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Journal of Chromatography A, 839 (1999) 101–107

JOURNAL OF CHROMATOGRAPHY A

Matrix solid-phase dispersion microextraction and determination by high-performance liquid chromatography with UV detection of pesticide residues in citrus fruit

A.I. Valenzuela*, R. Lorenzini, M.J. Redondo, G. Font.

Laboratori de Toxicologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrès Estellès s/n, 46100 Burjassot, València, Spain

Received 28 July 1998; received in revised form 2 December 1998; accepted 30 December 1998

Abstract

A multiresidue method based on matrix solid-phase dispersion (MSPD) microextraction was studied to determine the carbamate, benfuracarb, and urea insecticides, diflubenzuron, flufenoxuron hexaflumuron and hexythiazox, used in control of citrus pests. Optimisation of different parameters, such as the type of solid support for matrix dispersion, elution solvents and the clean-up step were carried out. The method used 0.5 g of orange sample, C_8 bonded silica as MSPD sorbent and dichloromethane as eluting solvent. Recoveries, at spiked concentrations below the maximum residue levels established by Spanish Government, were between 74 and 84% with relative standard deviations ranging from 2 to 4%. The limits of quantification were from 0.15 to 0.25 μg/g using high-performance liquid chromatography with UV detection at 200 nm. The method may be useful as a screening protocol for the determination of these newly developed pesticides in citrus samples.

Keywords: Citrus fruits; Pesticides; Benfuracarb; Diflubenzuron; Flufenoxuron; Hexaflumuron; Hexythiazox

1. Introduction

The use of carbamate and urea insecticides is fundamental to control pests in citrus fruits. The carbamate benfuracarb and ureas diflubenzuron, flufenoxuron, hexaflumuron and hexythiazox, are among the most widely used in citrus of the Valencian Community. At present, these provide an unquestionable benefit for citrus production, however the presence of residues in fruits can affect consumer health. Because of this, the regulatory authorities have established maximum residue limits (MRLs) for these insecticides [1].

Trace analysis of these substances requires techniques which allow detection of as many compounds as possible with only a few extraction and clean-up steps. Most protocols for analysis of pesticides in fruits and vegetables involve several extraction, purification and concentration steps, which make them expensive to perform and time consuming when many samples must be analysed [2,3].

Liquid–liquid extraction (LLE) methods have been used to extract carbamates and urea insecticides from several vegetable matrices. The most frequently employed solvents are dichloromethane [4] and different mixtures like dichloromethane–cyclohexane

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00055-2

[5] or dichloromethane–acetone [6]. However, large interferences are encountered when these techniques are used before chromatographic determination because the solvents are non-selective and therefore tend to extract endogenous material from the sample which interferes with the analysis. Because of this, a complex treatment including a purification step is needed to attain a satisfactory limit of detection. Various clean-up methods have been developed involving procedures using cartridges of C_{18} [6,7], Florisil [8], or activated carbon [9].

The sample size for residue analysis varies from a few grams to over 100 g and the volume of solvent for extraction ranges from 40 ml to several hundred millilitres. Considerable reduction of solvent consumption can be achieved by miniaturising the scale of sample extraction. Adoption of techniques such as MSPD can help to reduce considerably the size of sample and the solvent consumption [10,11]. MSPD isolation technology involves blending a small amount of sample with a solid support, followed by washing and eluting with a small amount of solvent to extract a wide range of compounds [2]. Kadenczki et al. [12] have demonstrated the applicability of Florisil to a large number of pesticide residues in fruits and vegetables. Other researches have also used Florisil [13,14] and C_{18} [15,16] to extract pesticides from fruits and vegetables.

Literature reports of the determination of urea insecticide residues in vegetables are very few, despite the extensive use of these pesticides. Reversed-phase columns with UV detection are predominantly employed for the determination of ureas and carbamates because they are, respectively, non-volatile and thermolabile. This methodology has been applied to the determination of benzoylureas in apples [4] and benzoylureas and carbamates in fruit pulp [6]. Mass spectrometry coupled with GC and HPLC is increasingly used [17]. Diflubenzuron has been determined by HPLC–thermospray (TSP)-MS [18], and by HPLC–atmospheric pressure chemical ionisation (APCI)-MS [5].

