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Effects of modifiers, adsorbents and eluents in supercritical fluid extraction of selected pesticides in soil[1]

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Abstract

The applicability of supercritical fluid extraction (SFE) in pesticide residue analysis of fenpropimorph, pirimicarb, parathion-ethyl, triallate and fenvalerate in soil was investigated. Fortification experiments were conducted to optimize the extraction procedure by varying modifiers, trap adsorbents and eluents. Best efficiency was achieved at 60°C extraction temperature and CO_2 pressure of $3.8 \cdot 10^7$ Pa by the use of 5% methanol as modifer, a diol-modified silica gel trap and ethyl acetate as eluent. Recoveries of the target compounds ranged from 93–104%. Additionally, real soil samples taken 3 and 31 days after application under field conditions were analyzed for fenpropimorph, pirimicarb and parathion-ethyl residues by SFE and slurry as well as Soxhlet extraction technique. For all extraction methods investigated comparably high extraction efficiency was achieved. SFE is the most rapid procedure considering especially that no time-consuming clean-up steps are necessary. However, the little sample size applicable in the instrument specific extraction thimbles is the limiting factor in the detection of pesticides at low concentrations. Therefore, SFE extracts of replicates have to be pooled and concentrated in order to yield detection limits as low as those of conventional extraction procedures. © 1997 Elsevier Science B.V.

Keywords: Pesticides; Soil; Environmental analysis; Extraction methods

1. Introduction

During the last few years the interest in analysis of pesticides in soils, plants and foodstuff by supercritical fluid extraction (SFE) has rapidly increased. The development and the widespread application range of carbon dioxide (CO_2) based SFE was referred to in several reviews [1–5]. But, the use of pure CO_2 in pesticide analysis is limited because CO_2 is considered as a nonpolar solvent with a liquid solubility equal to that of hexane. Therefore, the use of organic solvents as modifiers for extraction of, e.g. organochlorine and organophosphorous pesticides, triazines, polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) from different sample matrices is reported by several authors [6–13].

Trapping of extracted analytes is another important aspect. With respect to instrumentation, analyte sampling after the extraction step can be distinguished between two principles: solvent trapping and solid trapping. Using the solvent trapping method, analytes dissolved in CO_2 are directly depressurized in the solvent while in the second case analytes were

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Table 1
SFE parameter ranges tested in preliminary investigations.

Parameter	Range
CO_2 density	0.50–0.88 g/l
CO_2 pressure	$1.5–3.8 \cdot 10^7$ Pa
CO_2 flow	1–3 ml/min
Extraction temperature	40–100°C
Static extraction time	0–15 min
Dynamic extraction time	10–90 min

adsorbed on solid materials and subsequently eluted with low volumes of different solvents.

In the beginning of SFE most experiments were conducted on fortified samples. The results indicated that SFE was a sophisticated new technique. When SFE was performed with real environmental samples it became clear that SFE conditions developed for fortified samples often yielded low extraction efficiency because of stronger interactions between pesticides and sample matrices [14,15].

In this study, the applicability of SFE (Table 1) for residue analysis of selected pesticides in soil was investigated. Applying fenpropimorph (morpholine), pirimicarb (carbamate), parathion-ethyl (thio-phosphoric acid ester), triallate (thiocarbamate) and fenvalerate (pyrethroide), a wide spectrum of different physico-chemical properties was considered. First, different solvents were tested as modifiers by performing fortification experiments with sandy silt soil samples. Then, procedure efficiency of different trap adsorbents and trap eluents was determined. In order to exclude soil matrix interferences in the extraction procedure fortified sea sand samples were used. Finally, real soil samples were analyzed for fenpropimorph, pirimicarb and parathion-ethyl residues by comparing SFE efficiency with conventional solvent extraction conducting slurry and Soxhlet techniques.

2. Experimental

2.1. Standards

All reference substances were purchased from Riedel-de Haën (Seelze, Germany). Stock standard solutions (1 μg/μl) of fenpropimorph (FPM), triallate (TRI) and pirimicarb (PIR) were prepared in methanol while parathion-ethyl (PTE), fenvalerate (FEN) and dodemorph (DDM) were prepared in hexane. Hexabromobenzene (HBB) was dissolved in toluene. These solutions were stored at −20°C in the dark. Standard mixtures of target compounds were diluted with ethyl acetate to give concentrations of 50 to 5000 pg/μl and stored at 4°C in the dark. GC with nitrogen–phosphorous detection (GC–NPD) calibration of FPM, PIR, PTE was done on concentrations of 100, 500, 1000, 2500 and 5000 pg/μl with 1000 pg dodemorph/μl as internal standard. To ensure detection of TRI, PTE and FEN in the linear response range of ECD two four-point calibration curves with concentration levels of 50, 100, 500, 1000 pg/μl and 500, 1000, 2500, 5000 pg/μl, each time with 1000 pg hexabromobenzene/μl as internal standard, were recorded.

