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Rapid multiresidue screening method for the determination of pesticides in plant materials

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Abstract

A multiresidue screening method for pesticides in plant materials is presented. Aliquots (10 g) of samples are extracted with acetone–water, the extract is concentrated by reversed-phase solid-phase extraction and cleaned up on Florisil. The pesticides are analyzed by gas chromatography in combination with electron-capture detection or mass spectrometry in the negative chemical ionization mode. The method has been tested for 23 pesticides in 19 different plant materials at a level of 0.02–3 ppm. The advantages of mass spectrometric detection with negative chemical ionization for the determination of pesticides are discussed.

Keywords: Environmental analysis; Plant materials; Multiresidue methods; Food analysis; Pesticides

1. Introduction

Over the last years, several multiresidue methods have been reported that allow the screening for one or more of the major classes of pesticides in plant materials like fruits and vegetables. Generally, these methods involve an extraction step with a water-miscible solvent such as acetone [1–6], acetonitrile [7] or methanol [8], followed by partitioning into an organic solvent with a limited miscibility for water such as *n*-hexane [3,4], dichloromethane [1,2,6], benzene [4], toluene [5,8] or petroleum ether [1]. Most of these methods require additional clean-up steps like gel-permeation chromatography (GPC) [3,6,9] or chromatography on materials like silica [2,4,6], Florisil [8,9], carbon–cellulose [8] or alumina [2]. Other methods used for the removal of interfering co-extractives include precipitation or coagulation steps, sweep co-distillation, saponification, supercritical fluid extraction (SFE) or semi-preparative HPLC [10]. All these procedures are time-consuming and, therefore, only of limited use for quick screening of a large number of samples.

Some attempts have been made to avoid extensive sample clean-up by using gas chromatography (GC) in combination with selective detection methods like electrolytic conductivity detection (ELCD) [1,7]. Nevertheless, GC with highly selective detection methods like thermoionic and flame photometric detection often requires clean-up of the primary extracts in order to maintain separation efficiency of the GC column.

In recent years, reversed-phase solid-phase extraction (RP-SPE) has found widespread use for the extraction of pesticides and other toxic substances from water [11]. Recently, this technique has also

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been used for screening wine samples for a range of pesticides [12]. On the other hand, applications of RP-SPE in multiresidue methods for the analysis of plant materials are still not very common. Newsome and Collins [5] reported a method for fungicides, which seems to be the only method published so far that uses the advantages of RP-SPE for multiresidue analysis in plants, such as minimum solvent requirement, rapidity, ease of automation, cost effectiveness and minimal requirements with respect to apparatus and glassware [11].

In this paper, the development of a rapid multiresidue screening method for the determination of 23 pesticides, mainly organochlorine pesticides, is reported. RP-SPE is used to concentrate the pesticides from the primary acetone–water extract and at the same time allows the removal of water and many co-extractives. SPE on Florisil or precipitation of barium sulfate from the primary acetone–water extract are investigated as eventually necessary additional clean-up steps. GC with electron-capture detection (ECD) or mass spectrometric detection with negative chemical ionization (NCI-MS) were chosen for the final determination step. Nineteen different plant materials were used to investigate the general applicability of this multiresidue method.

2. Experimental

2.1. Gas chromatography

A Carlo Erba (Milan, Italy) Model HRGC 5300 Mega Series gas chromatograph equipped with an ECD 400 electron-capture detector, an on-column injector and an HP5 column (50 m×0.32 mm I.D., film thickness 0.17 μm) was used for GC–ECD measurements. The instrumentation for GC–MS consisted of a Hewlett-Packard (Palo Alto, CA, USA) Model HP 5890 gas chromatograph equipped with a split/splitless injector and a HP 5 column (25 m×0.25 mm I.D., film thickness 0.25 μm) which was coupled to a Hewlett-Packard HP 5989 MS-Engine quadrupole mass spectrometer. Negative chemical ionization was used with methane as the reagent gas and an ionization energy of 240 eV.

