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Application of solid-phase partition cartridges in the determination of fungicide residues in vegetable samples

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ABSTRACT

Disposable, ready-to-use cartridges filled with a macroporous diatomaceous material are used to extract in a single step fungicide residues with dichloromethane from aqueous acetone extracts of vegetables. This procedure takes the place of some functions (such as separating funnel partition, drying over anhydrous sodium sulphate and clean-up) usually performed by separate steps in classical schemes. Fourteen fungicides (dichloran, vinclozolin, chlorthalonil, triadimefon, dichlofluanide, procymidone, hexaconazole, captan, folpet, ditalimfos, iprodione, captafol, pyrazophos and fenarimol) were determined using the described procedure with recoveries between 83 and 107% at spiking levels ranging for the different compounds from 0.04 to 0.40 mg/kg. Crops subjected to the described procedure included lettuce, strawberry, apple, yellow pepper and peach, and gave extracts containing a mass of co-extractives between 5 and 30 mg. Compared with classical schemes, the described procedure is simple, less labour intensive, allows parallel handling of several extracts and does not require preparation and maintenance of equipment. Troublesome emulsions such as those frequently observed in separating funnel partitioning do not occur.

INTRODUCTION

Polar, water-miscible solvents, such as acetone [1–6], acetonitrile [7–10] or methanol [11] are the most frequently used solvents for the extraction of pesticide residues from vegetable samples in multi-residue procedures. With these solvents, pesticide residues are usually separated from crude aqueous acetone (or aqueous acetonitrile or aqueous methanol) extracts by dilution with salt solution and multiple separating funnel partitions into dichloromethane to remove hydrophilic unwanted co-extractives. Under these conditions, a wide range of both polar and non-polar pesticide residues can be recovered [2–6]. The dichloromethane is dried by passage through a column of anhydrous sodium sulphate and subjected to clean-up before the final determination. In multi-residue procedures, the clean-up is generally based on one or a combination of basic clean-up steps, such as size-exclusion chromatography [4,5,12–18], sweep co-distillation [19–23], column chromatography on Florisil [24–26], alumina [27,28], silica gel [4,29,30] or charcoal and its mixtures [2,7,31]. For certain crops and/or levels of determination, the crude dichloromethane extracts can be used without further clean-up [6].

In general, the drawbacks of the above-mentioned and other similar procedures are the amounts of solvents and reagents required, the washing and preparation of glassware, the occurrence of troublesome emulsions in the aqueous acetone–dichloromethane partition stage with certain vegetables, the preparation and maintenance of costly apparatus and, most important, the number of handling operations, which strongly affect the throughput of the residue laboratory.

On the basis of our experience in the control of vegetables for pesticide residues, a major part of residues occurring especially on leafy vegetables are

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attributable to fungicides. Therefore, we sought a non-conventional procedure, applicable to several fungicide compounds, for the rapid separation of these residues from crude aqueous acetone extracts, which could avoid the use of a separating funnel and provide at the same time a certain clean-up.

As we have reported previously on the advantages of the use of solid-phase partition cartridges in the field of pesticide residue determination [32–37], we tried a similar approach for the determination of different classes of fungicides in vegetable samples.

EXPERIMENTAL

Reagents

Dichloromethane and acetone were analytical-reagent grade solvents redistilled from an all-glass apparatus. Extrelut-20 cartridges obtained from Merck (Darmstadt, Germany) were washed with 6 *M* HCl until yellow colour was no longer eluted from the column and washed with water until neutrality. The water remaining in the column was removed with 100 ml of acetone and the acetone was removed with an upward stream of nitrogen at 1 l/min. Pesticide reference standards from the collection in this laboratory were kindly supplied by the main manufacturer of the pesticides and were >99% pure.

Apparatus

GC analyses were carried out on a Hewlett-Packard Model 5890 gas chromatograph with electron-capture detection (ECD). An HP 17 wide-bore, fused-silica column (cross-linked 50% phenyl–50% methylsilicone) was used. The gas flow-rates were carrier gas (helium) 7 ml/min, split vent 9 ml/min and septum purge vent 1 ml/min, the column head pressure was 10.5 kPa and the auxiliary gas to the detector was nitrogen at 60 ml/min. The column oven temperature programme was 50°C held for 2 min, increased to 180°C at 10°C/min, then to 270°C at 5°C/min and finally held at 270°C for 20 min. The injector was splitless, temperature 240°C and purge-off time 60 s. The detector temperature was 300°C. Quantification was carried out by the external standard method.

