ELSEVIER

Journal of Chromatography A, 754 (1996) 437–444

JOURNAL OF CHROMATOGRAPHY A

Optimization of a matrix solid-phase dispersion method for the analysis of pesticide residues in vegetables

E. Viana, J.C. Moltó*, G. Font

Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of València, C/Vicent Andrès Estellès s/n, 46100 Burjassot, València, Spain

Abstract

A multiresidue method based on matrix solid-phase dispersion (MSPD) is studied to determine chlorfenvinfos, chlorpyrifos, fenarimol, iprodione, procimydone, propiconazole, tetradifon, triadimefon and vinclozolin in artichokes, green beans, lettuces and tomatoes. Alumina, silica and Florisil were assessed as extracting phases, and the extracts from Florisil were the cleanest. To facilitate manual extraction, sand was added to the sample together with the dispersing phase. Three eluting systems were then studied, and dichloromethane proved to be the best. Further purification can be performed using solid-phase cleanup after diluting extracts with aqueous solutions. Octyl- and octadecyl-silica, modifications of the aqueous diluted extracts and several eluting solvents were studied. Determination was done by capillary gas chromatography (GC) with electron-capture detection, and confirmed by GC–MS using the electron impact mode and the selected ion monitoring. The proposed MSPD method was used to analyze 48 samples taken in the course of a year. Procymidone, vinclozolin, chlorpyrifos and chlorfenvinfos were identified in 10 samples at levels below the maximum residue levels allowed by the Spanish Government.

Keywords: Vegetables; Food analysis; Matrix solid-phase dispersion; Sample preparation; Pesticides

1. Introduction

The Valencian Community (Spain) has many agricultural areas where pesticides are used intensely. In these areas, vegetable production is very important, and therefore large amounts of acaricides, insecticides and fungicides are applied on these crops.

A large number of the pesticides now on the market include heterogeneous compounds [1] and have been found as contaminants in ground water [2]. For the present study, nine frequently utilized pesticides (chlorfenvinfos, chlorpyrifos, fenarimol, iprodione, procymidone, propiconazole, tetradifon, triadimefon and vinclozolin) were selected.

Strict control is necessary to protect the consumer from the harmful impact of pesticide residues. However, most known screening protocols involve tissue preparation and several extraction, purification and concentration steps, which make them expensive to perform when many samples must be analysed.

To eliminate some of the difficulties associated with solvent extraction of pesticide residues, a multiresidue technique called matrix solid-phase dispersion (MSPD) has recently been developed [3]. The mechanisms of the MSPD include sample homogenisation, cellular disruption, exhaustive extraction, fractionation and purification in a simple process. In MSPD, a small amount of sample (0.1–5

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00538-9

g) is blended together with the selected solid-phase, and their mixture is used to fill a chromatographic column. The elution of the MSPD column with a solvent or solvent sequence can normally provide clean extracts, which, if necessary, can be further purified.

Analyses of extracts are generally performed by gas chromatography (GC) with selective detectors such as electron-capture (ECD) [4–6], electrolytic conductivity [4], flame photometric [4–7] or nitrogen–phosphorus detection [5]. GC–MS is the most useful technique in environmental organic analysis [5,8,9]. The use of the electron impact mode (EI) is usually sufficient to reach good sensitivity and reveal structural information.

MSPD has been reported to extract chlorinated pesticides from beef fat [10], fish [11] and shell fish [12,13], chlorinated and other pesticides from milk and meat [14], and fruits or vegetables [5,15]. Few data are available in the literature on the extraction of the selected pesticides from vegetables using MSPD. In the present report, the solid-phase, eluting and volume solvent used in the MSPD extraction have been assessed. Octyl- and octadecyl-silica [16], and chemical reagents [17] were employed to purify pesticide extracts and real samples have been analyzed using the MSPD method.

2. Experimental

2.1. Reagents and standards

The selected pesticides chlorfenvinfos, chlorpyrifos, fenarimol, iprodione procymidone, propiconazole, tetradifon, triadimefon and vinclozolin, with purities between 95 and 99%, were purchased from Dr. Ehernstorfer Lab. (Promochem, Wesel, Germany).

