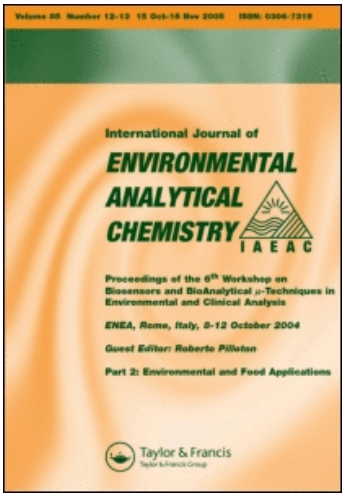

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Polyxeni P. Georgiou[a]; Konstantinos S. Liapis[b]; George E. Miliadis[b]; Panayotis A. Siskos[a]
[a] Environmental Analysis Group, Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Zographos, Athens, Greece [b] Pesticide Residues Laboratory, Benaki Phytopathological Institute, Kifissia, Greece

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Solid-phase extraction cleanup of tomato samples for the determination of pesticide residues by gas chromatography-electron capture detection

POLYXENI P. GEORGIOU*†, KONSTANTINOS S. LIAPIS‡, GEORGE E. MILIADIS‡ and PANAYOTIS A. SISKOS†

†Environmental Analysis Group, Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupoli, 157 7, Zographos, Athens, Greece
‡Pesticide Residues Laboratory, Benaki Phytopathological Institute, 145 61, Kifissia, Greece

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Extracts of tomato samples, obtained using acetone solvent followed by liquid–liquid partition with a mixture of dichloromethane and light petroleum (40–60°C), were subjected to cleanup with solid-phase extraction (SPE) columns for the simultaneous multiclass determination of 12 pesticides with different physicochemical properties ($\log P_{ow} = 0.7$ to >6). Silica, aminopropyl (NH_2), graphitized carbon black (GCB), octadecyl (C-18) with GCB and the mixed-mode SAX/PSA (SPE) columns were evaluated. The sample cleanup provided by these columns was evaluated using gas chromatography with electron capture detection. The mixed-mode SAX/PSA columns were found to provide the most effective cleanup, along with the (NH_2) columns, removing the greatest number of sample matrix interferences. The GCB sorbents also remove pigments but do not remove noticeable chromatographic interferents. The silica columns did little to eliminate the matrix effect for the compound dimethoate. Likewise, the C-18 in combination with the GCB sorbents did little to eliminate matrix interferences.

Keywords: Pesticides; Solid-phase extraction; Tomato; Food analysis

1. Introduction

Multiresidue methods (MRMs) that determine pesticide residues in agricultural products are needed to evaluate food quality. Multiresidue procedures typically consist of extraction with a water-miscible organic solvent (e.g. acetonitrile or acetone), removal of water, and GC determination using element-selective detectors. Many laboratories perform cleanup of the organic solvent extract prior to GC determination for many reasons, such as: (1) to prevent the deterioration of the GC column due to sample matrix coextractants, (2) to avoid interferences in the determination

*Corresponding author. Fax: +30-210-8078917. Email: bpipest@otenet.gr

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of pesticides at trace levels, and (3) to eliminate the matrix-induced enhancement effect [1, 2].

Solid-phase extraction (SPE) is being increasingly used in food analysis, mainly for the cleanup of samples. SPE is a simple preparation technique based on the separation of liquid chromatography, where the solubility and functional group interactions of sample, sorbent, and solvent are optimized to effect the retention and elution [3, 4]. Typically, SPE is used instead of liquid–liquid partition as a sample preparation tool because of its benefits: high recoveries, purified extracts, ease of automation, and reduction in the consumption of organic solvents [5–7].

The sorbents used in SPE are similar to those used in liquid chromatography including: reversed-phase sorbents such as octadecyl (C-18), bonded normal phase such as aminopropyl (NH_2), unbonded normal phase such as silica, adsorbents such as graphitized carbon black (GCB), and ion exchange such as the SAX/PSA mixed-mode column consisting of two sorbents: SAX quaternary amine (strong anion exchange sorbent) and PSA ethylenediamine-*N*-propyl (primary/secondary amine).

This study was designed for determining the efficiency of sample cleanup obtained using the above-mentioned SPE columns and their combinations.

