



ELSEVIER

Journal of Chromatography A, 788 (1997) 87–94

JOURNAL OF
CHROMATOGRAPHY A

Study of the feasibility of the use of activated carbon membranes for the on-line cleanup of vegetable extracts in the determination of N-methylcarbamate pesticides by liquid chromatography

Luis E. Sojo

ASL Laboratories Ltd., 1988 Triumph St., Vancouver, BC, Canada V5L 1K5

Received 4 March 1997; received in revised form 25 June 1997; accepted 26 June 1997

Abstract

Series of tests were carried out to assess the feasibility of using activated carbon membranes in the off-line and on-line cleanup of vegetable (green peppers) extracts for the analysis of N-methylcarbamate pesticides by HPLC with post-column derivatization and fluorescence detection. The approach was based on retaining the vegetable matrix co-extracted compounds on the carbon membranes, while allowing the pesticides to pass through. In the off-line method 10 ml of toluene-modified acetonitrile extracts were passed through a 22 cm activated carbon membrane, the eluent was collected, concentrated and solvent exchanged to chloroacetic acid buffer at pH 1 to a final volume of 5 ml, prior to injecting 20 μ l into the HPLC system. For the on-line method, a 20 μ l volume of toluene-modified acetonitrile extract was injected directly into the HPLC system. It was found that the membranes were effective in retaining sample interferences and providing off-line and on-line clean extracts. The recovery of the analytes at a 0.25 ppm spike level varied from 87 to 99% for the off-line method and from 56 to 93% for the on-line method. The lower recoveries for the on-line method were due to the retention of some of the pesticides (carbaryl and methiocarb) on membranes during the initial step of the chromatographic gradient. © 1997 Elsevier Science B.V.

Keywords: Vegetables; Food analysis; Sample handling; Membranes; Pesticides; Carbamates

1. Introduction

The determination of N-methylcarbamate pesticides in vegetables is usually carried out by solvent extraction with acetonitrile followed by open column liquid chromatographic cleanup [1–4]. The cleaned extract, after solvent exchange and concentration, is analyzed by high-performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection. Solid-phases such as carbon–Celite [1,3], C₁₈ [4] and aminopropyl [2] have been used for off-line cleanup. An automated method incorporating solid-phase cleanup to the HPLC de-

termination has been recently developed by de Kok and Hiemstra [5]. This method makes use of a commercial automated solid-phase extraction (SPE) cleanup (ASPEC) apparatus, which is connected on-line to the HPLC system. But even in this case, solvent exchange and extract concentration are necessary.

It may be possible to further simplify this analysis by selecting solid-phases which will remove matrix interferences present in the original extract, without having to exchange the extract solvent prior to cleanup. We have recently demonstrated the feasibility of using activated carbon membranes for the

cleanup of vegetable extracts in the determination of pesticide residues by gas chromatography (GC) with mass selective detection [6]. In the case of N-methylcarbamates, which are analyzed by HPLC with post-column derivatization and fluorescence detection, removal of matrix interferences is critical since the analyst does not have the advantage of selecting analytes unique detection signals, as is the case with mass selective detection.

This paper extends the applications of activated carbon membranes for the cleanup of vegetable extracts to the determination of the N-methylcarbamate pesticides, by using a single step on-line solid-phase cleanup with HPLC with post-column derivatization and fluorescence detection.

2. Experimental

2.1. Materials

N-Methylcarbamate pesticides and some of their metabolites including aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, 3-OH-carbofuran, aldicarb, propoxur, carbofuran, carbaryl and methiocarb were obtained from Ultra Scientific (North Kingston, RI, USA), in the form of a 100 ppm methanol cocktail. This solution was diluted accordingly with methanol to produce calibration and spiking solutions. Acetonitrile, toluene and methanol were all of pesticide-grade and obtained from BDH (Vancouver, Canada). *o*-Phthalaldehyde (OPA), OPA diluent, thiofluor and NaOH hydrolysis solution reagents were purchased from Thermo Analytical Solutions (North Vancouver, Canada). Activated carbon mem-