Solid-phases used for MSPD were (I) neutral aluminium oxide (70/230 mesh, activity I) from Merck, Darmstadt, Germany, II) Florisil (60–100 mesh) from Aldrich-Chemie, Steinheim, Germany, and III) silica (230–400 mesh) from Scharlau, Barcelona, Spain. Acid washed sand was obtained from Scharlau.

Solid-phases used for cleanup were (I) octyl-silica Bondapak (37-55 μm), and (II) octadecyl-silica Bondapak (37–55 μm), both purchased from Millipore, Waters Chromatography Division, Milford, MA, USA.

Acetone, dichloromethane (DCM), dimethylformamide (DMF), ethyl acetate, *n*-hexane (b.p. 40–60°C) and methanol, all free from interfering residues as tested by GC–ECD (concentration 100:1) were purchased from Promochem.

Stock (1000 μg/ml) and working solutions of pesticides were prepared in methanol and preserved at 4°C.

2.2. Apparatus

For routine analysis, a Konik KNK 2000C gas chromatograph (Sant Cugat del Vallés, Barcelona, Spain) equipped with a ^{63}Ni electron-capture detector and a Spectra-Physics SP 4290 integrator was utilised. For confirmation purposes, a Fisons 8000 series gas chromatograph coupled with a quadrupole mass spectrometer TRIO 1000 (Fisons Instruments, Milan, Italy) was used.

In both gas chromatographs, a fused-silica capillary column DB-5 (5% phenyl-methylsiloxane), 0.25 μm thickness film, 30 m×0.25 mm I.D., provided by J&W Scientific (Folsom, CA, USA) was used.

2.3. Gas chromatographic conditions

In both gas chromatographs, injectors operated in splitless mode (0.7 min) at 285°C. Oven temperature was programmed as follows: initial temperature 50°C (0.8 min) was increased at 30°C/min to 140°C and was held for 2 min, then increased at 5°C/min to 280°C, and held for 12 min. Helium was used as a carrier gas at a flow-rate of 2.8 ml/min. In the KNK 2000C, the ECD temperature was set at 300°C. In the Trio 1000, source and interface transfer line temperatures were set at 200°C and 250°C respectively. Electron impact ionisation mode with 70 eV electron energy was selected. To obtain complete mass spectra of selected pesticides, the mass range 60–370 u was scanned. From real samples, ions summarised in Table 1 were monitored using the selected ion monitoring (SIM) mode.

Table 1
Pesticide retention times, molecular masses, and selected ions for performing GC–MS (SIM)

Pesticide	Retention times (min)	Molecular mass	Selected ions, *m/z*
Chlorfenvinfos	25.40	358	267[a], 269, 323
Chlorpyrifos	23.65	350	197, 199
Fenarimol	38.70	331	107, 139[a], 319
Iprodione	31.42	330	187, 314[a]
Procymidone	25.77	283	96[a], 283
Propiconazole	30.02 and 30.35	342	173, 175, 259
Tetradifon	38.33	354	159[a], 227, 356
Triadimefon	23.95	294	128, 208[a]
Vinclozolin	21.89	285	198, 212, 285

[a] Corresponding to the most abundant ion within the range 60–370 u.

2.4. Extraction and purification columns

Extraction was carried out in glass chromatographic columns (40 cm×3 cm I.D.) containing 0.1 g of silanized glass-wool at the bottom. Purification was conducted in glass mini-columns (100 mm×9 mm I.D. with coarse frit No. 3) containing 0.5 g of C_8 covered with a silanized glass-wool plug. These mini-columns were activated by passing 10 ml of methanol and 10 ml of water.

2.5. Method

2.5.1. Extraction

Approximately 200 g of vegetable sample was chopped to obtain a material which was easy to mix. Five grams of a representative portion of the chopped sample, 10 g of Florisil and 8 g of acid washed sand were transferred into a glass mortar and was gently ground with a pestle to obtain a homogeneous flowing powdered sample. The mixture was transferred into the extraction column and covered with 0.1 g of silanized glass-wool. Mortar and pestle were rinsed with 50 ml of dichloromethane and the solvent was poured into the extraction column. Another 50 ml of DCM was added, the column was eluted and the extract was concentrated to ca. 10 ml in a Kuderna-Danish concentrator at 60°C. One ml of methanol was added and concentration was continued under gentle stream of nitrogen at 45°C in a thermal block to 1 ml. Two μl of the methanol extract was analyzed on GC.