2.2. Sample material

Field experiments were performed at test sites of the Federal Agricultural Research Centre (FAL) in Braunschweig, Germany. Spray application of FPM, PIR and PTE was done on a 720 m^2 test plot into a summer wheat stand. Application rates were according to 1.5 kg FPM/ha, 0.6 kg PIR/ha and 0.2 kg PTE/ha in 600 l water/ha. At 3 and 31 days after application, 20 single soil samples were taken in the superficial layer (0–5 cm) and combined to mixed samples. Soil texture of the Cambisol was a sandy silt with: 35.2% sand, 52.0% silt, 12.8% clay, $C_{org.}$: 0.8%, pH (0.01 *M* $CaCl_2$): 6.1 and maximum water capacity: 23.7%. Subsequently, samples were sieved to <2 mm and stored at −20°C till analysis. For fortification experiments untreated soil was sampled from a corresponding control plot.

2.3. Reagents

All solvents used were of residue analysis grade: acetone, cyclohexane, ethyl acetate, methanol and *n*-hexane (Baker, Griesheim, Germany). Further, distilled water, analytical-reagent grade anhydrous sodium sulphate, analytical-reagent grade sodium chloride, sea sand (Riedel-de Haën) and hydromatrix (Varian, Darmstadt, Germany) were used. Carrier, make up and detector gases for gas chromatography were helium 4.6, nitrogen 5.0, synthetic air and

hydrogen 5.0. CO_2 in qualities of 4.5 and technical grade (Linde, Hannover, Germany) was used for SFE.

2.4. Instrumentation

The following instruments were employed: rotary evaporator with 30–40°C bath temperature (Büchi, Göppingen, Germany), gel permeation chromatographic system (GPC) (Gilson/Abimed, Langenfeld, Germany) with Bio-Beads SX-8 (200–400 mesh) (BioRad, Munich, Germany), column diameter: 2.5 cm, gel bed length: 39 cm, flow-rate: 5 ml/min; supercritical fluid extractor HP 7680T with modifier pump HP 1050 (Hewlett–Packard, Waldbronn, Germany); gas chromatographs: HP 5890 with autosampler HP 7673, split/splitless injector, NPD and ^{63}Ni electron-capture detection (ECD) (Hewlett–Packard). For GC–ECD analysis a DB 5 fused-silica capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness (J&W Scientific, Folsom, USA) was used with helium as carrier gas (1.0 ml/min) and nitrogen as make up gas (60 ml/min). Temperature settings were: injector: 280°C, detector: 330°C, oven temperature program: 60°C (3 min)→20°C/min→160°C→4°C/min→280°C (15 min), injection volume: 1 μl splitless, with the splitter closed for 1 min. The GC–NPD determination was carried out with following parameters: DB 17 fused-silica capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness (J&W Scientific), carrier gas: helium (1.0 ml/min), synthetic air (100 ml/min), hydrogen (4 ml/min). Temperature settings were: injector: 280°C, detector: 280°C, oven temperature program: 60°C (1 min)→20°C/min→210°C (10 min)→25°C/min→280°C (2 min), injection volume: 1 μl splitless, with the splitter closed for 1 min.

2.5. Extraction procedures

2.5.1. Supercritical fluid extraction

In fortification experiments, 4 g sample material were spiked in a 50 ml beaker with 4 μg of each target compound to give concentrations of 1 mg/kg soil. According to Lopez-Avila et al. [16], Wuchner et al. [6] and Lehotay and Eller [17], 40 μl spiking standard in ethyl acetate (100 ng/μl) were distributed on the sample surface. Then, samples were homogenized by mixing and shaking for 2 min. After evaporation of solvent (approx. 15 min), spiked samples were mixed with 0.8 g hydro matrix which improved homogenous CO_2 flow through soil samples with different water contents [18] and filled into the extraction thimbles (7 ml).