branes (400–450 mg and 2 cm wide) made of acid-washed coconut charcoal were a gift from Susan M. Price of 3M Company (St Paul, MN, USA). These membranes consist of immobilized charcoal particles on a PTFE mesh with approximately 90% (w/w) of carbon per membrane, a surface area greater than 1000 m²/g and a nominal particle size of 15–20 μm. The membranes were cleaned by flushing them with 10 ml of toluene followed by 10 ml of methanol. Green peppers were bought from a local market and analyzed for incurred residues of N-methylcarbamate pesticides using the method described in Ref. [1]. No incurred residues were detected.

2.2. Equipment

The on-line cleanup HPLC system consisted of a Waters 600 quaternary pump equipped with a manual Rheodyne injection valve, a 20 μl sample loop, 4 mm in diameter stainless-steel on-line filter (5 μm) from Alltech Associates (Deerfield, IL, USA), a C₁₈ guard column and a C₁₈ (25 cm×0.1 mm I.D., 5 μm particle size) reversed-phase column both from Pickering Labs. (CA, USA). A Pickering PCX-5100 post-column derivatization module and a Waters 416 scanning fluorescence detector were used for post-column derivatization and detection of analytes.

Activated carbon membrane were cut to fit the size of the 5 μm micro-filter and positioned on top of it. The filter-membrane combination was encased in the filter cartridge as shown in Fig. 1. The filter-membrane cartridge was connected to the injection port through a 6-port Rheodyne switching valve. This valve was in turn connected to a Gilson liquid chromatographic pump Model 302. This pump was

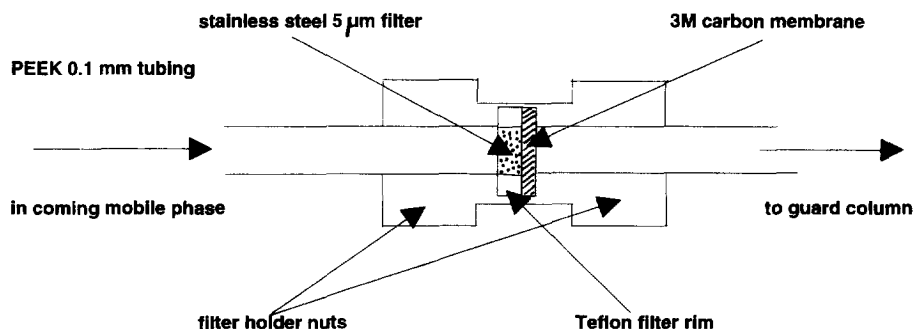


Fig. 1. Activated carbon membrane placement inside on-line micro-filter cartridge.

used to back-flush the on-line filter-membrane cartridge after each extract injection as described in earlier work [6]. The stainless-steel filter side of the filter-membrane cartridge was facing the incoming mobile phase. This configuration was selected since it was found that direct mobile phase pressure on the membrane may result in its rupture.

2.3. HPLC determinations

HPLC determinations were carried out on-line or off-line by manual injections. The mobile phase gradient consisted of 1.7 min of 100% water, followed by a jump (0.01 min) to water–methanol (80:20) with a gradient to water–methanol (25:75) in 44 min. This was followed by a jump (0.01 min) to 100% methanol for 5 min, followed by a 10 min equilibration period with 100% water. Mobile phase flow-rate was kept at 1 ml/min. For on-line determinations, a 30 s methanol “pulse” was added at the beginning of the gradient. Post-column derivatization of N-methylcarbamate pesticides was carried out by the Pickering post-column derivatization PCX-5100 unit, delivering 0.3 ml/min of hydrolysis reagent (0.05 M NaOH) and 0.3 ml/min OPA solution into the HPLC effluent. Detection of pesticides as fluorescent isoindole derivatives was carried out using 340 and 455 nm for excitation and emission wavelengths, respectively. Data were acquired using Chromperfect software from Justice Innovations (CA, USA). Calibration solutions were run daily. Sample concentrations were automatically calculated by the software using the external calibration method.