2.2. Materials for solid-phase extraction

Bakerbond spe C_{18}-modified silica, prepacked extraction columns (J.T. Baker, Phillipsburg, NJ, USA); silicagel 60 H, particle size 5–40 μm (Merck, Darmstadt, Germany); Spe-ed aminopropyl-modified silica, prepacked disposable extraction columns (Applied Separations, Lehigh Valley, PA, USA); Spe-ed Florisil super, prepacked disposable extraction columns (Applied Separations); polystyrene–divinylbenzene (PS–DVB) particles, without and with surface modifications, 4.5 μm particle size, synthesized and modified according to a procedure published elsewhere [13].

Those SPE materials that had been obtained in the form of prepacked disposable plastic columns were removed from the columns and packed into glass columns (60×10 mm I.D.) equipped with glass fiber frits (Macherey-Nagel, Düren, Germany) in order to avoid contamination of the extract with plasticizers.

2.3. Reagents

All organic solvents were of Ultra Resi Analyzed quality (J.T. Baker). Double distilled water was used throughout this work. Pesticide standards were obtained from Riedel de Haen (Seelze, Germany). Stock solutions were prepared by dissolving the pesticides in ethyl acetate to obtain concentrations of 1 mg/ml. Dilutions were made with ethyl acetate. All other chemicals used were of analytical reagent grade quality. Celite 545 was obtained from Fluka (Buchs, Switzerland).

2.4. Sample preparation

A 10-g aliquot of the sample was blended with 66 ml of acetone. Water was added to obtain a total mixture volume of 100 ml. The mixture was chopped and homogenized with a Ultra Turrax T25 (Ika, Staufen, Germany). A 3-g amount of Celite was added and the extract was filtered through a Buchner funnel (fitted with fast speed filter paper) with the aid of a gentle vacuum.

A 5-ml volume of the extract were diluted with water to 80 ml, yielding an acetone content of approximately 4%, and passed through the solid-phase extraction column containing 150–500 mg of a

reversed-phase solid-phase material. The flow-rate was 5 ml/min. Prior to use, the column had to be conditioned with 2–3 ml each of ethyl acetate, methanol and water.

Some of the residual water was removed from the column by blowing nitrogen through the bed for 2 min at a pressure of 1 bar. Afterwards, the pesticides were eluted with 2 ml of ethyl acetate. The eluate was dried by passing through an additional column containing 1 g of anhydrous Na_2SO_4.

The eluate was then concentrated to 1 ml under a gentle stream of nitrogen. A 1-ml volume of *n*-hexane was added and the extract was passed through a column containing 750 mg of Florisil conditioned with 3 ml each of ethyl acetate and ethyl acetate–*n*-hexane (1:1, v/v). The column was washed with 2 ml of ethyl acetate–*n*-hexane (1:1). The eluates were combined and used for GC.

Further experiments on additional clean-up steps included the precipitation of $BaSO_4$ in the primary extract. Precipitation was carried out by adding 2 ml of a concentrated Na_2SO_4 solution to 5 ml of the primary extract and precipitating the sulfate by adding a solution of saturated $BaCl_2$ until the precipitation was complete. Alternatively, the primary extract was passed through a column containing aminopropyl-modified silica before RP-SPE.

2.5. GC–ECD and GC–NCI-MS

The determination by GC–ECD was carried out by injecting 1 μl of the final extract (4 ml) directly onto the column. For quantitation, pentabromoethylbenzene was added as internal standard after the last clean-up step. In some cases the final extract had to be diluted to remain within the linear range of the detector.

The same internal standard was used for the determination by GC–NCI-MS. The 4-ml final extract was concentrated to 0.5 ml under a stream of nitrogen and 2 μl were injected using the splitless injection technique. The measurement was carried out in the multiion monitoring mode (MIM) using at least one specific ion for each pesticide. The chromatogram was divided into segments containing 1–4 pesticide peaks each (see Table 1).