Preliminary, basic extraction conditions were investigated. Ranges of parameters tested are shown in Table 1. Consequently, the following extraction conditions were used for further investigations: CO_2 flow, 2 ml/min; pressure, $3.8 \cdot 10^7$ Pa; extraction temperature, T_{Ex}=60°C; modifier amount, 5%; nozzle temperature (according to the modifier), 60–70°C; trap temperature, T_{Tr}=40–70°C.

Additionally, extraction was conducted in two steps in order to control extraction efficiency:

1. 12 min static, 30 min dynamic extraction, eluted twice with solvent (1 ml, flow-rate: 0.5 ml/min)
2. 2 min static, 10 min dynamic extraction, eluted twice with solvent (1 ml, flow-rate: 0.5 ml/min), two rinse substeps with different solvents (5 ml, flow-rate: 2 ml/min).

Trap sorbents used in these investigations were octadecyl- (ODS), cyano- (CN) and diol- (DIOL) modified silica gel, tenax (TNX) and stainless steel balls (SSB). Modifiers were *n*-hexane, acetone and methanol. Eluents used for removal of analytes from the trap were: *n*-hexane, ethyl acetate, acetone and methanol. The extracts were sampled in GC vials, 100 μl internal standard were finally added (10 ng/μl) and, without any clean up step, directly analyzed by GC–ECD and GC–NPD.

2.5.2. Slurry extraction

According to the principles of the usually applied DFG S19 multi method [19] and the on-line extraction method reported by Steinwandter [20], field moist soil was extracted for 12 h with an acetone–water mixture (2:1, v/v) on a horizontal shaker. For analysis, 50 g soil sample, 100 ml acetone and, according to the water content (x) of soil, $50-x$ g water were used. Then, approximately 15 g sodium chloride and, according to Koinecke et al. [21], 100 ml cyclohexane were added and shaken for another hour. The upper phase containing the organic solution was decanted and dried over anhydrous sodium sulphate. An aliquot of the organic phase (100–150 ml) was rotary evaporated under vacuum at 40°C and

Table 2
Recoveries of fenpropimorph (FPM), pirimicarb (PIR), parathion-ethyl (PTE), triallate (TRI) and fenvalerate (FEN) in spiked silty sand soil samples (1 mg/kg) using CO_2 with different modifiers (5%), ODS trap material and *n*-hexane as eluent

CO_2	Recoveries (%)				
	FPM	PIR	PTE	TRI	FEN
Without modifier	n.d.	n.d.	71	85	68
+*n*-Hexane	n.d.	n.d.	94	71	63
+Acetone	n.d.	57	107	91	80
+Methanol	87	84	98	81	109

dissolved in 5 ml cyclohexane–ethyl acetate (1:1, v/v) for GPC clean up. The analyte containing GPC fraction (80–180 ml) was concentrated and filled up to 1–5 ml with ethyl acetate including the internal standard (1000 pg/μl). Subsequently, target compounds were determined by GC–ECD and GC–NPD analysis.

2.5.3. Soxhlet extraction

According to Alzaga et al. [22], 50 g soil were extracted with 200 ml methanol in a Soxhlet apparatus for 16 h. The organic phase was dried over anhydrous sodium sulphate and rotary evaporated under vacuum at 40°C, followed by resolving in cyclohexane–ethyl acetate (1:1, v/v) for GPC clean up. The analyte containing GPC fraction (80–180 ml) was concentrated and filled up to 1–5 ml with ethyl acetate and internal standard (1000 pg/μl). Subsequently, the residues were determined by GC–ECD and GC–NPD analysis.

3. Results and discussion

3.1. Modifier test

As shown in Table 2, solvating power of pure CO_2 was too low for exhaustive extraction of the pesticides investigated. While FPM and PIR were not detectable in the eluates, recoveries of PTE, TRI and FEN ranged between 68–85%. To enhance SFE extraction efficiency CO_2 was modified with *n*-hexane, acetone and methanol. For this purpose, fortification experiments were carried out by using sandy

Table 3
Recoveries of fenpropimorph (FPM), pirimicarb (PIR), parathion-ethyl (PTE), triallate (TRI) and fenvalerate (FEN) of spiked sea sand samples (1 mg/kg), using methanol modifed CO_2 (5%), octadecyl- (ODS), cyano- (CN) and diol- (DIOL) modified silica gel, tenax (TNX) and stainless steel balls (SSB) as trap materials and different eluents ($n=4$)