2.4. Test solutions and spiked green pepper extract preparation

Test, calibration solutions and spikes were prepared at the 0.25 ppm level for all analytes in the selected solvent systems. Green peppers were washed with tap water and homogenized to a pulp in a blender; 5 g portions (wet mass) of the homogenate were spiked with appropriate volume of N-methylcarbamate spiking solution to give a final concentration of 0.25 ppm. Spiked green peppers were extracted with 10 ml of solvent and 1 g of NaCl per 5 g sample, by shaking in a capped centrifuge

tube, followed by centrifugation at 3000 rpm. The supernatants were dried over sodium sulphate before aliquots were taken for HPLC analysis.

2.5. Off-line experiments

Off-line experiments consisted of passing solutions or green pepper extracts of the N-methylcarbamate pesticides (5 ml of 0.25 ppm) through activated carbon membranes (22 mm in diameter) as described earlier [2]. Before HPLC analysis the eluents were concentrated to near dryness under a flow of nitrogen and reconstituted to 5 ml with chloroacetic acid buffer at pH 2. Volumes of 20 μ l of the processed eluents were injected into the HPLC system. Control solutions of the same concentration as in test solutions, not flushed through the membranes, were also analyzed. The purpose of the off-line experiments was to study pesticides interactions with the activated carbon membranes without complications arising from the on-line set-up.

2.6. On-line experiments

On-line experiments involved the analysis of N-methylcarbamate pesticides solutions or extracts. The on-line cleanup HPLC system was operated manually. Volumes of 20 μ l of the extracts were injected into the HPLC system with the membrane in the on-line position for 30 s, after which it was manually switched to the off-line position. Back-flush of the membrane with methanol was then carried out for a couple of minutes while the chromatographic run was in progress [7]. For comparison purposes the extracts from spiked green pepper samples were also cleaned and analyzed as described in Ref. [1].

3. Results and discussion

Off-line membrane filtration tests in acetonitrile indicate that most of the N-methylcarbamates go through without major retention on the activated carbon membrane as shown in Fig. 2. Carbaryl and methiocarb were the only pesticides fully retained. The breakthrough of all the other analytes was not due to membrane saturation but to lack of retention since the pesticides exhibit a preference for the