3. Results and discussion

Acetone was used for the primary extraction step because it has been demonstrated to be suited for the extraction of both non-polar pesticides and pesticides of medium polarity. Prior to RP-SPE, the polarity of the solvent must be increased in order to allow the adsorption of the pesticides to the RP material. This was achieved by adding water to the extract.

In a preliminary series of experiments, four different materials for RP-SPE were investigated with respect to quantitative preconcentration. Water was spiked with a standard mixture of the 23 pesticides to achieve concentrations between 0.05 and 0.1 ppm and extracted by SPE on the four different RP materials. These materials included PS–DVB particles, 4.5 μm, both unmodified and modified with

Table 1
Segments with pesticides and ions measured therein for the determination by GC–NCI-MS in the multi-ion monitoring mode

Segment number	Start time	Stop time	Pesticides	Ions measured (*m/z*)
1	8.00	10.30	α-Hexachlorocyclohexane, hexachlorobenzene	71, 255, 284
2	10.30	14.00	β-Hexachlorocyclohexane, lindane, quintozene, δ-hexachlorocyclohexane	71, 255, 265
3	14.00	16.50	Chlorpyriphosmethyl, heptachlor	212, 266, 300
4	16.50	20.00	Aldrin, malathion	35, 157, 237
5	20.00	22.50	Heptachloroepoxide, captan, folpet	35, 146, 150
6	22.50	26.50	*o,p*-DDE, α-endosulphan	35, 246, 406
7	26.50	30.00	Dieldrin, *p,p*-DDE, *o,p*-DDD, endrin	35, 248, 346
8	30.00	31.80	β-Endosulphan	404, 406, 408
9	31.80	33.80	*p,p*-DDD, *o,p*-DDT	35, 246, 248
10	33.80	35.00	Internal standard	35, 79, 81
11	35.00	40.00	*p,p*-DDT	35, 71, 248

GC temperature program: 130–174°C at 8 C°/min, 174°C 1 min, 174–203°C at 1 C°/min, 203–300°C at 30 C°/min.

C_{18} or carboxylic acid groups (capacity of 0.68 mequiv/g) as well as C_{18}-modified silica, 40 μm. Although lower recoveries had to be expected for the particles modified with the carboxylic acid groups because of their increased polarity, these particles were used to investigate the influence of the PS–DVB matrix in the case of a polar surface modification. The mean recoveries from three measurements each are given in Table 2. The unmodified PS–DVB particles and the C_{18}-modified PS–DVB particles yielded the best overall results, the other two materials gave slightly lower recoveries. The rather small decrease of the recoveries for the particles with the carboxylic acid groups showed the predominant influence of the PS–DVB matrix. It should be mentioned that this series of experiments was carried out with a standard in water containing 7% acetone. Reducing the acetone content to 4% led to an increase of the recovery of most of the pesticides with the exception of captan, folpet, *o,p*-DDE and α-endosulphan, as can be seen by comparison of Table 2 (data for samples in 7% acetone) and Table 3 (data for samples in 4% acetone). Therefore, real sample extracts were always diluted with water to 4% acetone.

Experiments with real sample extracts revealed that C_{18}-modified silica yielded considerably lower recoveries than observed for standards, whereas the recovery on PS–DVB particles remained virtually the same. For this reason, all further work on real samples was done with unmodified PS–DVB particles. One should take into account that the Si-C_{18} particles had a mean diameter of 40 μm, whereas the PS–DVB materials consisted of much smaller particles (mean diameter 4.5 μm). Apparently, the small particles cause an additional filtration effect, so that in the uppermost layer of the solid-phase all suspended particles were retained that had been produced when chopping the plant material. Pesticides adsorbed on very fine suspended particles might pass through the bed of the C_{18}-modified silica but not through the fine PS–DVB material.

