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Application of Different Extraction Methods for the Determination of Selected Pesticide Residues in Sediments

Magdalena Kvíčalová · Pavla Doubravová · Roman Jobánek · Marcela Jokešová · Věra Očenášková · Hana Süssenbeková · Alena Svobodová

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Abstract Using several extraction methods including the QuEChERS approach, samples of both model and natural sediments were prepared. For the isolation of the target group of pesticides, two variants of two complementary extractions had to be used. Resulting extracts were analysed with LC/MS/MS. Selected methods furnishing the best results were validated in the terms of linearity and repeatability. Their limits of detection ranged from 0.1 to 2 ng/g, their limits of quantification from 1 to 6 ng/g and their recovery percentage varied between 46 % and 102 %.

Keywords Pesticide · QuEChERS · Luke method · Sediment · LC/MS/MS

Pesticides are chemical compounds extensively applied to prevent the loss of agrarian cultures, food and feed supplies controlling undesirable plants, insects and fungi. During the long period of their frequently oversized use, they contaminated all environmental components. Because aquatic environment belongs to the most affected, pesticides can post a formidable hazard to living organisms and primary sources of drinking water.

The presence of pesticides in food undergoes regular inspections. Their determination involves many different approaches to extraction, purification and final analysis of real matrices. Among the newest approaches to sample preparation, QuEChERS (quick, easy, cheap, effective, rugged, safe) (Anastassiades et al. 2003) represents the method employing the extraction with acetonitrile, which is subsequently salted out from an aqueous matrix using $MgSO_4$ and NaCl and finally purified by dispersive SPE cleanup with anhydrous $MgSO_4$ and primary secondary amine (PSA). This preparation step is commonly coupled with GC/MS or LC/MS, which are nowadays the most frequent choices for the determination of trace amounts of pesticides (Lehotay et al. 2005; Pizzuti et al. 2007). Although QuEChERS is a widely spread procedure in the analysis of biological and food matrices (Cunha et al. 2009; Koesukwiwat et al. 2010; Botitsi et al. 2011; Romero-González et al. 2011), its use for the pesticide determination in sediments or soils is quite rare (Lesueur et al. 2008; Brondi et al. 2011).

The aim of our work was to test QuEChERS and its various modifications for the extraction of selected pesticides from sediments. This method was compared with two other analytical procedures: (a) the Luke method (Luke et al. 1975), based on extraction of the matrices with the mixture of three solvents of different polarity, which was used in Dutch laboratories for the determination of pesticides in various food matrices (Diez et al. 2006; Hiemstra and de Kok 2007); and (b) the extraction of sediment samples under basic conditions using the mixture of ammonia, water and acetonitrile (Rosales-Conrado 2009).

Materials and Methods

Certified analytical standards of carbendazim, clomazon, phenmedipham and spiroxamine were supplied by Accu-Standard. Standards of carboxin, cypermethrin, fenpropidin, fluroxypyr and deuterated internal standard of *trans*-cypermethrin-d_6 were purchased from Dr. Ehrenstorfer, chloridazon was obtained from Sigma-Aldrich. The stock

M. Kvíčalová (✉) · P. Doubravová · R. Jobánek · M. Jokešová · V. Očenášková · H. Süssenbeková · A. Svobodová
T. G. Masaryk Water Research Institute, p.r.i., Podbabská 30, 160 00 Prague 6, Czech Republic
e-mail: magdalena_kvicalova@vuv.cz

and working solutions were prepared by dissolving standards in acetone for spiking and in mobile phase for LC/MS/MS analyses. Acetone (SupraSolve for GC), *n*-hexane (SupraSolv for GC) and dichloromethane (SupraSolv for GC) were purchased from Merck, methanol (gradient grade) from J. T. Baker and acetonitrile (Chromasolv for HPLC) from Sigma-Aldrich. Methanol (LC/MS grade) was obtained from Scharlau, acetic acid (99 %, p. a.), NH_4OH solution (25 %, p. a.), NaCl (p. a.) and anhydrous Na_2SO_4 (p. a.) were obtained from Lachner. Anhydrous $MgSO_4$ was purchased from Merck, anhydrous sodium acetate (p. a.) from Chemos. PSA was supplied by Supelco. Ultrapure water was prepared in our laboratory using Elix and Milli-Q (Millipore) purification systems. Nitrogen used for evaporations (purity 5.0) was supplied by Linde.

The pesticides studied were selected from the complex of non-regularly monitored compounds in the Czech Republic, considered as moderately hazardous (WHO) with low ADI (acceptable daily intake). Their annual usage in the Czech Republic exceeds 10,000 kg. This group includes fluroxypyr, carboxin, chloridazon, carbendazim, cypermethrin, clomazon, spiroxamine, phenmedipham and fenpropidin (Table 1), which significantly differ in their chemical, physical and biological properties.