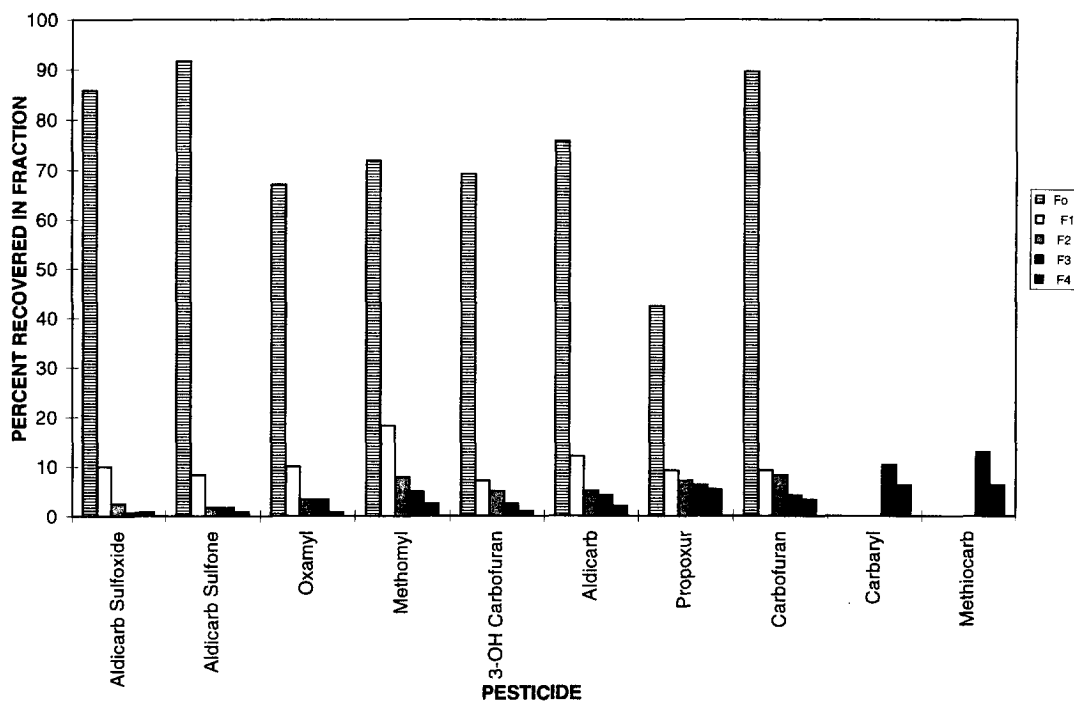


Fig. 2. Recoveries of N-methylcarbamate pesticides after filtering an acetonitrile solution through an activated carbon membrane in the off-line mode. (F0) Acetonitrile solution after passing through the membrane, (F1) first acetonitrile elution (5 ml), (F2) second acetonitrile elution (5 ml), (F3) first toluene elution (5 ml), (F4) second toluene elution (5 ml).

acetonitrile phase instead of the carbon phase. Subsequent flushing of the membranes with acetonitrile (fractions F1 and F2) removed traces of the pesticides still remaining on the membranes, with the exception of carbaryl and methiocarb, which required the use of toluene. But even in this case, these two latter analytes were not fully desorbed from the membrane.

The fraction of the N-methylcarbamate pesticides that remained on the membrane after F0 is likely to be bound to active sites on the carbon and/or diffused into the pores within the carbon particles. In the case of carbaryl and methiocarb, this represented 100% of their initial concentrations. The interactions between these pesticides and the carbon material are stronger than those of the rest of the compounds studied, and seem to be irreversible. Their nature likely being a combination of electrostatic and possible sorption to active sites on the carbon

since this pesticide was expected to behave very much like oxamyl. Slobodnik et al. [8] found that both methomyl and oxamyl showed similar recoveries from these membranes when using dichloromethane–methanol (80:20) mixtures for elution after sorption from water samples, while the recoveries for methomyl were significantly lower than those for oxamyl when using acetonitrile–methanol (80:20) and tetrahydrofuran–methanol (80:20) mixtures for elution. At this point we have no explanation for this behaviour.

Previously we have shown [6] that recovering pesticides containing benzene rings from activated carbon membranes requires the use of toluene as eluting solvent. Using toluene as a solvent modifier improved the recoveries of all pesticides especially of carbaryl and methiocarb as can be seen in Table 1. The fact that these analytes were significantly recovered ($\geq 80\%$) when dissolved in toluene–acetonitrile

Table 1
Effect of solvent composition on the recoveries of N-methylcarbamate pesticides after passing through an off-line activated carbon membrane

Pesticide	Recovery (%)	
	100% ACN	Toluene-ACN (25:75)
Aldicarb sulfoxide	83	109
Aldicarb sulfone	90	104
Oxamyl	80	98
Methomyl	59	84
3-OH-Carbofuran	79	100
Aldicarb	89	102
Propoxur	58	100
Carbofuran	89	104
Carbaryl	nd	79
Methiocarb	nd	81

Pesticides listed in order of chromatographic elution. ACN=acetonitrile.

Average of triplicate determinations. R.S.D. ranged from 5 to 50%.

supports the assumption that the sorption of these two pesticides has some degree of irreversibility or hysteresis. Oxygen containing active sites have been identified in other types of activated carbons [9,10]. Such sites would interact strongly with electron donor groups such as benzene rings present in the molecules of some of the pesticides studied here. There is evidence in the literature [9–11] that these sites can be deactivated by conditioning the carbon with ascorbic acid solutions. We tried such approach with no success. Slobodnik et al. [8] treated these

membranes with an ascorbic acid solution before they carried out their tests, although the authors do not mention whether this procedure was actually useful.