Table 2
Comparison of recoveries achieved with different RP-SPE materials, determination with GC–ECD

Pesticide	Recovery (%)			
	Si-C_{18}	PS–DVB unmodified	PS–DVB C_{18}-modified	PS–DVB modified with carboxylic acid groups
α-Hexachlorocyclohexane	87	89	81	89
Hexachlorobenzene	89	88	95	83
β-Hexachlorocyclohexane	82	96	90	98
Lindane	81	94	84	95
Quintozene	83	93	98	101
δ-Hexachlorocyclohexane	86	93	91	94
Chlorpyriphosmethyl	78	87	79	91
Heptachlor	69	92	91	79
Aldrin	85	89	92	76
Malathion	69	89	92	106
Heptachloroepoxide	81	93	95	86
Captan	77	99	103	84
Folpet	82	105	107	83
o,p-DDE	73	90	91	75
α-Endosulphan	82	93	95	92
Dieldrin	83	92	96	89
p,p-DDE	75	89	94	80
o,p-DDD	77	91	99	86
Endrin	88	93	103	93
β-Endosulphan	92	98	101	89
p,p-DDD	79	91	101	86
o,p-DDT	77	83	94	85
p,p-DDT	81	85	100	92

Table 3
Recoveries for horsetail herbs fortified with 23 pesticides at concentrations from 0.05 to 0.1 ppm, RP-SPE with PS–DVB unmodified, determination with GC-NCI-MS

No.	Pesticide	Relative retention time (lindane=1)	Recovery (%)	R.S.D.[a]
1	α-Hexachlorocyclohexane	0.876	100	3.3
2	Hexachlorobenzene	0.904	92	2.2
3	β-Hexachlorocyclohexane	0.975	96	1.7
4	Lindane	1.000	105	1.8
5	Quintozene	1.021	98	6.7
6	δ-Hexachlorocyclohexane	1.104	104	4.8
7	Chlorpyriphosmethyl	1.312	107	4.2
8	Heptachlor	1.342	101	1.1
9	Aldrin	1.544	94	2.7
10	Malathion	1.561	96	6.6
11	Heptachloroepoxide	1.856	99	2.3
12	Captan	1.890	98	2.5
13	Folpet	1.942	98	2.7
14	*o,p*-DDE	2.073	79	6.1
15	α-Endosulphan	2.096	83	5.5
16	Dieldrin	2.319	99	2.6
17	*p,p*-DDE	2.355	99	4.4
18	*o,p*-DDD	2.424	94	7.0
19	Endrin	2.504	99	3.6
20	β-Endosulphan	2.601	99	3.4
21	*p,p*-DDD	2.750	102	2.5
22	*o,p*-DDT	2.770	101	6.6
23	*p,p*-DDT	2.981	97	6.7

The small particle size of the PS–DVB particles led to some backpressure that allowed the use of only 150 mg material in order to maintain a flow-rate of 5 ml/min at a pressure of 1.2 bar. The C_{18}-modified silica particles allowed the use of 500 mg without any backpressure problems. Nevertheless, the mean recoveries from real samples were quite satisfactory for PS–DVB particles (data are given in Table 3) so that 150 mg of this material seem to be sufficient.

For clean-up steps before or after the RP-SPE step, materials such as Florisil, silica and aminopropyl-bonded silica were investigated. Florisil gave the best results for removing the colored co-extractives after RP-SPE. The results were also satisfactory when using silica, while the aminopropyl-bonded material did not give comparable results.

Clean-up by precipitation of $BaSO_4$ before RP-SPE did not yield satisfactory results. A reduction of the colored co-extractives was possible, but at the same time losses of the pesticides were observed with recoveries in the range of 50–60%. The reason could be the fact that the fine suspended plant particles were co-precipitated with the $BaSO_4$ and the adsorbed pesticides thereby were trapped and lost. Therefore, clean-up by precipitation procedures was not investigated further.

In Table 3, recovery data are given for horsetail herbs (which had previously been found to be free of pesticide residues) fortified with 23 pesticides at a level of 0.05–0.1 ppm. The spiking of the sample with a mixture of the pesticides was done just before homogenization by the Ultra Turrax. The mean recoveries were in the range of 92 to 107%, with standard deviations below 7% for 21 of the 23 pesticides tested. α-Endosulphan and *o,p*-DDE showed 83 and 79% recovery, respectively.

