with moderate organic and suspended particulate matter content was still adequate for environmental analysis.

Table 2
Standards: metolachlor spiked water by SPME–GC–ECD

Conc. (μg/l)	Mean	S.D.	R.S.D. (%)
180	592 604	32 110	5
20	57 155	3743	7
18	55 345	3008	5
2	7847	52	1
0.2	3817	173	5
0.02	4943	88	2
0.002	459	35	8

4.6. Septum coring

Coring of the GC septum caused by the SPME device led to an approximately 50% increased frequency for septum replacement. The greater diameter of the septum piercing needle and its square cut end compared to the sharp design of GC syringe needles were the likely cause of this coring.

4.7. Rapid analytical technique

It has been demonstrated that SPME combined with GC–ECD can quickly, accurately, and effectively detect metolachlor at environmentally significant levels in runoff and tile drainage water from an agricultural watershed. This makes SPME a promising candidate for environmental monitoring procedures. As concern over ground water contamination increases and world-wide use of agricultural pesticides increases, the need for an effective, low-cost and reliable analytical technique is imperative. SPME could prove to fill many of these analytical requirements. The low cost and simplicity of the SPME fibre assembly combined with future developments using portable GC systems may make this method suitable for on-site testing for environmental contaminants.

4.8. Comparison

A major difference of SPME methods from more conventional extraction methods is that SPME does not require the use of organic solvents, where other methods do. The elimination of the use of organic solvents in analytical techniques reduces the amount of hazardous wastes produced which decreases costs involved. The simplicity of the SPME method also eliminates the need for sample clean-up (Fig. 4), which can be time consuming as well as a source of analyte loss and experimental error. Extraction time is reduced from hours or days to minutes, and extraction occurs within the sample vial. The lowest detectable quantity at a signal-to-noise ratio of three was 0.003 μg/l. The lowest concentration of analyte reproducibly analysed using this method was 0.002 μg/l. This concentration is below all other reported limits of reproducibility, and even below other reported limits of detection [9,12,13]. These factors

combined with the reproducibility and detection limits observed in this study make SPME a promising analytical technique for similar pesticide residues in the future.

References

[1] J.D. Gaynor, D.C. MacTavish and W.I. Findlay, J. Environ. Qual., 24 (1995) 246.
[2] R. Frank, B.S. Clegg, C. Sherman and N.D. Chapman, Arch. Environ. Contam. Toxicol., 19 (1990) 319.
[3] E.M. Thurman, D.A. Goolsby, M.T. Meyer and D.W. Kolpin, Environ. Sci. Technol., 25 (1991) 1794.
[4] G. Chesters, G.V. Simsiman, J. Levy, B.J. Alhajjar, R.N. Fathulla and J.M. Harkin, Rev. Environ. Contam. Toxicol., 110 (1989) 1.
[5] B.P. Krawchuk and G.R.B. Webster, Water Poll. Res. J. Canada, 22 (1987) 129.
[6] D.J. Munch and C.P. Febis, Environ. Sci. Technol., 26 (1992) 921.
[7] Determination of Organic Compounds in Drinking Water; PB-89-220461/AB; U.S. Environmental Protection Agency, National Technical Information Service: Springfield, VA, December 1990.
[8] D.J. Munch and C.P. Febis, Environ. Sci. Technol., 26 (1992) 921.
[9] D.J. Munch, R.L. Graves and R.A. Maxey, Environ. Sci. Technol., 24 (1990) 1446.
[10] Manual for Chemical Methods for Pesticides and Devices Second Edition; U.S. Environmental Protection Agency, Office of Pest Programs, Analytical Chemistry Branch: Beltsville MD, 1987.
[11] Methods Manual for Chemical Analysis of Trace Organics and Pesticides in Environmental Samples; AECV92-M2; Alberta Environmental Centre, Vegreville, AB, 1992.
[12] A. Di Corcia and M. Marchetti, Environ. Sci. Technol., 26 (1992) 66.
[13] R.K. Lankow and J.H. Rittenburg, Immunoassays: The Future of Analysis, Quantix Systems, Cinnaminson, NJ, 1992.
[14] J.D. Gaynor, D.A. Cancilla, G.R.B. Webster, L.P. Sarna, K.N. Graham and H.Y.F. Ng, J. Agric. Food Chem., in press.
[15] Z. Zhang, M.J. Yang and J. Pawliszyn, Anal. Chem., 66 (1994) 844.
[16] C.L. Arthur and J. Pawliszyn, Anal. Chem., 62 (1990) 2145–2148.
[17] C.L. Arthur, L.M. Killam, K.D. Buchholz and J. Pawliszyn, Anal. Chem., 64 (1992) 1960–1966.
[18] C.L. Arthur, D.W. Potter, K.D. Buchholz, S. Motlagh and J. Pawliszyn, LC·GC, 10 (1992) 656–661.
[19] D. Louch, S. Motlagh and J. Pawliszyn, Anal. Chem., 64 (1992) 1187.
[20] G.R.B. Webster, L.P. Sarna and K.N. Graham, 29th Annual Pesticide and Trace Analysis Seminar for Western Canada, Vancouver, B.C., Canada, May 3–5, 1994, Paper No. 13.
[21] R.E. Shirey, G.D. Wachob and J. Pawliszyn, Supelco Reporter, 12 (1993) 8.
[22] G.R.B. Webster, L.P. Sarna and K.N. Graham, in G.K. Ostrander (Editor), Techniques in Aquatic Toxicology, Lewis Publishers, Chelsea, MI, 1995, in press.