The purpose of this report was to develop a MSPD method for the microextraction of four urea insecticides and benfuracarb in oranges with the potential of more efficient processing of samples. Different parameters were studied to optimise the extraction method and HPLC determination for their use as screening protocol.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile, methanol, acetone, ethyl acetate and dichloromethane, all HPLC grade, were supplied by Merck (Darmstadt, Germany) and Baker (Deventer, Netherlands). Ultra pure water was prepared by ultrafiltration of distilled water with a Milli-Q system (Millipore, Bedford, MA, USA).

Solid phases used for MSPD were C_8 and C_{18} bonded silica (40–60 μm) from Analysis Vínicos (Tomelloso, Spain), cellulose microcristalline and silica (230–400 mesh) from Merck.

The standards carbamate benfuracarb (88.2%) and urea insecticides diflubenzuron (99.7%), flufenoxuron (99.3%), hexaflumuron (98%), hexythiazox (99.3%), were supplied by Promochem (Wesel, Germany).

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

2.2. Extraction procedure

Citrus samples (200 g of whole fruit) were prepared using a food processor and mixed thoroughly, according to the directive 79/700 (CEE). An aliquot (0.5 g) of the sample was placed into a mortar (50 ml capacity) and 0.5 g of the C_8 sorbent were added and gently blended for 5 min using a pestle, to obtain a homogeneous mixture. This mixture was introduced into a 100×9 mm I.D. glass chromatographic column with a coarse frit (No. 2) and covered with a plug of silanized glass wool at the top. The dispersion was washed and conditioned with 10 ml of distilled water. Vacuum by water aspiration was applied to obtain a constant flow. The dispersion was then dried by drawing room air through the column using a vacuum. Pesticide residues were eluted with 15 ml of dichloromethane. The eluate was evaporated to dryness with air at 50°C. A 0.5-ml volume of acetonitrile was added and

throughly mixed in ultrasonic bath for 5 min. The extract was filtered with acrodisk (0.2 μm) of PTFE and 20 μl were injected into the liquid chromatograph.

Recovery studies were carried out by spiking fresh samples (0.5 g) of orange with the insecticide fortification solution at different levels: 10 μg/g for the optimisation assays and at low level (1 μg/g for benfuracarb, 0.5 μg/g for diflubenzuron, 0.3 flufenoxuron and hexaflumuron and 0.2 μg/g for hexythizox) to calculate the accuracy and reproducibility of the proposed method below the legislated MRLs.

The extraction procedure described above is based on the data obtained from different optimisation assays. They involved the study of different solid supports for matrix dispersion in which cellulose, silica, C_8 and C_{18} were tested, and also different elution solvents (dichloromethane, methanol, acetonitrile, ethyl acetate and acetone). Moreover, the optimisation of the procedure included an assessment of an additional purification step with columns filled with different solid phases like celite, C_2, cellulose, silica, GCB and cyanopropil.

2.3. HPLC analysis

A Shimadzu (Kyoto, Japan) SCL-6A System liquid chromatograph equipped with two LC 6A pumps, a Rheodyne Model 7125 injector (20 μl loop), a detector Merck Hitachi 4250 UV–vis and a Shimadzu C-R4A Chromatopac data processor was used.

The analytical column was a reversed-phase Kromasil C_{18}, 250×4.6 mm I.D., 5-μm particle size, and a guard cartridge C_{18} of 30×4.6 mm I.D. The separation of the selected insecticides was by gradient elution. The mobile phase was acetonitrile–water, delivered at a flow-rate of 0.5 ml/min, with a composition gradient acetonitrile–water increasing from 88% acetonitrile to 90% over 13 min, decreasing from 90 to 88% over 2 min, and finally re-equilibrating at 88% for 15 min.

The insecticide concentrations in the final extract were calculated by comparing the peaks areas for each compound with those obtained from standard solutions.

3. Results and discussion

A satisfactory resolution was achieved on an HPLC reversed-phase column packed with C_{18} using gradient elution with acetonitrile–water. The time of analysis did not exceed 20 min. Linearity was verified in triplicate with six concentrations ranging from 1.0 to 0.03 μg/g (1, 0.500, 0.250, 0.125, 0.060 and 0.030 μg/g). The regression coefficients were between 0.9995 and 0.9997.