The effect of increasing concentrations of toluene before off-line cleanup on the recoveries of the carbamate pesticides is summarized in Table 2. The toluene-acetonitrile ratio was varied from (0:100) to (50:50). The recovery of all the analytes, in particular carbaryl and methiocarb, significantly improved with increasing concentration of toluene. Toluene solutions of no less than 16% were tested, and although smaller concentrations of toluene may still provide acceptable recoveries, 25% toluene was adopted since it permitted direct comparison with the method using carbon-Celite columns for cleanup [1].

3.1. On-line cleanup of green pepper extracts

One of the aims of the on-line method is to be able to carry out cleanup and HPLC analysis in a single step. The efficiency of the activated carbon membrane in removing matrix interferences is shown in Fig. 3. Although a significant removal of early eluting interferences is accomplished (Fig. 3A,B), it was found that membrane efficiency is highly dependent of its solvent the initial mobile phase composition and the membrane history. The chromatogram in Fig. 3C, which was run immediately after

Table 2
Effect of solvent composition on the recoveries of N-methylcarbamates from spiked green peppers after filtering through an activated carbon membrane in the off-line configuration.

Pesticide	Recovery (%)		
	Toluene-ACN (16:84)	Toluene-ACN (25:75)	Toluene-ACN (50:50)
Aldicarb sulfoxide	71	102	86
Aldicarb sulfone	73	102	84
Oxamyl	62	66	67
Methomyl	101	95	93
3-OH-Carbofuran	71	95	91
Aldicarb	96	97	94
Propoxur	72	91	87
Carbofuran	77	85	87
Carbaryl	71	85	91
Methiocarb	72	77	87

Pesticides listed in order of chromatographic elution. ACN=acetonitrile.

Average of triplicate determinations. R.S.D. ranged from 5 to 50%.

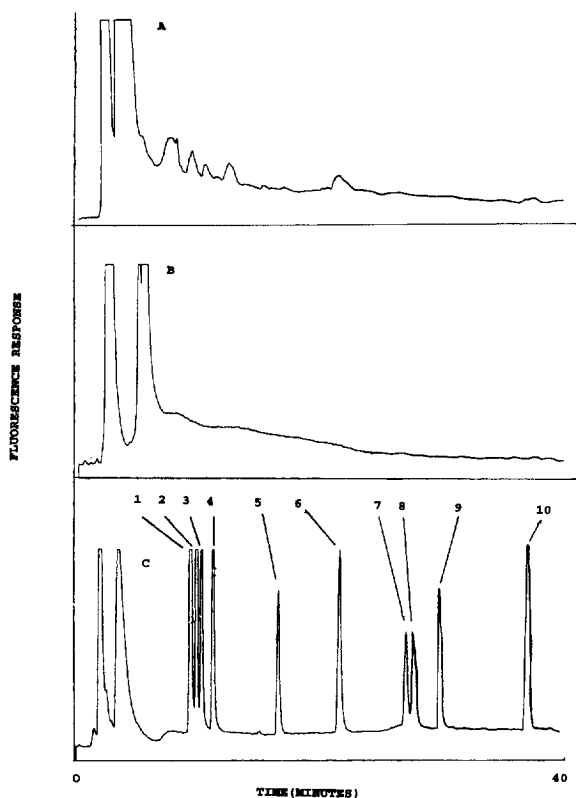


Fig. 3. HPLC chromatograms of (A) a green pepper blank extract without cleanup, (B) the same extract as in (A) but after on-line cleanup with the activated carbon membranes and (C) an extract of a spiked green pepper sample at 0.2 ppm level after on-line cleanup with the activated carbon membrane. HPLC conditions as described in Section 2.3. Recorder attenuation 5 for all injections. Peaks: (1) aldicarb sulfoxide, (2) aldicarb sulfone, (3) oxamyl, (4) methomyl, (5) 3-OH-carbofuran, (6) aldicarb, (7) propoxur, (8) carbofuran, (9) carbaryl and (10) methiocarb.