Procedure

Prepare aqueous acetone extracts of fruits and vegetables by homogenizing 100 g of vegetable with 200 ml of acetone, filtering and diluting with acetone washings to 350 ml according to ref. 2. Take an aliquot of 20 ml of the extract equivalent to *ca.* 5.7 g of crop and transfer it on to the top of an Extrelut-20 column. Allow the liquid to drain and wait 10 min to obtain an even distribution on the filling material. Pass through the column, from bottom to top, a nitrogen flow of 1 l/min for 20 min. Disconnect the Extrelut-20 column from the gas line, attach to the column outlet a 32 × 0.70 mm I.D. Luer-lock needle (supplied with the column) as a flow restrictor and elute the column with five 20-ml portions of dichloromethane. Concentrate to a small volume using a rotary evaporator (40°C; reduced pressure), then to dryness by manually rotating the collecting flask. Dilute to a suitable volume with acetone and analyse by GC–ECD.

For recovery experiments, add suitable amounts of standards to the chopped vegetables in the homogenization jar. Allow the solvent to evaporate, then proceed with the extraction.

RESULTS AND DISCUSSION

As discussed in the Introduction, liquid–liquid partitioning is used to separate compounds of interest from the bulk of the crude extract. In conventional procedures the liquid–liquid partitioning is carried out by shaking the two phases in a separating funnel, and repeating this step several times to attain completeness of the transfer process. Some drawbacks are associated with this kind of operation.

Basically the same operation can be performed on solid-phase, ready-to-use, disposable cartridges filled with a macroporous diatomaceous earth, which is used to hold one of the liquid phases (the crude aqueous acetone extract) while the other (the partition solvent) is simply poured in portions on to the cartridge and allowed to drain. This type of cartridge is commercially available from different manufacturers. We used Extrelut-20 cartridges that can hold *ca.* 20 ml of crude aqueous acetone extract, leaving *ca.* 1 cm of the bed at the bottom unwetted.

Before running the dichloromethane (the partitioning solvent) through the column, acetone is partially removed with an upward stream of nitrogen. This reduces the acetone in the partition

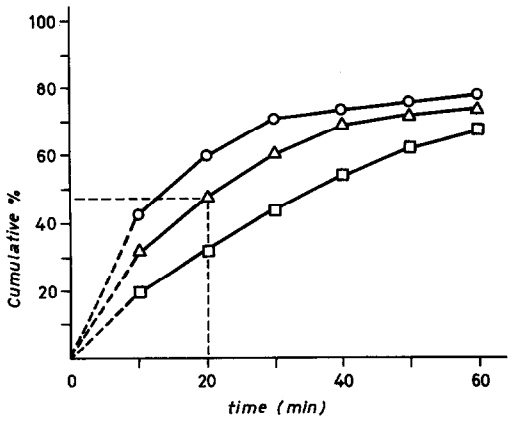

Fig. 1. Cumulative percentage mass loss (y-axis) of Extrelut-20 cartridges loaded with 20 ml of aqueous acetone extract using different nitrogen flow-rates: ○ = 2; △ = 1; □ = 0.5 l/min.

solvent, thus preventing the carryover of water and, by reducing the eluting strength of the draining mixture, improving the clean-up. Different combinations of flow and time parameters have been tried (see Fig. 1). In the first application we reported [32], we used a nitrogen flow of 2 l/min for 30 min, which removed a great proportion of acetone and consequently green pigments of vegetables were retained on the column, when it was eluted with low-polarity solvents (light petroleum or 25% dichloromethane in light petroleum of b.p. 40–60°C). However, as we are interested in a general purpose procedure, we now use conditions (1 l/min for 20 min) that leave more acetone on the column (*ca.* 50% of the original content), thus allowing the recovery of more polar compounds, such as the fungicides under consideration. Under these conditions the mass of material remaining in the extract after the solid-phase partitioning is in the range 5–30 mg, having loaded on to the column the equivalent of 5.7 g of different fruits and vegetables. This mass range is of the same order as that obtained by subjecting the aqueous acetone extract to the classical, time-consuming sequence of separating funnel partitioning into dichloromethane, drying over anhydrous sodium sulphate, solvent exchange and clean-up. However, compared with the classical schemes, the same performance with our method is obtained in a shorter time (*ca.* 60 min), with very simple operations, and by using only one disposable item and a reduced volume of solvent. Crops subjected to the described procedure included lettuce, strawberry, apple, yellow pepper and peach. Although the extract from lettuce contains green pigments, it is amenable to capillary GC with splitless or direct (in liner) injection techniques. Figs. 2–4 show chromatograms obtained from the analysis of representative crops containing incurred fungicide residues, which show a satisfactory clean-up of the crop extract. Fig. 5 shows a chromatogram of the standard mixture of fourteen fungicides.