2.5.2. Purification

The 1 ml methanol from the extraction step was diluted to 100 ml of water and the solution was forced through a C_8 purification mini-column under vacuum. The mini-column was washed twice with 10 ml of distilled water, and the washings were discarded. Adsorbed residues were then eluted with 5 ml of ethyl acetate. The extract was separated from the residual water with a Pasteur pipette and concentrated to 1 ml at 45°C under a gentle stream of nitrogen, and 2 μl was analyzed by GC.

2.5.3. Recovery studies

To study the accuracy of the extraction step, 5 g vegetable samples free of the selected pesticides [analyzed by GC–MS (SIM)] were spiked with 50 μl of the methanol working solution to produce samples containing selected pesticides at levels ranging from 0.01 to 0.5 μg/g. To calculate relative standard deviation (R.S.D.%), 5 replicate spikes were analysed.

The accuracy of the purification step was studied by spiking 1 ml of methanol extract obtained from clean crops with 50 μl of the methanol working solution, and then diluting to 100 ml with distilled water. Five replicate samples were analysed to calculate recovery means, and their respective R.S.D.s.

2.5.4. Modifications of the procedure

At the extraction step, some variations of the proposed method were studied. Alumina and silica were checked as solid supports for matrix dispersion and the proposed eluting agent DCM was modified to contain 10% of acetone or 10% of DMF.

At the purification step the following modifications were studied: (I) the dilution of methanol extract to 100 ml of water containing 1% and 10% methanol or DMF, (II) the use of C_{18} instead of C_8, and (III) the elution of the C_8 with ethyl acetate or with a mixture of DCM–hexane (85:15).

3. Results and discussion

3.1. Extraction

Because vegetable samples free of pesticide residues are not commercially available, crops on which synthetic compounds such as fertilizers, pesticides or additives are not used were employed as blanks. The use of ecological samples does not guarantee the absence of interfering compounds for there can be natural endogenous substances or residues of persistent pesticides remaining in the ecosystems. For this reason blank samples were first analysed by GC–MS (SIM) before being spiked, and none of the selected ions were found at the corresponding retention times of the selected pesticides (Table 1). The ECD profiles also showed that blanks were free of the selected pesticides.

The proposed extraction is a modification of that reported by Kadenczki and al. [15]. Recoveries and their respective R.S.D. obtained following the described extraction method for the four vegetables are shown in Table 2. The mean recovery was 91%, and the limits of quantification obtained using tomato samples were 23 ng/g for chlorfenvinfos, 7 ng/g for chlorpyrifos, 29 ng/g for fenarimol, 39 ng/g for iprodione, 34 ng/g for procymidone, 40 ng/g for propiconazole, 8 ng/g for tetradifon, 34 ng/g for triadimefon and 7 ng/g for vinclozolin. Sand added at the extraction step does not influence recoveries, but it facilitates the mechanical dispersion of the sample, the column elution, and the emptying of the column content. Recoveries for most pesticides are simillar to those reported by Kadenczki and al. [15].

Fortification levels (see Table 2) were selected to reach the maximum residue limits (MRL) established by the Spanish Government [18], which range from 0.01 μg/g for fenarimol in artichokes to 10 μg/g for iprodione in lettuce.

The use of alumina or silica as solid-phases for microdispersion of tomato samples produces recoveries similar to those obtained with Florisil, except for fenarimol (120%) and iprodione (130%) when alumina was used. In these cases the high recoveries can be attributed to the presence of interfering endogenous substances, and purification is therefore recommended. Chromatographic profiles obtained from tomato MSPD with Florisil, alumina and silica are shown in Fig. 1; Florisil gave the cleanest chromatograms with lowest baselines. Since the extracts of artichokes and green beans obtained using alumina and silica showed some interfering peaks, Florisil was selected for MSPD. The nature of the interfering peaks is unknown; its mass spectra contain neither characteristic ions from organochlorine or organophosphorus compounds, nor from selected pesticides.