2. Experimental

2.1. *Chemicals and solvents*

Acetone, acetonitrile, and a mixture of ethyl acetate/hexane were used for preparation of stock and working standard solutions. Acetone, dichloromethane, and petroleum ether were used for extraction purposes. Hexane, acetonitrile, and a mixture of acetone/*n*-hexane were used for reconstituting the solutions before passing through SPE cartridges. Elution solvents for SPE cartridges were ethyl acetate, hexane, acetonitrile, toluene, and acetone. All solvents were of pesticide residue analysis grade and were obtained from Lab Scan (Ireland).

Pesticide standards of dichlorvos (97% purity), dimethoate (98.5%), methidathion (97.5%), β-endosulphan (99%), bifenthrin (99%), fenarimol (98.5%), permethrin (95.5%), and fenvalerate (98%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany); chlorpyriphos methyl (99.8%) was obtained from Dow Agrosciences (Greece); phosalone (99.5) was obtained from Rhone-Poulenc (Greece); and diazinon (99.5%) was obtained from Novartis.

Individual stock standard solutions (1000 $\mu g\,mL^{-1}$) for each pesticide standard were prepared in acetone and stored at $-20^{\circ}C$. A standard stock solution containing all compounds was prepared in acetone from the individual stock standard solutions. The concentration of each compound in this solution was: dichlorvos, dimethoate, fenvalerate, β-endosulphan, and phosalone 8.5 $\mu g\,mL^{-1}$; diazinon, chlorpyrifos methyl, fenarimol and permethrin 4.5 $\mu g\,mL^{-1}$; methidathion 2 $\mu g\,mL^{-1}$; iprodione 42 $\mu g\,mL^{-1}$ and bifenthrin 17 $\mu g\,mL^{-1}$. Working standard mixture solutions for measurement were prepared from this standard stock solution in the following solvents depending on the SPE cartridges used for cleanup: 50 : 50 v/v ethyl acetate/hexane for silica and aminopropyl SPE cartridges, acetonitrile for GCB and C-18 + GCB SPE cartridges, and acetone for SAX/PSA SPE cartridges. The concentrations of the working standard mixture solutions were 6, 8, 10, 12, and 14% and 6, 8, 10, 12, and 14‰

of the concentration of the standard work solution for the first and second fortification level, respectively.

2.2. *Gas-chromatographic system*

For the gas-chromatographic separation and determination, a Fisons HRGC 8560, series Mega 2 gas chromatograph was used. The GC was equipped with a splitless injector and an autosampler AS-800. The analytical column used was a 30 m × 0.32 mm i.d., 0.25 μm film thickness, DB-5 coated with a 5% diphenyl–95% dimethylsiloxane stationary phase. The temperature programme consisted of a 1.0 min hold at 50°C, ramp at 30°C min^{-1} to 180°C, 1.8°C min^{-1} to 230, 30°C min^{-1} to 260°C, and a final hold for 25 min. Instrument control, data acquisition, and integration of the compounds' peaks were performed using Chrom-Card software.

2.3. *Extraction procedure*

The extraction procedure was based upon an existing method [8]. According to this method, from the homogenized sample of tomato, an aliquot of 15 g was weighed into a 250 mL PTFE centrifuge bottle (Nalgene, Rochester, NY) and extracted with 30 mL of acetone for 30 s with an Ultra-Turrax T25 (IKA, Germany) at 8000 rpm. A 60 mL volume of dichloromethane : light petroleum (1 : 1) was added, and the mixture was extracted for a further 30 s. The mixture was then centrifuged at 4000 rpm for 5 min. An aliquot of 25 mL was concentrated to dryness in a water bath at 60°C.

For improving chromatography, five different cleanup procedures were evaluated. For this purpose, the residue was redissolved in 5 mL of *n*-hexane for the silica and aminopropyl SPE cleanup, acetonitrile for the GCB and C18 + GCB cleanup and a mixture of 5 : 95 v/v acetone/*n*-hexane for the SAX/PSA cleanup.

2.4. *Preparation of fortified samples*

Samples from untreated tomatoes were used as control samples and for the fortification experiments. These samples were homogenized and analysed in duplicate, and then 15 g subsamples were kept frozen until fortification. Subsamples were fortified at two different levels of each pesticide, each time using the appropriate working standard mixture solution of the 12 pesticides in the study, prepared after dilution of the standard stock solution in acetone. Fortified samples were left to stand for 3 h before analysis to allow pesticide absorption onto the matrix. Two fortification levels were selected, named the first and second level. At the second fortification level, the concentrations of all compounds were 10 times lower than in the first. The first fortification level was chosen to be equal to the MRL of the EU [9] for the compounds diazinon, chlorpyrifos methyl, iprodione, phosalone, and fenarimol, while for the compounds dichlorvos, bifenthrin, permethrin, and methidathion, the second fortification level was equal to the MRL of the EU [9]. For the rest of compounds (dimethoate, β-endosulphan, and fenvalerate), this study was conducted at different concentrations than the MRLs. Under this scheme, the majority of the compounds were studied at levels corresponding either with MRL and MRL/10 or with MRL and 10 times the LOD (as proposed by the EU).