The fourteen fungicides assayed with the described procedure were dichloran, vinclozolin, chlorthalonil, triadimefon, dichlofluanide, procymidone, hexaconazole, captan, folpet, ditalimfos, iprodione, captafol, pyrazophos and fenarimol. For recovery experiments, the pesticides were added to a vegetable in a homogenization jar, the extraction was carried out with acetone and a portion of the acetone extract was processed according to the described procedure. In Table I are presented the results of the recovery experiments. The recoveries of the fourteen fungicides were satisfactory (between 83 and 107%) when the solid-phase cartridge was eluted with 100 ml of

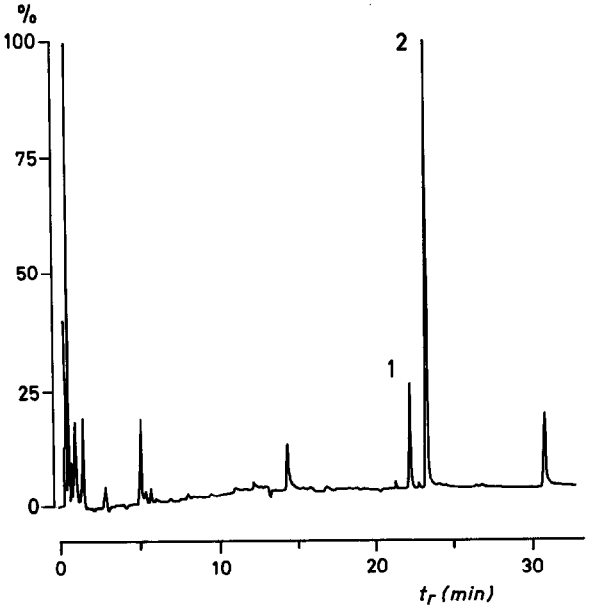

Fig. 2. GC–ECD of lettuce extract with incurred residues: 5.7 g in 250 ml, 1 μl injected. 1 = Vinclozolin (4.3 mg/kg); 2 = chlorthalonil (9.8 mg/kg).

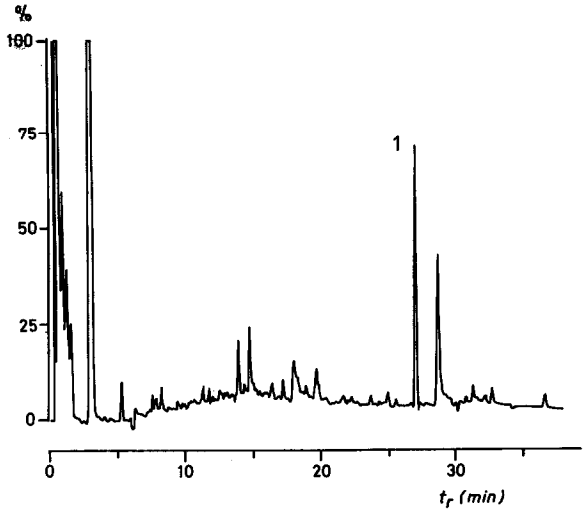

Fig. 3. GC–ECD of apple extract with incurred residues: 5.7 g in 25 ml, 1 μl injected. 1 = Procymidone (2.1 mg/kg).

dichloromethane at spiking levels ranging from 0.04 to 0.40 mg/kg for the different fungicides. In the development of the method consistently low recoveries of captan, folpet and captafol were observed when Extrelut-20 cartridges were used as supplied. The cause was attributed to the cartridges and, when the compounds could not be eluted with high volumes of dichloromethane, a reaction with or