To improve the selectivity of the eluting agent, the addition of a small proportion (10%) of a polar solvent, such as acetone or DMF, to the DCM was tested. The results obtained with tomato samples are shown in Table 3. DCM is a common solvent used for the extraction of pesticides from vegetables, and it was found here to be the most efficient single

Table 2
Average recoveries (R) and relative standard deviations (RSD) obtained by MSPD extraction of fortified vegetables

esticide	Artichoke		Green bean		Lettuce		Tomato		Level of spike (ng/g)
	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	
Clorfenvinfos	94	15	85	9	91	12	86	9	55
Chlorpyrifos	89	13	89	8	85	8	93	8	16
Fenarimol	90	10	104	9	97	9	92	7	65
Iprodione	80	8	91	7	85	7	97	5	95
Procymidone	90	16	95	14	87	15	90	11	90
Propiconazole	97	16	75	14	97	15	90	13	90
Tetradifon	95	14	80	11	87	11	87	10	18
Triadimefon	94	8	101	4	85	6	94	5	80
Vinclozolin	100	10	96	8	94	9	96	8	20

(n=5).

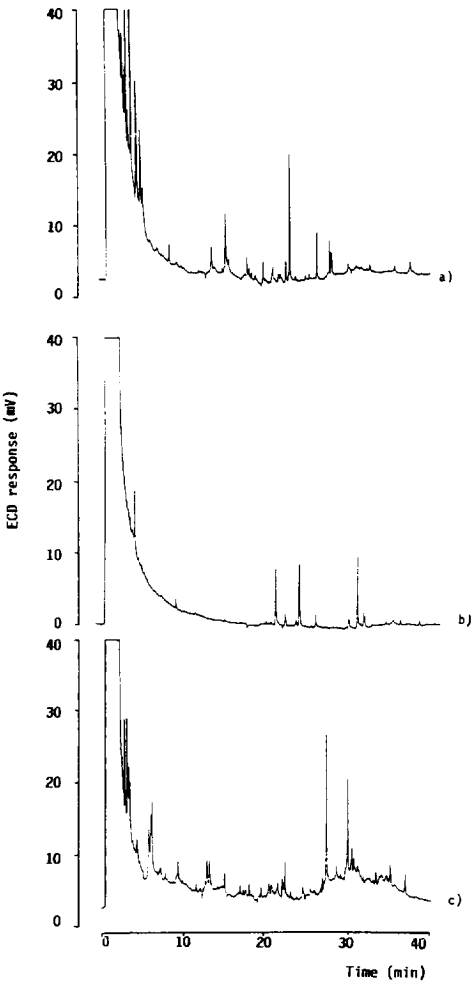

Fig. 1. ECD chromatograms obtained by MSPD of pesticide-free tomato samples using: (a) silica, (b) Florisil, and (c) alumina.

component solvent. When the polarity of the elution mixture was reduced, the extracts became cleaner but recoveries decreased.

The volume of eluting solvent required to obtain the highest recoveries was found to be 100 ml of DCM. When only 50 ml of it was used, the average recoveries diminished to 84%. Iprodione, which decreased to 73%, was the compound most affected.

3.2. Purification

Once the extraction method had been selected, a purification procedure based on the use of octyl-silica was studied. Generally when performing the selected extraction procedure, extracts were clear enough to allow direct quantification, but some artichoke or green bean extracts required additional purification. These extracts were diluted to 100 ml with distilled water and then purified on C_8.

Table 3
Average recoveries (R) and relative standard deviations (RSD) obtained from spiked tomato samples by modifying the proposed elution solvent (DCM) for MSPD extraction

Pesticide	DCM–acetone (9:1)		DCM–DMF (9:1)	
	R (%)	RSD (%)	R (%)	RSD (%)
Chlorfenvinfos	67	9	35	8
Chlorpyrifos	60	9	40	10
Fenarimol	65	10	83	9
Iprodione	66	11	45	10
Procymidone	68	13	71	13
Propiconazole	70	12	45	14
Tetradifon	65	10	42	11
Triadimefon	65	8	63	7
Vinclozolin	73	11	70	9

($n=5$).
Level of spike as in Table 2.