Nineteen different plant materials were investigated with respect to the applicability of the method described in this paper (Table 4). No interferences were observed for 16 of these matrices when using GC–ECD. A typical chromatogram obtained for marshmallow root is given in Fig. 1. Curcuma root, German chamomile and horsetail herbs turned out to

Table 4
Plant materials tested for the analysis by GC–ECD, RP-SPE with PS–DVB unmodified

Plant material	Trivial name	Determination possible with ECD
Betulae folia	Birch leaves	yes
Althaeae radix	Marshmallow root	yes
Quercus cortex	Oak bark	yes
Cardui mariae fructus	St. Mary's thistle fruits	yes
Curcuma xanth. rhiz.	Curcuma root	no
Chelidonii herba	Tetterwort root	yes
Cimizifuga rhiz.	Black snakeroot	yes
Boldo folia	Boldo leaves	yes
Rumicis herba	Sorrel	yes
Juglandis folium	Walnut leaves	yes
Anisi fructus contus	Anise seed	yes
Matricariae flos	German chamomile	no
Gentianae radix	Gentian root	yes
Taraxaci herba	Dandelion leaves	yes
Solidaginis herba	Golden rod wort	yes
Centaurii herba	Centaury tops	yes
Equiseti herba	Horsetail herbs	no
Primulae flos	Primrose flowers	yes
	Industrial wood wastes	yes

be extremely difficult matrices that could not be analyzed by GC–ECD in a concentration range of 0.05 to 0.1 ppm.

When using the MS detector in the selective and sensitive NCI mode and by concentrating the extract by blowing off most of the solvent, the pesticides

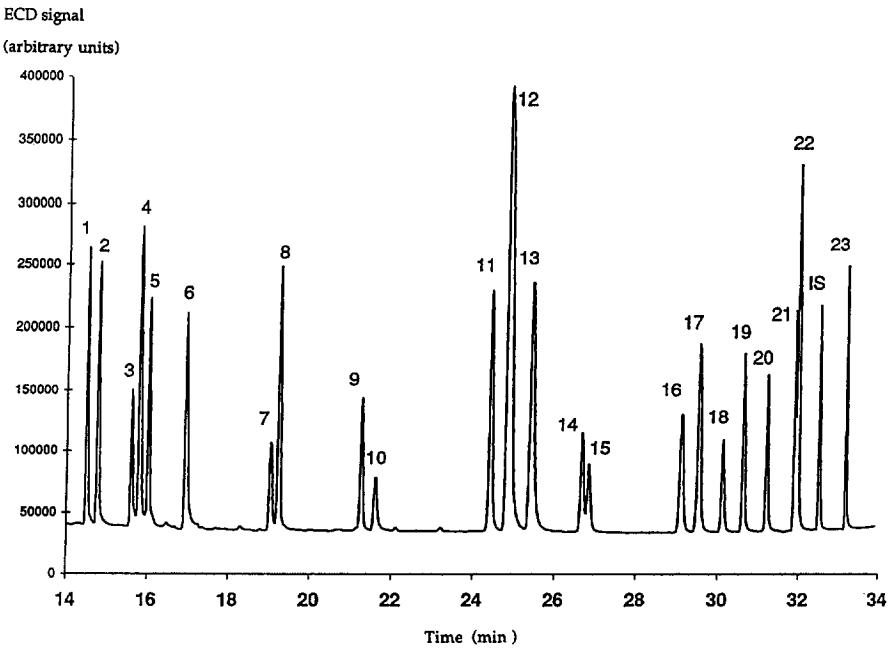

Fig. 1. ECD chromatogram of an extract from marshmallow roots fortified with 23 pesticides listed in Table 3. IS, internal standard pentabromoethylbenzene. For chromatographic conditions, see Experimental.

could easily be determined down to about 10 ppb in all of the 19 plant materials without any interferences with the exception of malathion and the endosulphans, which could be determined down to approximately 100 ppb. An improvement by a factor of 10 is possible if the extract is further concentrated to 50 μl. By proper selection of the segments and ions it was possible to determine all 23 pesticides in one run while measuring three ions in each segment (Fig. 2).