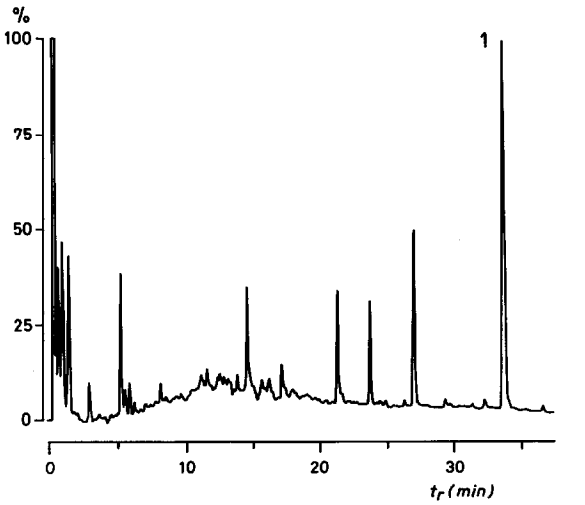

Fig. 4. GC–ECD of strawberry extract with incurred residues: 5.7 g in 40 ml, 1 μl injected. 1 = Iprodione (8.0 mg/kg).

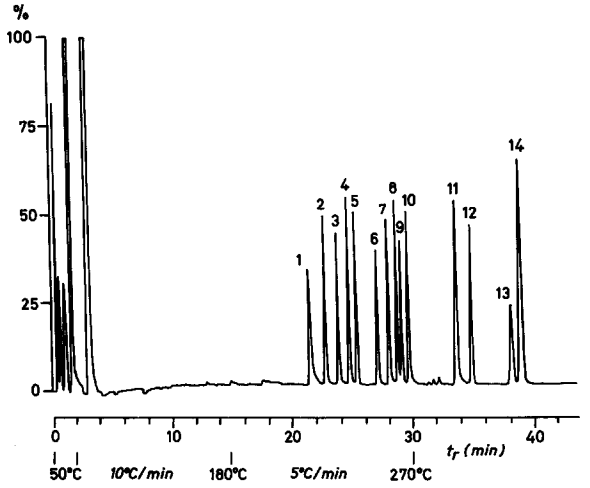

Fig. 5. GC–ECD of the standard mixture of fourteen fungicides. 1 = Dichloran (0.1 ng); 2 = vinclozolin (0.2 ng); 3 = chlorthalonil (0.1 ng); 4 = triadimefon (0.32 ng); 5 = dichlofluanid (0.50 ng); 6 = procymidone (0.7 ng); 7 = hexaconazole (0.30 ng); 8 = captan (0.24 ng); 9 = folpet (0.21 ng); 10 = ditalimfos (0.40 ng); 11 = iprodione (1.0 ng); 12 = captafol (0.39 ng); 13 = pyrazophos (1.00 ng); 14 = fenarimol (0.50 ng).

TABLE I

RECOVERIES OF FOURTEEN FUNGICIDES ADDED TO "BLANK" LETTUCE SAMPLES DETERMINED BY THE DESCRIBED PROCEDURE, AND COMPARISON BETWEEN ACID-WASHED AND NON-ACID-WASHED EXTRELUT-20 COLUMNS

Fungicide	Spiking level (mg/kg)	Mean reovery ± R.S.D. (%) ($n = 6$)	
		Acid-washed	Non-acid-washed
Dicloran	0.04	94 ± 6	92 ± 5
Vinclozolin	0.08	101 ± 8	107 ± 11
Chlorthalonil	0.04	107 ± 10	89 ± 10
Triadimefon	0.13	91 ± 10	87 ± 11
Dichlofluanid	0.20	88 ± 11	76 ± 12
Procymidone	0.28	93 ± 11	87 ± 12
Hexaconazole	0.12	92 ± 11	87 ± 11
Captan	0.10	85 ± 13	67 ± 13
Folpet	0.08	83 ± 24	67 ± 15
Ditalimfos	0.16	90 ± 19	78 ± 14
Iprodione	0.40	93 ± 17	88 ± 14
Captafol	0.16	92 ± 20	50 ± 17
Pyrazophos	0.40	94 ± 18	90 ± 16
Fenarimol	0.40	92 ± 17	89 ± 16

irreversible adsorption by the filling material was assumed. The results in Table I show that good recoveries for these three compounds could be obtained with acid-washed cartridges. The performance of the method was also tested by analysing crops containing incurred residues with both the described procedure and the conventional procedure of separating funnel partitioning. The results, given in Table II, show satisfactory agreement.