To improve the selectivity of the purification process, the diluted aqueous extract was added with a polar solvent such as methanol and DMF. As shown in Table 4, when 10% of methanol was present instead of 1%, a slight, non significant decrease in recoveries was observed. The use of 1% of DMF is, on the whole, equivalent to 1% of methanol, but the presence of 10% decreased the recoveries for most of the selected pesticides. The effect of purification on green bean extracts free of pesticides when directly analysed (a) and after clean-up with C_8 (b) is compared in Fig. 2.

The chromatographic profiles of extracts obtained after purification with C_{18} showed more peaks of endogenous substances than those obtained using C_8, since recoveries did not differ significantly, the use of C_8 was preferred. A similar effect has been observed when comparing C_8 and C_{18} for extracting organochlorine pesticides from medicinal infusions [19].

Two eluting systems for the C_8 purification column were examined, and the results are shown in Table 5. Ethyl acetate produced better mean recoveries (93%) than the DCM–hexane mixture (85:15) (72%).

Table 4
Average recoveries (R) and relative standard deviation (RSD) obtained for C_8 purification of spiked tomato extracts which were diluted to 100 ml of water containing different proportions of methanol or DMF

Pesticide	Methanol				DMF			
	1%		10%		1%		10%	
	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)
Chlorfenvinfos	75	7	72	9	89	9	90	8
Chlorpyrifos	73	8	69	7	92	8	80	7
Fenarimol	91	7	70	10	99	9	84	7
Iprodione	93	5	70	11	99	6	81	10
Procymidone	87	8	78	7	99	10	97	8
Propiconazole	90	10	82	10	87	12	89	9
Tetradifon	85	9	75	9	84	10	96	9
Triadimefon	86	5	75	8	89	7	89	9
Vinclozolin	90	5	83	11	99	7	96	10

($n=5$).
Levels of spike were equivalent to those from Table 2.

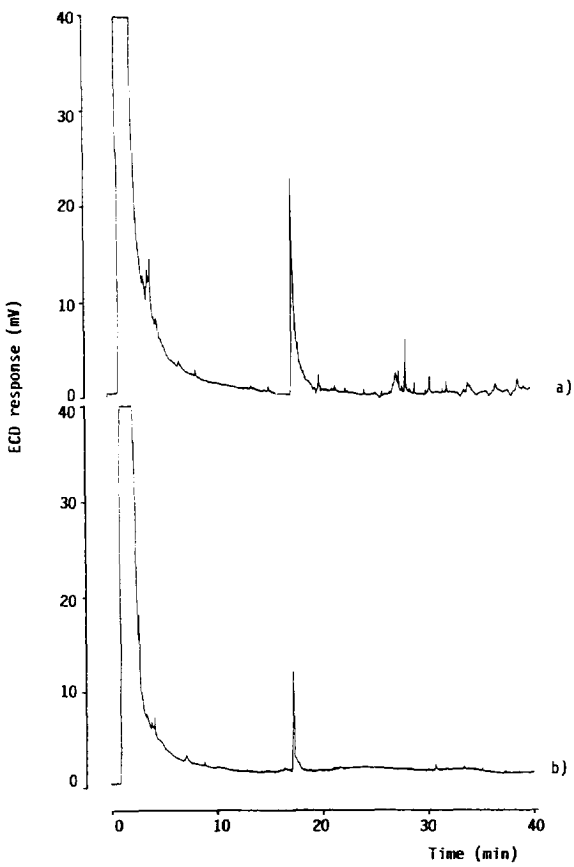

Fig. 2. ECD chromatograms of a pesticide-free green bean extract: (a) without any purification, and (b) after proposed C_8 purification.

Table 5
Average recoveries (R) and relative standard deviations (RSD) obtained with two elution systems for the C_8 purification of spiked tomato extracts

Pesticide	Ethyl acetate		DCM–hexane (85:15)	
	R (%)	RSD (%)	R (%)	RSD (%)
Chlorfenvinfos	89	9	80	8
Chlorpyrifos	92	8	83	8
Fenarimol	99	9	63	7
Iprodione	99	6	60	5
Procymidone	99	10	73	10
Propiconazole	87	12	90	9
Tetradifon	84	10	74	11
Triadimefon	89	7	66	7
Vinclozolin	99	7	63	9

($n=5$).
Levels of spike were equivalent to those from Table 2.