ECD was slightly more sensitive than MS detection when injecting standard solutions. Nevertheless, this disadvantage of MS was overcompensated by the much higher selectivity when measuring in the NCI mode as described above. To obtain the highest sensitivities for all pesticides the Cl^- ion (m/z 35) had to be used for determination in some segments. This led to some reduction in the selectivity as it becomes impossible to differentiate between chlorinated compounds with the same retention time, but this problem could be circumvented by using a second ion as a qualifier. Even when concentrating the final extract by a factor of 100 by evaporating the solvent with a gentle stream of nitrogen, which was possible in a few minutes without any discernible losses, no severe background interferences occurred during GC–MS analysis.

In a series of preliminary experiments, the behavior of more polar pesticides such as dimethoate was investigated. It turned out that a rather polar eluent such as ethyl acetate–acetone (7:3, v/v) was necessary to elute the polar pesticide from the Florisil column. In this case, some of the colored coextractives were also eluted, which caused additional interferences when using GC–ECD, especially in the case of golden rot wort and centaury tops. No interferences of any kind, however, were seen when using MS. Therefore, the method also seems to be applicable to the more polar organophosphorus pesticides.

This method was developed to screen for pesticide residues exceeding existing guideline values. The linearity of the method in the region of these guideline values had to be ascertained. Generally, a linearity in the range of one order of magnitude at the level of the guideline value of each pesticide seemed sufficient. This linear working range was checked according to ASTM procedures [14]. The method was found to be linear in the range given in

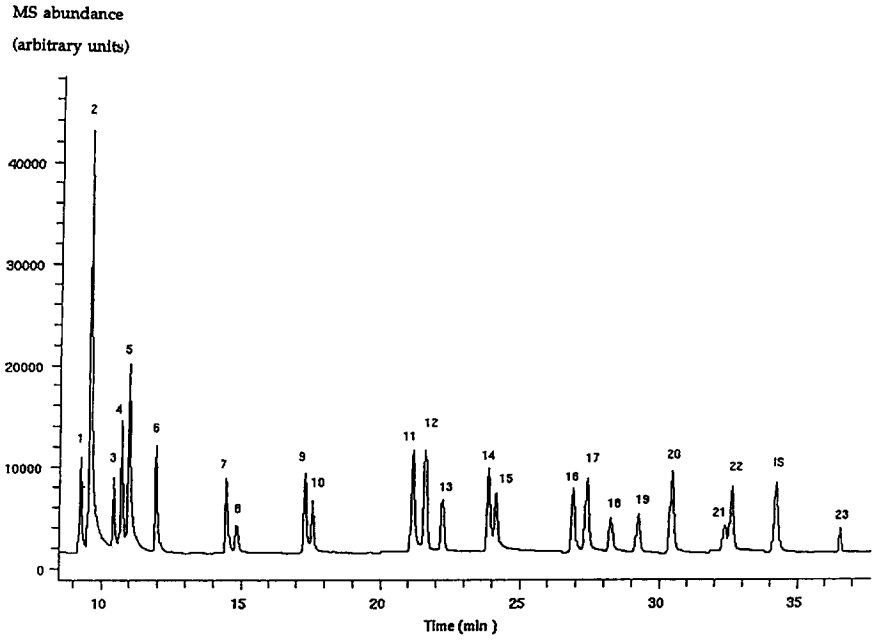

Fig. 2. NCI-MS chromatogram of an extract from marshmallow roots fortified with 23 pesticides listed in Table 3. IS, internal standard pentabromoethylbenzene. For chromatographic conditions, see Experimental.