The main feature of the described procedure is that the column appears to perform several functions in a single step, *viz.*, the removal of water and hydrophilic co-extractives, the transfer of pesticide residues into a low-boiling solvent and a low-activity adsorption clean-up. The last function is not easily recognized when the extract is analysed by GC–ECD, but it is more apparent when alkali flame ionization detection (AFID) is used, as can be seen from Fig. 6, where the GC–AFID (the GC conditions were the same as for GC–ECD) of an apple analysed for triazophos (an organophosphorus compound not considered in this work) by acetone extraction and separating funnel partitioning according to ref. 2 is compared with GC–AFID performed after acetone extraction and Extrelut-20 partitioning (the procedure described here). In classical schemes, the same functions are carried out through separate, time-consuming and labour- and glassware-intensive operations. Unlike the classical separating funnel partitioning, with the described procedure the extraction is rapid, emulsions do not occur and addition of salt solution and drying of the extraction solvent with anhydrous sodium sulphate are not necessary. In comparison with the described procedure, the procedure reported by Hopper [38] for partitioning of organophosphorus pesticide residues on a solid-phase partition column requires very large volumes of solvents and reagents to condition the column prior to use and appears lengthy. Compared with instrumental clean-up techniques (*e.g.*, size-exclusion chromatography and sweep co-distillation), the described procedure is very simple,

TABLE II

COMPARISON BETWEEN RESULTS OBTAINED IN THE ANALYSIS OF VEGETABLE SAMPLES WITH INCURRED FUNGICIDE RESIDUES USING THE CONVENTIONAL AND THE PROPOSED PROCEDURES

Sample	Fungicide	Residues (mg/kg)	
		Conventional procedure[a]	Proposed procedure
Apple	Procymidone	1.8	2.1
Celery	Vinclozolin	1.7	1.6
	Vinclozolin	0.06	0.05
	Iprodione	0.1	0.2
Lettuce	Vinclozolin	14.0	13.8
	Vinclozolin	2.6	4.3
	Chlorthalonil	4.6	3.8
	Chlorthalonil	11.6	9.8
	Procymidone	0.8	0.7
Strawberry	Chlothalonil	1.2	0.9
	Procymidone	3.1	2.6
	Procymidone	0.5	1.0
	Vinclozolin	1.1	1.0
	Iprodione	9.6	8.8

[a] Acetone extraction and separating funnel partitioning into dichloromethane according to ref. 2.

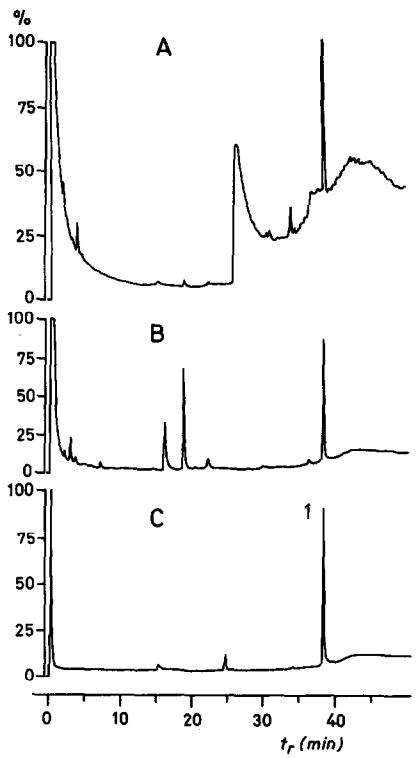

Fig. 6. GC–AFID of an apple extract obtained by (A) acetone extraction and partitioning into dichloromethane according to ref. 2, 25 g in 2 ml, 1 μl injected, and (B) acetone extraction and Etrelut-20 partitioning, 5.7 g in 1 ml, 2 μl injected. (C) Triazophos standard (2 ng). GC conditions as for GC–ECD.

rapid and inexpensive and does not require the preparation and maintenance of costly apparatus or skilled operators.

CONCLUSIONS

Unlike the classical procedures, separation of fungicide residues from hydrophilic co-extractives is carried out in a single step on ready-to-use, disposable cartridges filled with a macroporous diatomaceous earth. The essential features of this procedure include high efficiency of the process, lack of emulsions, reduced consumption of solvents, no reusable glassware, single-step and straightforward operations, low-cost items, reduced time and the possibility of parallel handling of several samples.