Since the selected pesticides have medium to low polarities, the use of a large volume of water to dilute extracts at the purification step can decrease recoveries and, on the other hand, can help to eliminate some hydrophilic interfering compounds which may also pass through the C_8 packing. For this reason, several volumes of water were therefore tested for diluting extracts (500, 100 and 50 ml), but no significant differences in recoveries and no evident improvement in chromatographic profiles were found regardeless of the volume used. Because the

purification time is prolonged unnecessarily when 500 ml is used, and since using 50 ml produces some precipitation of compounds, 100 ml was selected for purification.

Another way to purify extracts is to use chemical reagents such as potassium hydroxide in ethanol, 90% sulphuric acid, chromium (VI) oxide in acetic acid or trifluoroacetic acid that can selectively destroy interfering compounds. These reagents have been recommended in previous reports because of they are economical and effective for purification and confirmation of organochlorine [19], organophosphorus and other pesticides, and polychlorinated biphenyls (PCB) [17]. However, these procedures were useless when applied to extracts of vegetables containing chlorfenvinfos, chlorpyrifos, fenarimol, iprodione, procymidone, propiconazole, tetradifon, triadimefon or vinclozolin, since the selected pesticides were destroyed after the contact with these chemical reagents.

3.3. Analysis of samples

The optimized analytical procedure was used to analyze artichoke, lettuce, green bean and tomato samples obtained from local markets. Samples were taken from packing cases over a one-year period in groups of 24 units (green beans) and 12 units (tomatoes, lettuces and artichokes) each month. Samples were refrigerated at 4°C and extracted within a week. The extracts were kept at 4°C until the analysis was done.

Extracts were first quantified by GC–ECD and then confirmed by GC–MS (SIM). As shown in Table 6, residues of pesticides were found in 10 of the 48 analyzed sets of samples. Procymidone and vinclozolin were frequently present, and chlorfenvinfos, chlorpyrifos, triadimefon and iprodione were occasionally detected. The residues detected were always below the MRL permitted by the Spanish Government. Fig. 3 shows an ECD chromatogram of a green bean sample where procymidone was detected. The other unknown peaks were not recognized as pesticides by GC–MS.

In summary, MSPD using Florisil provides good recoveries and clean extracts for most samples. The recoveries using alumina and silica are similar, but their extracts contain more endogenous compounds than those coming from Florisil. Even when using Florisil, some artichoke and green bean extracts require additional purification. This can be done by diluting the methanol extract with water and re-extracting pesticides with a C_8 mini-column. The proposed procedures require small amounts of samples and solvents, involve few steps, and sample manipulation is simple. When the proposed pro-

Table 6
Results of market sample analyses

Month	Sample	Identified pesticide	Concentration (mg/kg)	MRLs (mg/kg) [18]
January	Artichokes	Chlorfenvinfos	0.172	0.5
		Vinclozolin	0.009	0.05
March	Tomatoes	Chlorpyrifos	0.065	0.5
		Triadimefon	0.173	0.5
April	Lettuces	Iprodione	0.168	10
		Fenarimol	0.140	0.2
May	Green beans	Procymidone	0.186	2
June	Tomatoes	Vinclozolin	0.060	3
		Procymidone	0.142	2
August	Lettuces	Procymidone	0.166	5
	Tomatoes	Chlorpyrifos	0.120	0.5
		Vinclozolin	0.012	3
		Procymidone	0.170	2
September	Tomatoes	Procymidone	0.188	2
October	Artichokes	Chlorfenvinfos	0.240	0.5
November	Artichokes	Chlorfenvinfos	0.356	0.5
		Triadimefon	0.189	0.5