Table 5
Concentrations for pesticides as used in the quality control experiments, RP-SPE with PS–DVB unmodified

Pesticides	Lower limit (ppm)	Upper limit (ppm)
Hexachlorocyclohexane, aldrin, dieldrin, heptachlor, heptachloroepoxide	0.02	0.2
Chlorpyriphosmethyl, endrin, hexachlorobenzene	0.04	0.4
DDE, DDD, DDT, lindane	0.06	0.6
Captan, folpet, quintozene	0.2	2
Malathion, endosulphan	0.3	3

Table 5. The criterion of homogeneity of the variances over the linear range was found to be fulfilled. By diluting or concentrating the final extract the linear range can easily be expanded. For the determination of recoveries the plant materials were fortified with a mixture of the pesticides in the range given in Table 5 just before homogenization with the Ultra Turrax. The recovery function was found to be linear in the range given above with no matrix interferences discernible.

4. Conclusions

The method described in this paper seems to be a rapid and efficient alternative to the existing multiresidue methods for screening fruits, vegetables and herbs for pesticide residues. Among the SPE materials investigated in this paper, unmodified and C_{18}-modified PS–DVB particles with a particle size of 4.5 μm yield the best results and therefore can be most recommended. The recoveries were as good as or better than most of those described in the literature, with a very short sample preparation time and minimum waste of organic solvents. The results agreed with those obtained by the reference method DFG-S19 [6].

The use of the MS in the NCI mode proved to be a highly recommendable alternative to several detectors described in the literature. The rather high standard deviations, which were observed at the beginning and which might be the reason why the NCI mode has not been used very often for routine analysis, could be reduced to less than 3% for three measurements after careful optimization of the MS parameters.

It should be possible to use this method for other plant materials as one can expect that very few plant materials would produce interferences worse than chamomile or horsetail herbs. An adjustment of the clean-up step for the determination of other pesticides is possible by using a slightly different eluent for the Florisil column and scanning for specific ions with the MS. For sample matrices with a high fat content an additional GPC clean-up step as described by Specht and Tillkes [6] or Holstege et al. [9] might be advisable.

References

[1] M.A. Luke, J.E. Froberg, G.M. Doose and H.T. Masumoto, J. Assoc. Off. Anal. Chem., 64 (1981) 1187.
[2] A. Ambrus, J. Lantos, E. Visi, I. Csatlos and L. Sarvari, J. Assoc. Off. Anal. Chem., 64 (1981) 733.
[3] J. Blaha and P.J. Jackson, J. Assoc. Off. Anal. Chem., 68 (1985) 1095.
[4] K. Sasaki, T. Suzuki and Y. Saito, J. Assoc. Off. Anal. Chem., 70 (1987) 760.
[5] W.H. Newsome and P. Collins, J. Chromatogr., 472 (1989) 416.
[6] W. Specht and M. Tillkes, Fresenius J. Anal. Chem., 301 (1980) 300.
[7] S.M. Lee, M.L. Papathakis, H.C. Feng, G.F. Hunter and J.E. Carr, Fresenius J. Anal. Chem., 339 (1991) 376.
[8] P.T. Holland and T.K. McGhie, J. Assoc. Off. Anal. Chem., 66 (1983) 1003.
[9] D.M. Holstege, D.L. Scharberg, E.R. Tor, L.C. Hart and F.D. Galey, J. Assoc. Off. Anal. Chem., 77 (1994) 1263.
[10] S.M. Walters, Anal. Chim. Acta, 236 (1990) 77.
[11] G. Font, J. Manes, J.C. Molto and Y. Pico, J. Chromatogr., 642 (1993) 135.
[12] P.T. Holland, D.E. McNaughton and C.P. Malcolm, J. Assoc. Off. Anal. Chem., 77 (1994) 79.
[13] E. Spanos, Ph.D. Thesis, University of Linz, Linz, Austria, 1995.
[14] Annual Book of ASTM Standards, Vol. 14.01, American Society for Testing and Materials, Philadelphia, PA, 1988.