2.5. *SPE cleanup*

Elution solvents for the SPE columns were as follows: 50 : 50 v/v ethyl acetate/*n*-hexane and ethyl acetate for silica and aminopropyl SPE columns (IST, 500 mg, 3 mL); acetonitrile and 3 : 1 v/v acetonitrile/toluene for GCB SPE columns (Supelco, 500 mg, 6 mL); and 3 : 1 v/v acetonitrile/toluene for C-18 (IST, 500 mg, 3 mL) + GCB SPE columns and acetone for SAX/PSA columns (IST, 1 g, 6 mL). When the C-18 + GCB cleanup was used, the extract first passed through the C-18, and the eluant was then cleaned with the GCB column. The SPE columns were always preconditioned with elution solvents. For columns containing 500 mg of sorbent, 5 mL of elution solvent was used, whereas for columns containing 1 g of sorbent, 10 mL was necessary. The flow rate was gravitational, and the eluates were collected in 10 mL conical centrifuge tubes. Once all the extract reached the sorbent bed, the columns were eluted with two 5 mL portions of elution solvent. The eluates were evaporated to 1.0 mL with a rotary evaporator. One microlitre of the final eluate was injected to the GC-ECD system.

3. Results and discussion

Each of the five methods with a different SPE cleanup procedure was evaluated by assessing the basic parameters, namely accuracy, precision, and sensitivity. Accuracy was assessed by calculating the attained recovery, whereas precision was assessed by calculating the relative standard deviation (RSD) values of three spiked tomato samples (four in the case of SAX/PSA cleanup) at each of the two spiking levels. The sensitivity of each method was assessed by the limits of detection (LOD) and quantification (LOQ), which were estimated to be equal to the concentration of the analyte producing a chromatographic peak with a signal-to-noise ratio of 3 and 10, respectively. Quantitation was performed by comparing the peak areas of the samples' solutions with the peak areas of two solutions of analytical standards bracketing the area of the sample and not differing in concentrations more than 20% from each other, without the use of a calibration curve. This proposed method of bracketing fits better with pesticide residue analysis, either for validation purposes or for real samples, because it does not suffer from absence of linearity, especially at very low concentrations, as in the case of the second fortification level.

The attained limits of detection and quantitation of methods (by using three and 10 times the requirement of the signal-to-noise ratio) are shown in table 1, along with the retention times of the 12 pesticides. In the case of permethrin and fenvalerate showing multiple peaks, due to the presence of isomers, the calculation of the limit of quantitation was applied for the isomer with a higher sensitivity, permethrin II and fenvalerate III. From the data given in table 1, it can be seen that the sensitivity of the methods is satisfactory, as the limits of quantitation range from 0.0002 to 0.1 mg kg^{-1}, values well below the established MRLs in tomato.

Recovery and precision data of the proposed methods are listed in tables 2 and 3 for two concentration levels. The recoveries achieved with various SPE columns at the first level were 54.5–117% with a relative standard deviation (RSD) of 3.03–8.47% for silica SPE columns (with the exception of one extreme value of 188%), 56.9–131% with RSD 2.99–12.9% for amino SPE columns, 69.6–131% with RSD 2.96–6.01% for

Table 1. Retention times (RT), limits of detection (LOD), limits of quantitation (LOQ), and maximum residue limits (EU MRLs for tomatoes) of the 12 pesticides studied.

Pesticide	RT (min)	LOD ($mg\,kg^{-1}$)	LOQ ($mg\,kg^{-1}$)	MRL ($mg\,kg^{-1}$)
Dichlorvos	6.09	0.04	0.1	0.1
Dimethoate	10.97	0.01	0.03	0.02
Diazinon	11.85	0.002	0.006	0.5
Chlorpyriphos methyl	13.83	0.0003	0.001	0.5
Methidathion	20.02	0.003	0.01	0.02
β-Endosulphan	25.07	0.0001	0.0002	0.5
Iprodione	32.77	0.003	0.01	5
Bifenthrin	33.80	0.0003	0.001	0.2
Phosalone	35.23	0.0001	0.0003	1
Fenarimol	36.65	0.0003	0.001	0.5
Permethrin	38.85–39.34	0.003	0.01	0.05
Fenvalerate	46.49–47.75	0.003	0.01	0.05

Table 2. Recovery and precision data ($n=3$) of the compounds determined in spiked tomato samples (first level) through each different cleanup.