chromatogram in Fig. 3B, shows a further reduction of the early eluting peaks and a shift to earlier retention times. This shift did not occur to the analytes retention times. Further injections of sample extracts resulted in similar chromatograms to the one shown in Fig. 3C. For this reason, membranes were conditioned overnight by soaking in methanol prior to their placement on-line and flushed with methanol after each injection. The removal of interferences are not only carried out to reduce the probability of false positive results, but also to reduce the likelihood of deteriorating the chromatographic column. Extracts from green peppers and other vegetables contain

large quantities of high-molecular-mass fatty acids which would affect chromatographic separation and require long column equilibration times.

The recoveries of N-methylcarbamates using direct injection of the extracts into the on-line cleanup HPLC system are shown in Table 3. The results of direct injections of acetonitrile solutions into the on-line system are also included for comparison. The recoveries from these solutions are in general lower than those from green peppers extracts. This recovery enhancement in the presence of matrix components is not due to co-eluting matrix interferences. This effect is probably due to matrix components interacting with active sorption sites on the membrane which otherwise would interact with the pesticides. As expected, the recoveries for most of the pesticides improved when toluene was used as modifier in the final extract with the exception of carbaryl and methiocarb which recoveries dropped down. At present we cannot explain this finding, since based on the off-line tests (Table 2), the recoveries for these compounds were expected to increase in the presence of toluene.

The amount of toluene injected into the chromatographic system is quite small. Sample injections were 20 μ l, which translates into 5 μ l of toluene for toluene-acetonitrile (25:75) extracts. This small amount of toluene had no effect on the chromatography and column performance even after a twenty consecutive injections.

3.2. Comparison of cleanup methods

Table 4 summarizes the results for spiked green peppers using off-line carbon-Celite column cleanup [1] and both off-line and on-line activated carbon membranes procedures. With the exception of carbaryl and methiocarb both membrane procedures compared favourably with the established carbon-Celite column procedure; in fact, they showed better reproducibility than the carbon-Celite column method. A significant advantage of the membrane method is that it takes less than a minute to achieve cleanup for the off-line approach and almost no time at all for the on-line configuration, in contrast to the carbon-Celite column method which can take up to a few hours. The membrane cleanup methods use very little solvent in comparison to the column method.

Table 3
Recoveries of N-methylcarbamate pesticides from spiked green pepper using activated carbon membranes in the on-line configuration

Pesticide	Recovery (%)		
	100% ACN ^a	100% ACN ^b	Toluene-ACN ^b (25:75)
Aldicarb sulfoxide	84	82	93
Aldicarb sulfone	69	74	90
Oxamyl	59	69	89
Methomyl	36	90	85
3-OH-Carbofuran	54	73	82
Aldicarb	47	55	85
Propoxur	35	47	73
Carbofuran	35	47	72
Carbaryl	nd	88	64
Methiocarb	nd	70	56

Pesticides listed in order of chromatographic elution. ACN=acetonitrile.

Average of triplicate determinations. R.S.D. ranged from 5 to 50%.

^a No matrix.

^b Green pepper matrix.

The amount of toluene used in the off-line procedure is approximately 2.5 ml per a total of 10 ml sample extract, while in the on-line configuration the amount of toluene used 0.250 ml per 1 ml of sample extract. The slightly better recoveries obtained with the off-line membrane cleanup method indicate that the on-line method still requires some refining. Changes to the HPLC gradient may be necessary to increase the recoveries of carbaryl and methiocarb.

The selection of one membrane procedure over the

other will depend on the application. For samples which cannot be analyzed immediately the off-line procedure may be preferred, since it permits sample extract preservation by exchanging the extract solvent to chloroacetic acid buffer, since some of the N-methylcarbamates may not stable in organic solvents for long periods of time [12]. In the on-line configuration, no solvent exchange was done prior to injection.