Extraction and clean-up conditions had to be carefully selected to achieve the highest recovery for the pesticides contained in the plant material while eliminating most of the interfering matrix components. It has been demonstrated that mixing biological samples with silica bonded supports provokes disruption of the sample structure by the mechanical blending, while the phase induces a lot of chemical interactions within the sample components. Furthermore chemical interactions between the matrix and the phase allow specific solvent elution of the compounds of interest [2]. The most suitable extraction conditions (type of solid phase, eluent and clean-up) were assessed. Silica, cellulose, C_8 and C_{18} were checked as solid supports for matrix dispersion. The results reported in Fig. 1 show that the best recoveries were obtained using C_8 or C_{18} for all compounds, using dichloromethane as eluent. C_8 and C_{18} proved to be better orange dispersants than the other solid supports assessed due to their hydrophobic characteristics which provided high affinity for non-polar compounds. The use of silica and cellulose failed to extract the studied pesticides. In particular, a poor recovery of the pesticides was observed when cellulose extraction was performed. For these reasons, only C_8 and C_{18} were used subsequently.

In trace analysis, when residue levels are close to the limit of sensitivity of the instrument, even trace matrix components can interfere with determination, so that the polarity of solid phases for the extraction and the elution solvents should be adequately selected. Dichloromethane, methanol, acetonitrile, ethyl acetate and acetone were tested as elution solvents (Table 1). Although the use of different eluting solvents produced similar recoveries, the dichloromethane was considered optimal for the extraction

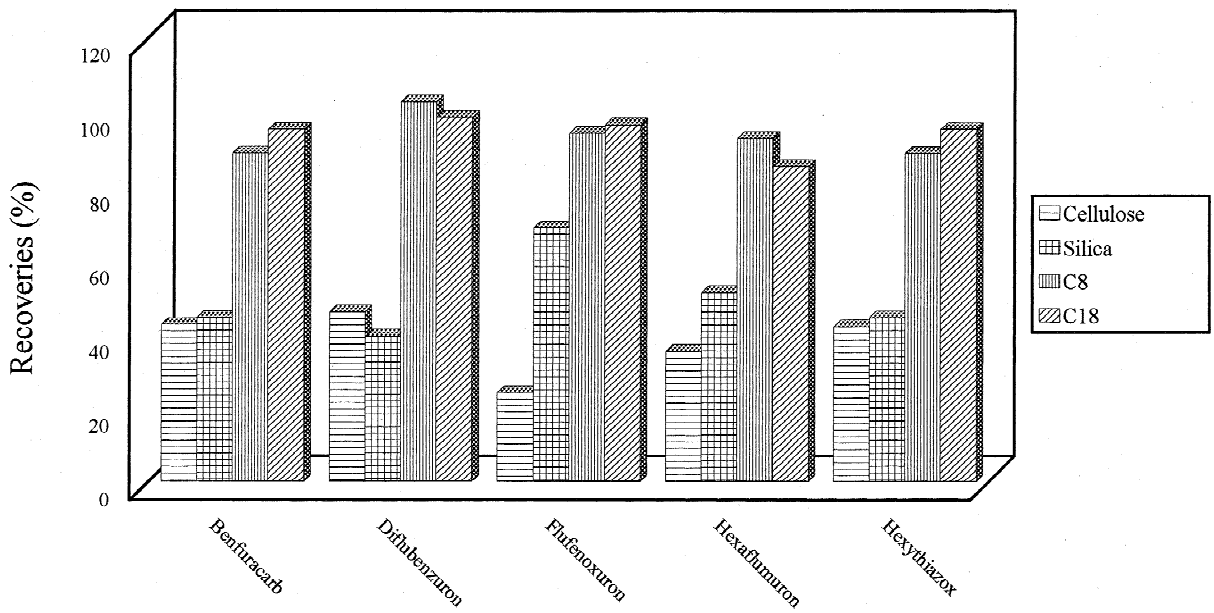

Fig. 1. Effect on the pesticide recoveries of different solid supports for matrix dispersion at spiked level of 10 μg/g.

because it gave the cleanest extracts. Elution of the MSPD column, prepared as described with C_8 or C_{18} solid supports, with methanol, acetonitrile, ethyl acetate or acetone produced large interferences, as evidenced by the colour of the residue observed after solvent evaporation and by the number and intensity of peaks recorded by HPLC analysis of the extracts. Using acetone as eluting solvent the high recoveries obtained for flufenoxuron can be attributed to the presence of interfering endogenous substances, and purification is therefore recommended.