		Silica cleanup		Aminopropyl cleanup		GCB cleanup		C18 + GCB cleanup		SAX/PSA cleanup	
Compound	Fortification level ($mg\,kg^{-1}$)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Dichlorvos	1	54.5	3.67	–	–	69.9	5.59	–	–	36.8	10.1
Dimethoate	1	188	3.03	107	6.65	108	4.90	102	5.87	120	3.12
Diazinon	0.5	93.1	5.16	104	8.68	88.4	4.00	86.6	5.30	113	3.44
Chlorpyriphos methyl	0.5	55.7	3.41	61.8	2.99	100	5.46	68.1	4.49	96.0	3.03
Methidathion	0.2	134	8.47	66.3	9.30	131	3.33	98.6	10.2	97.8	6.41
β-Endosulphan	1	91.8	5.89	119	3.74	96.3	2.96	74.5	4.35	98.0	2.80
Iprodione	5	78.8	7.83	56.9	12.9	182	3.03	69.2	3.65	106	3.24
Bifenthrin	2	111	7.15	118	3.19	98.8	5.31	82.8	4.96	104	4.61
Phosalone	1	116	5.76	120	3.79	120	6.01	89.7	4.59	115	3.37
Fenarimol	0.5	108	6.97	131	6.68	94.4	2.82	74.8	5.74	111	4.49
Permethrin I	0.5	116	3.25	101	3.97	94.4	3.34	78.9	5.14	109	4.29
Permethrin II	0.5	113	6.62	105	4.28	95.6	3.10	83.0	6.15	114	3.26
Fenvalerate	1	117	5.21	116	5.17	123	4.30	86.0	6.59	115	3.23

– : No data obtained at this level.

GCB SPE columns (with the exception of one extreme value of 182%), 68.1–102% with RSD 3.65–10.2% for C-18 + GCB SPE columns, and 96.0–120% with RSD 2.80–10.1% for SAX/PSA columns.

At the lower fortification level, the recoveries were: 65.4–108% with RSD 7.01–20.9% for silica SPE columns (with the exception of one extreme value of 207%), 54.4–137% with RSD 2.01–9.35% for amino SPE columns (except one value of 28%), 60.6–123% with RSD 2.08–8.23% for GCB SPE columns, 66.5–121% with RSD 3.10–10.2% for C-18 + GCB SPE columns and 76.9–116% with RSD 3.01–10.7% for SAX/PSA SPE columns. For validating residue methods, RSD values of 35, 30, 20, 15, and 10% are accepted at concentrations of $\leq$0.001, 0.001–0.01, 0.01–0.1, and $>1\,mg\,kg^{-1}$, respectively [10].

The extreme value of 188% (at the first fortification level) and 207% (at the second fortification level) for the compound dimethoate can be attributed to the

Table 3. Recovery and precision data ($n = 3$) of the compounds determined in spiked tomato samples (second level) through each different cleanup.

		Silica cleanup		Aminopropyl cleanup		GCB cleanup		C18 + GCB cleanup		SAX/PSA cleanup	
Compound	Fortification level ($mg\,kg^{-1}$)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Dichlorvos	0.1	65.6	19.3	–	–	67.4	3.24	–	–	–	–
Dimethoate	0.1	207	18.0	137	2.35	106	5.22	121	10.2	116	10.7
Diazinon	0.05	79.5	10.7	90.6	7.25	73.4	3.13	67.0	6.37	89.8	6.49
Chlorpyrifos methyl	0.05	67.2	20.9	83.2	4.50	104	3.30	79.4	3.26	97.9	3.33
Methidathion	0.02	75.4	11.9	54.4	9.35	60.6	8.23	66.5	8.68	76.9	6.21
β-Endosulphan	0.1	82.1	10.5	97.7	2.01	96.2	4.61	82.0	3.81	94.6	3.26
Iprodione	0.5	106	11.6	28.8	6.46	121	2.17	92.7	3.10	108	3.34
Bifenthrin	0.2	65.4	11.5	89.0	2.01	104	2.08	78.7	3.89	89.4	3.20
Phosalone	0.1	108	9.67	115	5.61	119	2.21	111	3.43	116	3.04
Fenarimol	0.05	89.5	7.01	98.5	6.54	104	2.13	80.0	4.28	107	3.11
Permethrin I	0.05	92.9	7.95	88.7	7.25	105	3.67	94.0	3.11	92.6	3.03
Permethrin II	0.05	85.0	9.73	101	3.79	107	2.34	84.8	3.15	103	3.36
Fenvalerate	0.1	96.1	8.83	102	2.03	123	2.04	87.2	3.29	105	3.01