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REFERENCES

1 J. A. R. Bates, *Analyst*, 90 (1965) 453.
2 G. Becker, *Dtsch. Lebensm.-Rundsch.*, 75 (1979) 148.
3 A. Ambrus, J. Lantos, E. Visi, I. Csaltos and L. Sarvari, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 733.
4 W. Specht and M. Tilkes, *Fresenius' Z. Anal. Chem.*, 322 (1985) 443.
5 J. J. Blaha and P. Jackson, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 1095.
6 M. A. Luke, J. E. Froberg, G. M. Doose and H. T. Matsumoto, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 1187.
7 R. W. Storherr, P. Ott and R. R. Watts, *J. Assoc. Off. Anal. Chem.*, 54 (1971) 513.
8 K. Helrich (Editor), *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC, Arlington, VA, 1990, 15th ed., Section 970.52.
9 J. Hild and H.-P. Thier, *Dtsch. Lebensm.-Rundsch.*, 73 (1977) 330.
10 S. M. Lee, M. L. Papathakis, H.-M. C. Feng, G. F. Hunter and J. Carr, poster presented at the *7th IUPAC International Congress on Pesticide Chemistry, Hamburg, August 5–10, 1990.*
11 D. Chaput, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 542.
12 L. D. Johnson, R. H. Waltz, J. P. Ussary and F. E. Kaiser, *J. Assoc. Off. Anal. Chem.*, 59 (1976) 174.
13 M. P. Seymour, T. M. Jefferies and L. J. Notarianni, *Analyst*, 111 (1986) 1203.
14 A. H. Roos, A. J. Van Munsteren, P. M. Nab and L. G. M. Th. Tuinstra, *Anal. Chim. Acta*, 196 (1987) 95.
15 J. A. Ault, C. M. Schofield, L. D. Johnson and R. H. Waltz, *J. Agric. Food Chem.*, 27 (1979) 825.
16 J. F. Lawrence, *Int. J. Environ. Anal. Chem.*, 29 (1987) 289.
17 D. M. Holstege, D. L. Scharberg, E. R. Richardson and G. Moller, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 395.
18 J. R. Patterson, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 1016.
19 R. W. Storherr and R. R. Watts, *J. Assoc. Off. Anal. Chem.*, 48 (1965) 1154.
20 J. Pflugmacher and W. Ebing, *Fresenius' Z. Anal. Chem.*, 263 (1973) 120.
21 M. Eichner, *Z. Lebensm.-Unters.-Forsch.*, 167 (1978) 245.
22 B. Luke and J. C. Richards, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 902.
23 S. L. Head and V. W. Burse, *Bull. Environ. Contam. Toxicol.*, 39 (1987) 848.
24 B. McMahon and J. A. Burke, *J. Assoc. Off. Anal. Chem.*, 61 (1978) 640.
25 T. Stijve and E. Cardinale, *Mitt. Geb. Lebensmittelunters. Hyg.*, 65 (1974) 131.
26 H. Beckman and D. Garber, *J. Assoc. Off. Anal. Chem.*, 52 (1969) 286.
27 A. V. Holden and K. Marsden, *J. Chromatogr.*, 44 (1969) 481.
28 P. A. Greve and W. B. F. Grevenstuck, *Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 40 (1975) 1115.
29 H. Steinwandter and H. Schluter, *Fresenius' Z. Anal. Chem.*, 286 (1977) 90.
30 H. Steinwandter and H. Schluter, *Dtsch. Lebensm.-Rundsch.*, 74 (1978) 139.
31 R. R. Watts, R. W. Storherr, J. R. Pardue and T. Osgood, *J. Assoc. Off. Anal. Chem.*, 52 (1969) 522.
32 A. Di Muccio, A. Ausili, I. Camoni, R. Dommarco, M. Rizzica and F. Vergori, *J. Chromatogr.*, 456 (1988) 149.
33 A. Di Muccio, M. Rizzica, A. Ausili, I. Camoni, R. Dommarco and F. Vergori, *J. Chromatogr.*, 456 (1988) 143.
34 A. Di Muccio, A. Santilio, R. Dommarco, M. Rizzica, L. Gambetti, A. Ausili and F. Vergori, *J. Chromatogr.*, 513 (1990) 333.
35 A. Di Muccio, A. Ausili, L. Vergori, I. Camoni, R. Dommarco, L. Gambetti, A. Santilio and F. Vergori, *Analyst*, 115 (1990) 1167.
36 A. Di Muccio, A. Ausili, R. Dommarco, D. Attard Barbini, A. Santilio, F. Vergori, G. De Merulis and L. Sernicola, *J. Chromatogr.*, 552 (1991) 241.
37 A. Di Muccio, I. Camoni, L. Vergori, R. Dommarco, D. Attard Barbini, F. Vergori, A. Ausili and A. Santilio, *J. Chromatogr.*, 553 (1991) 305.
38 M. L. Hopper, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 731.