Despite the fact that recoveries were similar when either C_8 or C_{18} was used as solid support and dichloromethane as eluent, the use of C_8 was preferred because it provided a clean-up chromatogram, although the possibility of carrying out an additional purification step was studied. The blended matrix (composed of orange and C_8) was introduced into a glass column containing 0.5 g of different solid phases like celite, C_2, cellulose, silica, graphitized carbon black (GCB) and cyanopropil (CN) at the bottom. The results shown in Fig. 2 demonstrated

Table 1
Average recoveries (R)±relative standard deviations (R.S.D.) obtained with different elution solvents by MSPD extraction of orange samples to spiked level 10 μg/g[a]

	Dichloromethane		Methanol		Acetonitrile		Ethyl acetate		Acetone	
	C_{18}	C_8	C_{18}	C_8	C_{18}	C_8	C_{18}	C_8	C_{18}	C_8
Benfuracarb	100±6	94±7	98±7	98±3	90±11	89±3	98±6	102±3	92±5	102±3
Diflubenzuron	98±3	102±2	98±5	101±5	102±3	97±7	87±4	100±6	100±8	103±3
Flufenoxuron	96±8	94±2	99±5	99±6	101±3	94±5	93±6	102±6	107±2	120±12
Hexaflumuron	85±3	93±8	89±9	86±2	88±4	88±5	80±3	86±3	88±4	90±3
Hexitiazox	95±9	90±7	94±6	84±5	92±5	93±7	85±3	89±4	98±7	88±7

[a] n=5.

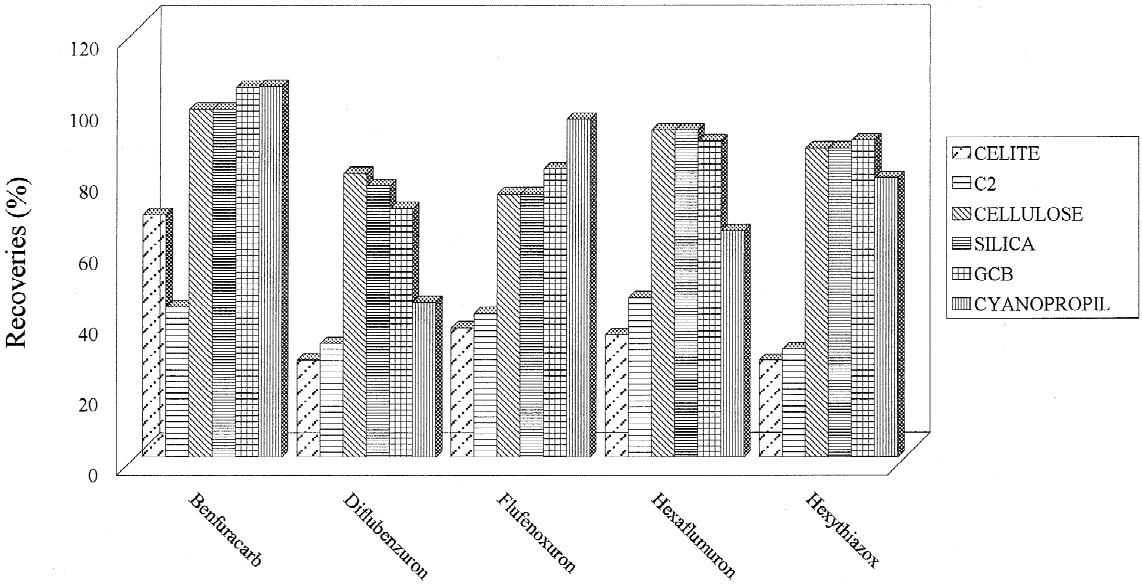

Fig. 2. Effect on the pesticide recoveries of different solid phases for clean-up procedures at spiked level of 10 μg/g.

that clean-up with celite and C_2 produced a stronger decrease in the recoveries of all analysed pesticides. The purification with cyanopropil also showed a decrease in the recoveries, more pronounced for diflubenzuron, hexaflumuron and hexythiazox.