– : No data obtained at this level.

matrix-enhancement effect, which was not eliminated by the cleanup with silica SPE columns, and caused excessively high recovery results for some pesticides in food [11]. The matrix-induced effect is influenced by many factors such as a pesticide's chemical structure, type of matrix, state of the chromatographic system, and compound/matrix concentration [12]. For a validated method, recoveries of 70–110% are acceptable; in the case of a routine analysis, the accepted recoveries range between 60 and 140% [10]. Podhorniak *et al.* [13] proposed that acceptable recoveries range from 50 to 150% for low concentration levels. The compound dichlorvos was not determined when cleanup was performed with either the SPE amino or the C-18 + GCB and SAX/PSA (only at the lower fortification level) cartridges. This can be attributed either on insufficient elution of the compound or on the loss of dichlorvos during the evaporation, because this compound has the highest vapour pressure of the 12 compounds [14].

Acetonitrile, *n*-hexane, or acetone extracts of tomato samples were eluted through normal phase (silica), bonded normal phase (aminopropyl), carbon (GCB), mixed mode SAX/PSA and the combination of C-18 and GCB SPE columns. The SPE sorbents retained matrix co-extractants while allowing the pesticides to elute. This has been reported previously [15], and we have confirmed the existence of interference compounds in the final eluants from the SPE columns.

The relative cleanup achieved with the various SPE columns was evaluated by GC/ECD analysis. Figure 1 shows that the mixed-mode SAX/PSA columns achieve the most effective removal of matrix coextractants The GCB and aminopropyl SPE columns also achieve a relatively satisfactory removal of interferences. However, the combination of C-18 and GCB columns and the silica columns alone did little to remove interferences producing chromatograms of a poor quality.