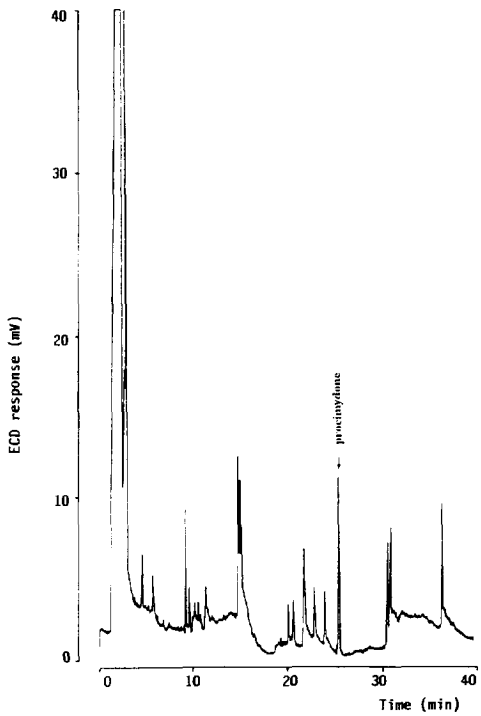

Fig. 3. ECD chromatogram of a green bean sample containing 0.186 μg/g of procymidone.

cedures were applied to 48 real market samples, residues were detected in 10 samples at levels below the allowed MRL.

Acknowledgments

The authors wish to thank the CITYT (AMB93-1215) for financial support for this project, and the Science and Education Ministry for a grant to E. Viana.

References

[1] Y. Nakamura, Y. Tonogai, Y. Sekiguchi, Y. Tsumura, N. Nishida, K. Takakura, M. Isechi, K. Yuasa, M. Nakamura, N. Kifune, K. Yamamoto, S. Terasawa, T. Oshima, M. Miyata, K. Kamura and Y. Ito, J. Agric. Food Chem., 42 (1994) 2508.
[2] Y. Picó, J.C. Moltó, M.J. Redondo, E. Viana, J. Mañes and G. Font. Bull. Environ. Contam. Toxicol., 53 (1994) 230.
[3] S.A. Barker, A.R. Long and C.R. Short, J. Chromatogr., 475 (1989) 353.
[4] H.M. Pylypiw, J. Assoc. Off. Anal. Chem., 76 (1993) 1369.
[5] C.M. Torres, Y. Picó and J. Mañes, Chromatographia, 41 (1995) 685.
[6] D.M. Holstege, D.L. Scharberg, E.R. Tor, L.C. Hart and F.D. Galey, J. Assoc. Off. Anal. Chem., 77 (1994) 1263.
[7] M. Miyahara, Y. Okada, H. Takeda, G. Aoki, A. Kobayashi and Y. Saito, J. Agric. Food Chem., 42 (1994) 2795.
[8] W. Liao, T. Joe and W.G. Cisick, J. Assoc. Off. Anal. Chem., 74 (1991) 554.
[9] D. Barceló, Anal. Chim. Acta., 263 (1992) 1.
[10] A.R. Long, M.M. Soliman and S.A. Barker, J. Assoc. Off. Anal. Chem., 74 (1991) 493.
[11] A.R. Long, M.D. Crouch and S.A. Barker, J. Assoc. Off. Anal. Chem., 74 (1991) 667.
[12] H.M. Lott and S.A. Barker, J. Assoc. Off. Anal. Chem., 76 (1993) 67.
[13] H.M. Lott and S.A. Barker, J. Assoc. Off. Anal. Chem., 76 (1993) 663.
[14] S.A. Barker, A.R. Long and M.E. Hines, J. Chromatogr., 629 (1993) 23.
[15] L. Kadenczki, Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi, G. Reese and W. Ebing, J. Assoc. Off. Anal. Chem., 75 (1992) 53.
[16] Y. Picó, J.C. Moltó, J. Mañes and G. Font, J. Microcol. Sep., 6 (1994) 331.
[17] E. Viana, J.C. Moltó, J. Mañes and G. Font, J. Chromatogr. A, 678 (1994) 109.
[18] RD 280/1994, B.O.E. No 58, Madrid, 9 March 1994.
[19] J.C. Moltó, B. Lejeune, P. Prognon and D. Pradeau, Int. J. Environ. Anal. Chem., 54 (1994) 81.