The use of cellulose, silica and GCB seemed useful, because the extracts were less coloured than those obtained by dispersion with C_8. However the recoveries decreased, mainly for diflubenzuron and flufenoxuron, and no evident improvement in chromatographic profiles was found, so these solid phases were not considered further.

The conditions, which yielded the maximum recoveries of the studied pesticides included C_8 as solid support for micro-dispersion and dichloromethane as the eluting solvent. Accuracy was calculated as the percentage of recovery and reproducibility, expressed as R.S.D. (%) are shown in Table 1 at level of fortification of 10 μg/g.

Recovery experiments were also carried out at a low fortification level (between 0.2 and 1 μg/g, depending on the selected pesticide) to calculate the accuracy and reproducibility of the proposed method below the MRLs. The recoveries were 80% for benfuracarb, 78% for diflubenzuron, 84% for flufenoxuron, 74% for hexaflumuron and 75% for hexythiazox, with R.S.D. values ranging from 2 to 4%. Fig. 3 shows the HPLC–UV chromatograms, obtained by the MSPD procedure described, for a non-fortified citrus sample and for a citrus sample fortified at a level below the MRLs.

The limits of quantification were established for the MSPD procedure with C_8, after the whole processing of orange samples. They were 0.25 μg/g for diflubenzuron, flufenoxuron and hexaflumuron, and 0.15 μg/g for benfuracarb and hexythiazox. They were below the MRLs set by the Spanish government for these pesticides in citrus samples which are 2 μg/g for benfuracarb, 1 μg/g for diflubenzuron and hexyithiazox and 0.3 μg/g for flufenoxuron and hexaflumuron [1].

4. Conclusions

The described microextraction procedure is very simple, rapid and requires only small sample sizes and solvent volumes. This method constitutes a significant advance in simplicity and efficiency, that makes it possible to screen many samples and use for routine monitoring. Future research is being con-