Trap	Eluent	Recoveries (%)±R.S.D. (%)				
		FPM	PIR	PTE	TRI	FEN
ODS	*n*-Hexane	102±9	57±20	105±13	96±2	94±4
	Ethyl acetate	67±6	86±8	89±13	92±4	99±8
	Acetone	2±2	93±0	93±1	100±1	97±7
	Methanol	92±6	106±4	116±3	113±8	77±5
CN	*n*-Hexane	55±40	90±8	106±5	114±5	106±2
	Ethyl acetate	3±6	101±8	115±11	109±5	121±4
	Acetone	10±5	90±1	97±2	110±4	108±5
	Methanol	55±4	91±2	100±2	114±8	101±24
SSB	*n*-Hexane	93±1	77±7	94±7	19±2	98±1
	Ethyl acetate	94±7	83±1	98±2	21±8	79±5
	Acetone	85±8	69±8	86±6	26±3	92±2
	Methanol	96±6	74±5	95±6	33±4	33±3
TNX	*n*-Hexane	102±4	86±3	96±2	106±5	90±4
	Ethyl acetate	97±3	90±2	100±6	112±13	97±2
	Acetone	99±3	92±3	101±2	101±8	94±11
	Methanol	87±5	85±6	69±25	50±3	7±7
DIOL	*n*-Hexane	102±1	93±4	95±15	111±3	79±2
	Ethyl acetate	102±3	93±3	104±3	99±2	100±5
	Acetone	108±3	98±2	108±4	105±7	111±5
	Methanol	103±4	93±3	100±8	104±7	99±15

Table 4
Efficiency of *n*-hexane and ethyl acetate as eluents of DIOL trap

Pesticide	Eluent	Elution step	Recovery (%)
FPM	*n*-Hexane	1st	102
		2nd	0
	Ethyl acetate	1st	102
		2nd	0
PIR	*n*-Hexane	1st	8
		2nd	83
	Ethyl acetate	1st	93
		2nd	0
PTE	*n*-Hexane	1st	57
		2nd	38
	Ethyl acetate	1st	104
		2nd	0
TRI	*n*-Hexane	1st	111
		2nd	0
	Ethyl acetate	1st	99
		2nd	0
FEN	*n*-Hexane	1st	30
		2nd	49
	Ethyl acetate	1st	100
		2nd	0

Fenpropimorph (FPM), pirimicarb (PIR), parathion-ethyl (PTE), triallate (TRI) and fenvalerate (FEN) were extracted from spiked sea sand samples (1 mg/kg) using methanol modified CO_2 (5%).

silt soil spiked with target compounds (1 mg/kg), modifier amount of 5%, ODS as trap material and *n*-hexane as eluent. Using hexane as modifier yielded results similar to those of pure CO_2, only the extracted amount of PTE increased. Using acetone modified CO_2, recovery of PIR increased to 57% but without any increase in recoveries of FPM. Recoveries of 81–109% for all target compounds, however, were achieved only by the use of methanol. Based on these results, methanol modified CO_2 was applied in further experiments.

3.2. Variation of trap adsorbents and eluents

Method optimization was continued by a systematical examination of the applicability of different traps and eluents. Trap adsorbents tested were ODS, CN and DIOL as well as TNX and SSB. Eluents used for this investigation were *n*-hexane, ethyl acetate, acetone and methanol. In order to exclude interferences between pesticides and soil matrix, fortifications (1 mg/kg) were performed to less sorptive sea sand samples ($n=4$). Recoveries and relative standard deviation (R.S.D.) values are presented in Table 3. This general survey clearly shows that the applicability of several adsorbents and eluents was limited because of the wide spectrum of physico-chemical properties of target compounds. FPM was incompletely released when the ODS trap was eluted with ethyl acetate or acetone. Application of hexane or methanol was inappropriate because of decreasing recoveries of PIR and FEN. A universal application of CN and SSB traps was excluded due to little recoveries of FPM and TRI. Applying a TNX trap with methanol as eluent, recoveries of FEN were only 7% which emphasized low efficiency of this adsorbent/eluent combination.