Some operational differences between configurations are obvious. In the off-line configuration, approximately a 20 cm² of membrane cross-section is exposed to the incoming sample extract, while in the on-line configuration was approximately 2 cm². The off-line procedure used 10 ml of extract in contrast to the on-line procedure, which used 20 µl. It may be possible to increase sample size for both methods, but this will depend on the membrane capacity for matrix co-extractives. Membrane capacity may be increased by placing more than one membrane in series as shown by Slobodnik et al. [8]. The same authors also found that the membranes could be re-used after appropriate cleanup. We analyzed up to twenty extracts with a single membrane, after back-flushing the membrane in between samples, before membrane deterioration was evident. This was noticed in the on-line configuration, in which shifts in retention times and bad peak shape, in particular for early eluting compounds, were evident.

Table 4
Comparison of off-line and on-line cleanup methods for N-methylcarbamate pesticides in spiked green peppers

Pesticide	Recovery (%)		
	Activated carbon membrane		Carbon-Celite column Off-line
	Off-line	On-line	
Aldicarb sulfoxide	88±2	93±5	94±5
Aldicarb sulfone	87±1	90±5	98±31
Oxamyl	68±8	89±2	97±28
Methomyl	99±2	84±6	101±50
3-OH-Carbofuran	92±1	82±6	99±41
Aldicarb	99±5	85±11	92±32
Propoxur	89±2	73±13	96±31
Carbofuran	83±7	72±10	99±25
Carbaryl	91±7	62±2	97±54
Methiocarb	88±9	56±2	84±13

Pesticides listed in order of chromatographic elution. Average of triplicate determinations.

4. Conclusions

The data presented in this study shows that single activated carbon membranes can be used for normal-phase off-line and on-line cleanup of vegetable extracts in the determination of N-methylcarbamate pesticides by HPLC with post-column derivatization. These carbon membranes effectively removed matrix interferences from the extracts, although their efficiency is very dependent of membrane conditioning and history. Membrane capacity could also be increased by coupling two or more membranes in series. Both on-line and off-line procedures seem to give acceptable recoveries from green peppers spikes at the 0.25 ppm level.

Acknowledgements

The author would like to thank Susan M. Price, Richard Pieper and Craig Markell of 3M Company (St. Paul, MN, USA) for providing the carbon activated membranes.

References

- [1] J. Fillion, R. Hindle, M. LaCroix, J. Selwyn, *J. Off. Assoc. Anal. Chem.* 78 (1995) 1252.
- [2] D.M. Holstege, D.L. Schergerg, E.R. Tor, L.C. Hart, F.D. Galey, *J. Off. Assoc. Anal. Chem.* 77 (1994) 1263.
- [3] C. Daniel, *J. Off. Assoc. Anal. Chem.* 71 (1988) 542.
- [4] M.J. Page, M. French, *J. Off. Assoc. Anal. Chem.* 75 (1992) 1073.
- [5] A. de Kok, M. Hiemstra, *J. Assoc. Off. Anal. Chem.* 75 (1992) 1063.
- [6] L.E. Sojo, A. Brocke, J. Fillion, S.M. Price, *J. Chromatogr.* 788 (1997) 141.
- [7] L.E. Sojo, D.S. Gamble and D.W. Gutzman, *J. Agric. Food Chem.*, (1997) in press.
- [8] J. Slobodnik, O. Oztezkizan, H. Lingeman, U.A.Th. Brinkman, *J. Chromatogr. A* 750 (1997) 227.
- [9] M. Battista, A. DiCorcia, M. Marchette, *Anal. Chem.* 61 (1989) 935.
- [10] R. Jonge Robert, A.M. Breure, J.G. van Andel, *Water Res.* 30 (1996) 883.
- [11] A. DiCorcia, S. Marchese, R. Samperi, *J. Chromatogr.* 642 (1993) 163.
- [12] S. Chiron, A. Valverde, A. Fernandez-Alba, D. Barceló, *J. Assoc. Off. Anal. Chem.* 78 (1995) 1346.