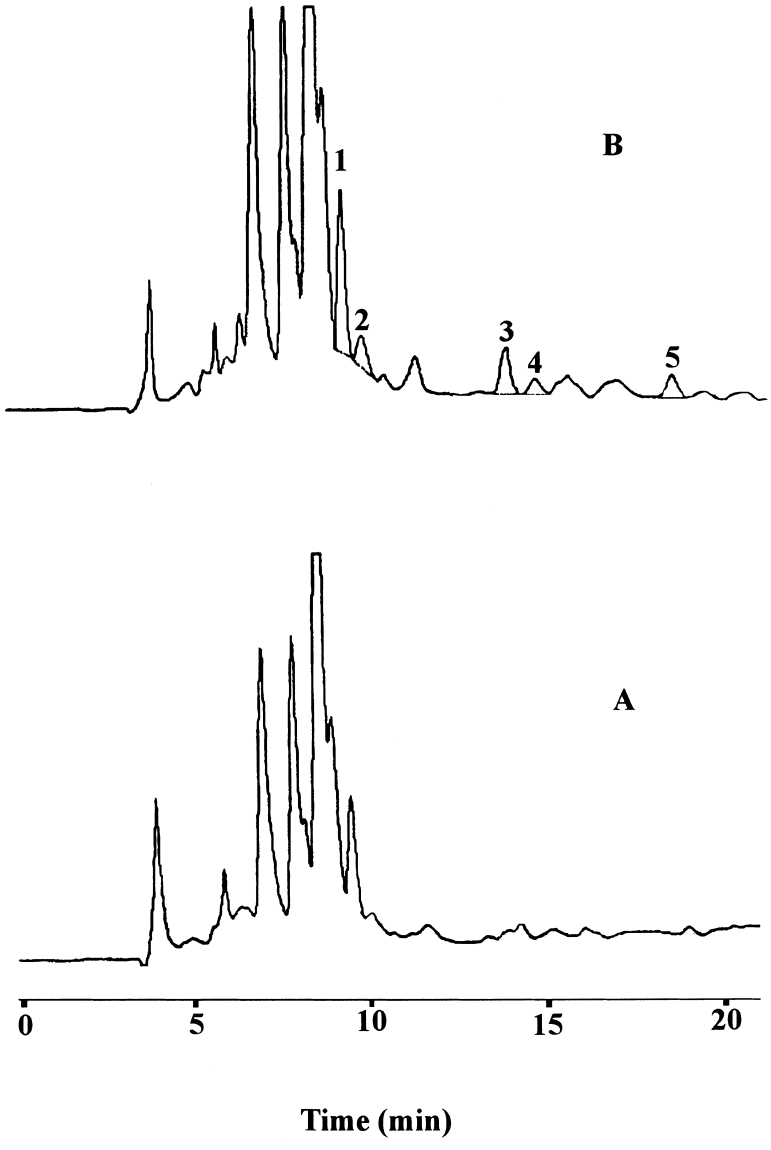

Fig. 3. (A) Chromatogram of a non-fortified orange sample. (B) Chromatogram showing the separation of the pesticides in a fortified orange sample (level of fortification): 1=Diflubenzuron (0.5 μg/g), 2=Hexaflumuron (0.3 μg/g), 3=Benfuracarb (1 μg/g), 4=Hexythiazox (0.2 μg/g) and 5=Flufenoxuron (0.3 μg/g).

ducted using HPLC–MS for unambiguous identification of these pesticides in difficult extracts.

Acknowledgements

The authors thank the Generalitat Valenciana (GV 97-AG-03-13) for financial support for this project, and the Agencia Española de Cooperación Internacional for the grant received by A.I. Valenzuela.

References

[1] Orden Ministerial, B.O.E. No 298, Madrid, 11 December 1996.

[2] Y. Picó, J.C. Moltó, J. Mañes, G. Font, J. Microcol. Sep. 6 (1994) 331.
[3] J. Sherma, J. Assoc. Off. Anal. Chem. Int. 80 (1997) 283.
[4] T. Tomsej, J. Hajslova, J. Chromatogr. A 704 (1995) 513.
[5] K.A. Barnes, R.J. Fussell, J.R. Startin, S.A. Thorpe, S.L. Reynolds, Rapid Comm. Mass Spectrom. 9 (1995) 1445.
[6] C. Bicchi, C. Balbo, A. Binello, A. D'Amato, J. High Resolut. Chromatogr. 19 (1996) 105.
[7] C. Bicchi, C. Balbo, A. D'Amato, O. Panero, Chromatographia 43 (1996) 439.
[8] A. Di Muccio, D.A. Barbini, T. Generali, P. Pelosi, A. Ausili, F. Vergori, I. Camoni, J. Chromatogr. A 765 (1997) 39.
[9] L.E. Sojo, A. Brocke, J. Fillion, S.M. Price, J. Chromatogr. A 788 (1997) 141.
[10] J. Sherma, J. Anal. Chem. 65 (1995) 1R.
[11] H.B. Wan, M.K. Wong, J. Chromatogr. A 754 (1996) 43.
[12] L. Kadenzcki, Z. Arpad, I. Gardi, A. Ambrus, L. Gyorfi, G. Reese, W. Ebing, J. Assoc. Off. Anal. Chem. Int. 75 (1992) 53.
[13] E. Viana, J.C. Moltó, G. Font, J. Chromatogr. A 754 (1996) 437.
[14] Y.C. Ling, I.P. Huang, J. Chromatogr. A 695 (1995) 75.
[15] C.M. Torres, Y. Picó, M.J. Redondo, J. Mañes, J. Chromatogr. A 719 (1996) 95.
[16] C.M. Torres, Y. Picó, J. Mañes, J. Chromatogr. A 778 (1997) 127.
[17] J.L. Tadeo, in: L.M.L. Nollet (Editor), Handbook of Food Analysis, Marcel Dekker, New York, 1996, Ch. 30, p. 1291.
[18] J.P.G. Wilkins, Anal. Proc. 30 (1993) 306.