The best results for all target compounds were obtained using a DIOL trap. The recoveries for all eluents tested were between 79 and 111% with a maximum R.S.D. of 15%. Particularly, recoveries of fenvalerate were depending on eluent selected. For the use of methanol, less repeatibility was revealed by R.S.D. of 15% while hexane only released 79% of the amount spiked. Distinguishing between first and second elution step, differences in elution efficiency of hexane and ethyl acetate became obvious (Table 4). While using ethyl acetate, all analytes were exhaustively eluted in the first step. Applying hexane, however, elution of PTE, PIR and FEN re-

Table 5
Efficiency of slurry extraction (SLU), Soxhlet extraction (SOX) and SFE [methanol modified CO_2 (5%), DIOL trap and ethylacetate as eluent] for fenpropimorph (FPM), pirimicarb (PIR) and parathion-ethyl (PTE) in real soil samples (3 and 31 days after application)

Pesticide	Concentration (μg/kg)					
	3 days after application			31 days after application		
	SLU	SOX	SFE	SLU	SOX	SFE
FPM	435	381	411	136	138	135
PIR	105	82	85	47	45	42
PTE	62	55	60	18	16	14

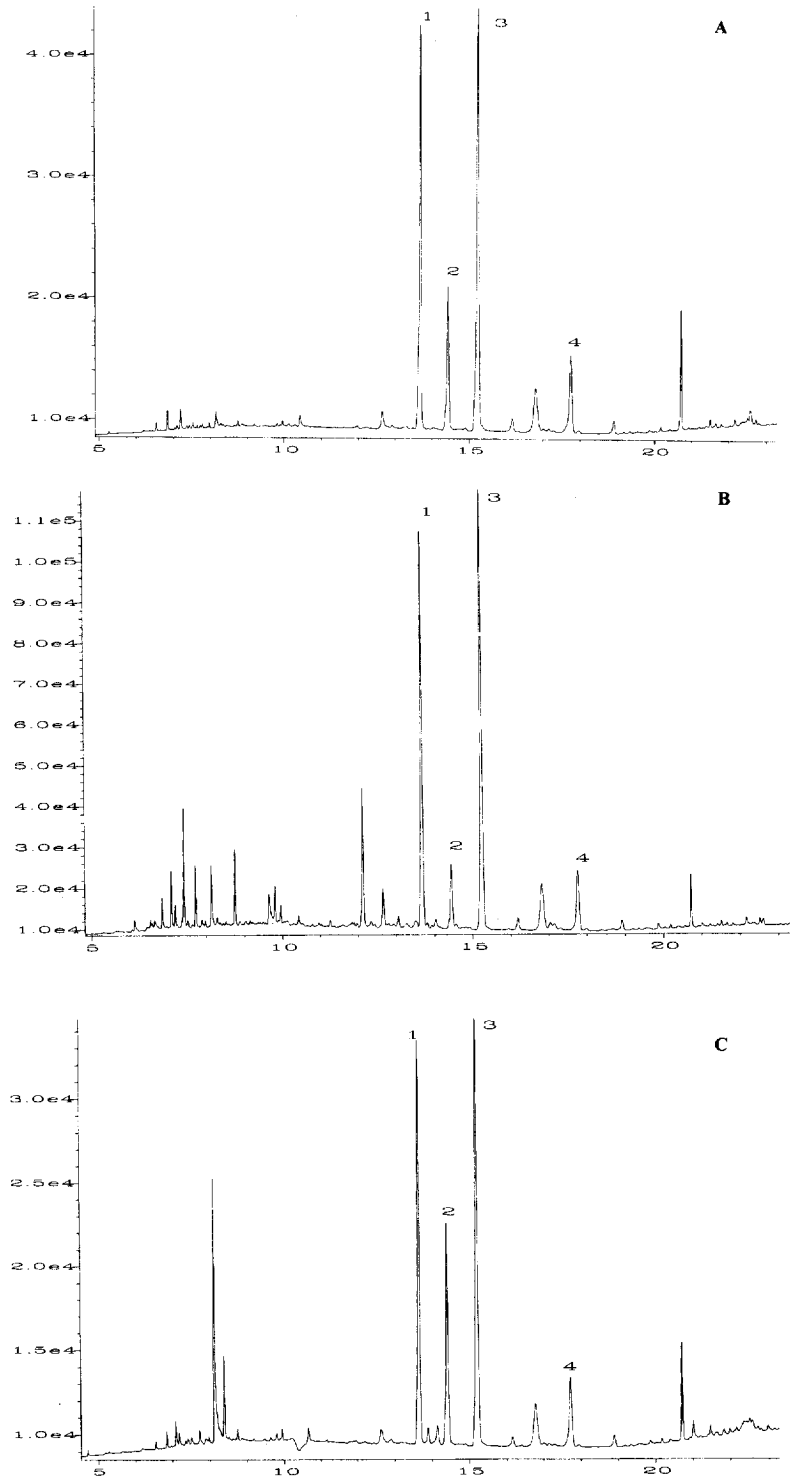

Fig. 1. GC–NPD chromatograms of real soil samples after slurry (A) and Soxhlet extraction (B), both cleaned up by gel permeation chromatography, and SFE (C) without clean up. [1: fenpropimorph, 2: dodemorph (internal standard), 3: pirimicarb, 4: parathion-ethyl].