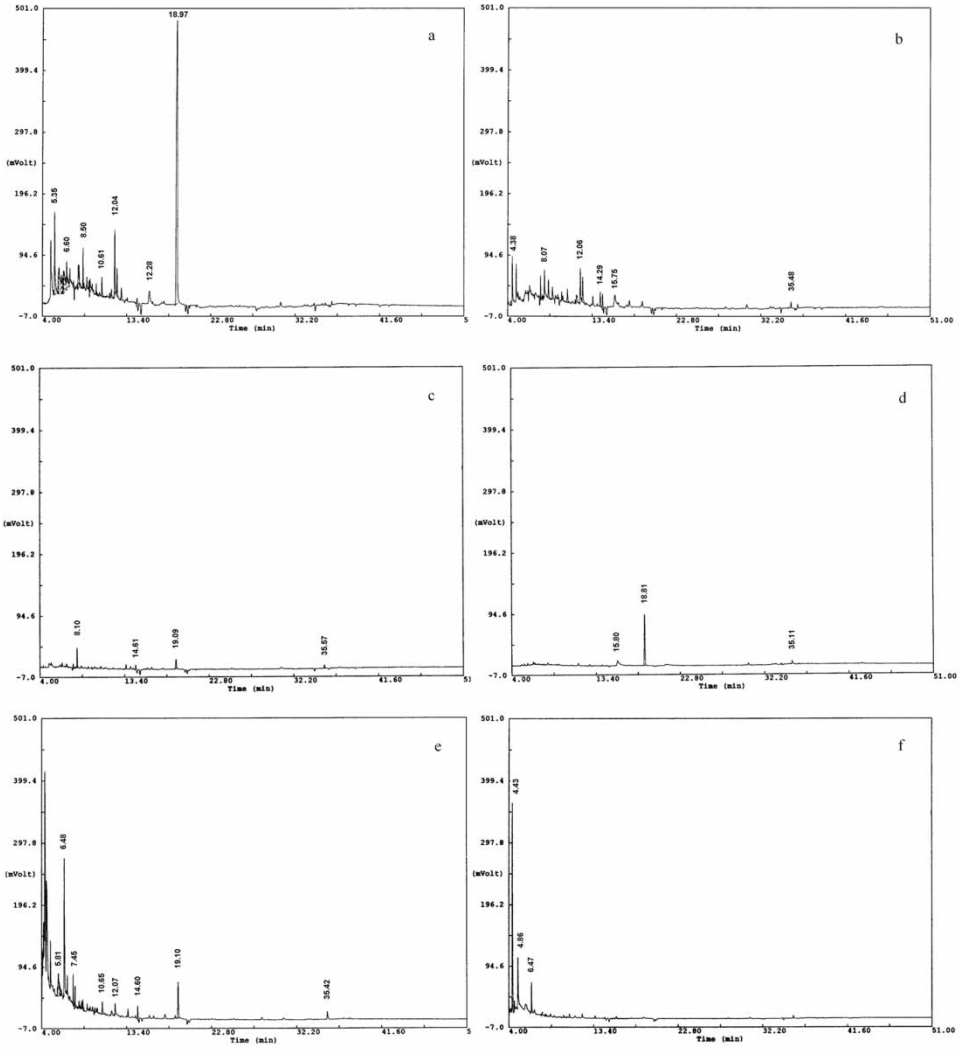

Figure 1. GC/ECD chromatograms of a blank tomato extract: (a) before SPE cleanup; (b) after SPE cleanup with silica columns; (c) after SPE cleanup with aminopropyl columns; (d) after SPE cleanup with GCB columns; (e) after SPE cleanup with C-18 and GCB columns; and (f) after SPE cleanup with SAX/PSA columns.

4. Conclusions

The main conclusion of this study is that the use of the mixed-mode SAX/PSA SPE column can help to achieve excellent cleanup of tomato extracts for multiresidue pesticide analysis. The GCB and aminopropyl sorbents can help to achieve an acceptable cleanup but to a lesser degree. The silica SPE columns do not succeed in removing all the matrix interferences and do not eliminate the matrix effect for the compound dimethoate. Finally, the use of C-18 and GCB columns produces poor chromatographic results.

References

[1] D.R. Erney, A.M. Gillespie, D.M. Gilvydis, C.F. Poole. *J. Chromatogr*., **638**, 57 (1993).
[2] F.J. Schenck, S.J. Lehotay. *J. Chromatogr. A*, **868**, 51 (2000).
[3] E.M. Thurman, M.S. Mills. *Solid-Phase Extraction – Principles and Practices*, *Chemical Analysis Series*, *147*, p. 2, Wiley, New York (1998).
[4] N.J.K. Simpson. *Solid-Phase Extraction – Principles, Strategies and Applications*, Marcel Dekker, New York (1998).
[5] J.S. Fritz. *Analytical Solid-Phase Extraction*, Wiley, New York (1999).
[6] D. Barcelo, M.-C. Hennion. *Techniques and Instrumentation in Analytical Chemistry*, *19*, p. 262, Elsevier, Amsterdam (1997).
[7] Y. Odanaka, N. Tomiyama, Y. Koma, O. Mantano, S. Goto. *J. Pestic. Sci*., **16**, 756 (1992).
[8] P. Van Zoonen (Ed.) *Analytical Methods for Pesticide Residues in Foodstuffs: Multi-Residue Method 1*, 6th Edn, p. 4, Ministry of Public Health, Welfare and Sport, Bilthoven, The Netherlands (1996).
[9] Informal Coordination of MRLs Established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC and 90/642/EEC. Available online at: http://europa.eu.int/comm/food/plant/protection/resources/mrl_pesticide.pdf (accessed 4 March 2005).
[10] European Commission. *Quality Control Procedures for Pesticide Residue Analysis: Guidelines for Residue Monitoring in the European Union* (European Commission, 2003), Document No. SANCO/10476/2003.
[11] J.L. Bernal, M.-J. del Nozal, J.J. Jimenez, J.M. Rivera. *J. Chromatogr. A*, **778**, 111 (1997).
[12] J. Hajslova, K. Holadova, V. Kocourek, J. Poustka, M. Kodula, P. Cuhra, M. Kempny. *J. Chromatogr. A*, **800**, 283 (1998).
[13] I.V. Podhorniak, J.F. Negron, F.D. Griffith. *J. Assoc. Off. Anal. Chem*., **84**, 873 (2001).
[14] C. Tomlin. *The Pesticide Manual*, 10th Edn, p. 2, Crop Protection Publication and Royal Chemical Society, Alton, Hampshire, UK (1995).
[15] G.A. Junk, M.J Avery, J.-J. Richard. *Anal. Chem*., **60**, 1347 (1988).