mained incomplete and a second step was necessary. Therefore, most efficient trapping of these target compounds was achieved by using a DIOL trap and ethyl acetate as eluent.

3.3. Analysis of real soil samples

Finally, SFE was compared with conventional slurry and Soxhlet extraction. Table 5 shows the results for real soil samples taken at 3 and 31 days after application under field conditions. SFE gave comparable results with both other extraction procedures. The advantage of SFE was that extracts were immediately ready for GC analysis because no clean up steps were necessary. This effect is examplified in chromatograms of slurry and Soxhlet extracts, both cleaned up by GPC, and of SFE extracts (Fig. 1). Limits of SFE were pointed out by sample size applicable depending on the volume of instrument specific extraction thimbles. According to a thimble volume of only 7 ml, 4 g soil mixed with 0.8 g hydromatrix can be filled in. Considering a minimum concentration of 100 pg FPM, PIR or PTE/μl which was detectable by GC–NPD, a detection limit of only 25 μg/kg soil was calculated for SFE. In contrast to the slurry and Soxhlet techniques, which are usually based on a sample size of 50 g dry soil and which yield detection limits of 4 μg/kg soil, it was necessary to pool and concentrate SFE eluates of replicates to get detectable amounts ≥10 μg pesticide/kg soil.

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References

[1] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A–642A.
[2] S. Bøwadt, S.B. Hawthorne, J. Chromatogr. A 703 (1995) 549–571.
[3] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 66 (1994) 106R–130R.
[4] I.J. Barnabas, J.R. Dean, S.P. Owen, Analyst 119 (1994) 2381–2394.
[5] T.L. Chester, J.D. Pinkston, D.E. Raynie, Anal. Chem. 68 (1996) 487R–514R.
[6] K. Wuchner, R.T. Ghijsen, U.A.Th. Brinkman, R. Grob, J. Mathieu, Analyst 118 (1993) 11–16.
[7] E.G. van der Velde, M. Dietvorst, C.P. Swart, M.R. Ramlal, P.R. Koostra, J. Chromatogr. A 683 (1994) 167–174.
[8] T.M. Fahmy, M.E. Paulaitis, D.M. Johnson, M.E.P. McNally, Anal. Chem. 65 (1993) 1462–1469.
[9] V. Janda, G. Steenbeke, P. Sandra, J. Chromatogr. 479 (1989) 200–205.
[10] J.M. Levy, L.A. Dolata, R.M. Ravey, E. Storozynsky, K.A. Holowczak, J. High Resolut. Chromatogr. 16 (1993) 368–371.
[11] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 66 (1994) 909–916.
[12] R. Alzaga, J.M. Bayona, D. Barceló, J. High Resolut. Chromatogr. 19 (1996) 23–26.
[13] N. Alexandrou, J. Pawlisyn, Anal. Chem. 61 (1989) 2770–2776.
[14] V. Lopez-Avila, N.S. Dodhiwala, W.F. Beckert, J. Chromatogr. Sci. 28 (1990) 468–476.
[15] F. David, M. Verschuere, P. Sandra, Fresenius J. Anal. Chem. 344 (1992) 479–485.
[16] V. Lopez-Avila, N.S. Dodhiwala, J. Agric. Food Chem. 41 (1993) 2038–2044.
[17] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821–830.
[18] D. Plate, R. Giersberg, GIT Fachz. Lab. 1 (1996) 38–41.
[19] DFG pesticide comission, Manual of Pesticide Residue Analysis, VCH, Weinheim, 1987.
[20] H. Steinwandter, Fresenius J. Anal. Chem. 332 (1985) 752–754.
[21] A. Koinecke, R. Kreuzig, M. Bahadir, J. Siebers, H.-G. Nolting, Fresenius J. Anal. Chem. 349 (1994) 301–305.
[22] R. Alzaga, J.M. Bayona, D. Barceló, J. Agric. Food Chem. 43 (1995) 395–400.