As a matrix for model samples, river sandy sediment was used. It was first predried, sieved to maximum particle size smaller than 1 mm and dried at 400°C. This material was spiked to concentration 4 or 10 ng/g by the solution of all pesticides in acetone, shaked manually to achieve homogeneity and air-dried to constant weight. Before analysis, it was stored at room temperature.

Natural sediment samples were collected from several Czech and Moravian rivers (Berounka, Bilina, Dyje, Labe, Ohre, Ostravice and Zelivka's supply, Martinicky potok). These samples were lyophilized, pulverized in a ball mill, sieved, mixed and stored as described above.

For the extraction of selected compounds, the QuEChERS and Luke (Dutch) methods were first used. Both methods were modified by the addition of different amounts of water to the samples. The QuEChERS was also varied by the addition of several salt mixtures and the change of pH of the extraction solvent (see Table 2). Because both above methods led to low recovery of spiroxamine, third procedure, the extraction under basic conditions with the acetonitrile/water/ammonia mixture (80:20:1, v/v/v), was additionally employed.

QuEChERS procedure (Q): A sample (4 g) in a 50 mL PE centrifuge tube with optionally added water (0–20 mL)

Table 1 Molecular structures, CAS numbers, and molecular weights of the target analytes

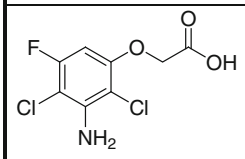
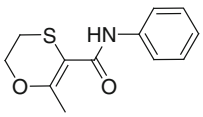
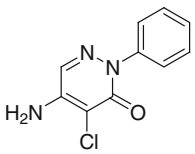
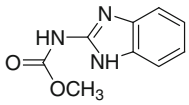
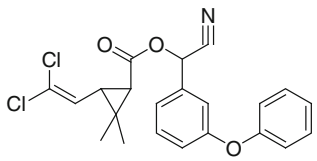
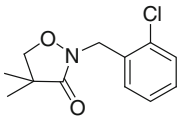
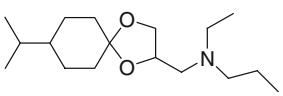
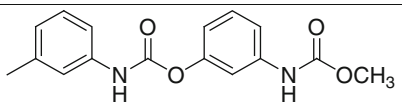
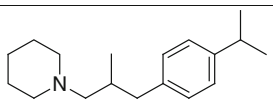
Pyridinecarboxylic acids	Carboxamides	Pyridazinones	Benzimidazoles
			
Fluroxypyr (69377-81-7), MW: 255.03	Carboxin (5234-68-4), MW: 235.3	Chloridazon (1698-60-8), MW: 221.6	Carbendazim (10605-21-7), MW:191.21
Pyrethroids	**Oxazolidinones**	**Spiroketalamines**	
			
Cypermethrin (5231-07-8), MW: 416.3	Clomazon (81777-89-1), MW: 239.7	Spiroxamine (118134-30-8), MW: 297.5	
Carbamates	**Morpholines**		
			
Phenmedipham (13684-63-4), MW: 300.31	Fenpropidin (67306-00-7), MW: 273.46		

Table 2 Overview of extraction methods

Method number	Salt mixtures	Sample to water ratio (w/w)	Extraction solvent or solvent mixtures
Q1A	$NaCl + MgSO_4$	–	ACN
Q1B	$NaCl + MgSO_4$	1:2	ACN
Q1C	$NaCl + MgSO_4$	1:5	ACN
Q2A	$CH_3COONa + MgSO_4$	–	ACN
Q2B	$CH_3COONa + MgSO_4$	1:2	ACN + 1 % CH_3COOH
Q2C	$CH_3COONa + MgSO_4$	1:5	ACN + 1 % CH_3COOH
Q2D	$CH_3COONa + MgSO_4$	1:2	ACN + 1 % NH_3
L1	–	–	Acetone/hexane/dichloromethane
L2	–	1:2	Acetone/hexane/dichloromethane
AM	–	–	Acetonitrile/water/ammonia

was shortly hand-shaked and left 1 h to soak. Then, 10 mL of acetonitrile containing optionally 1 % of additive and 2 g of selected salt mixture was added. The sample was extracted for 10 min using mechanical horizontal shaker at 250 rpm and centrifuged at 2,500 rpm for 10 min. The upper organic layer was transferred to a 15 mL PE centrifuge tube with 2 g of anhydrous magnesium sulfate. Then the sample was again centrifuged under the same conditions and the organic layer was evaporated by nitrogen stream in a TurboVap apparatus at 32°C to near dryness. The residual solvent was removed by standing in the air. The residue was redissolved in 1 mL of methanol/water mixture (1:1, v/v), the initial mobile phase for the LC/MS/MS analysis.

Luke method (L): A sample (4 g) in the 50 mL PE centrifuge tube with optionally added water (0–8 mL) was shortly hand-shaked and left 1 h to soak. Then 10 mL of the extracting mixture of acetone, hexane and dichloromethane (1:1:1, v/v/v) and subsequently 15 g of anhydrous sodium sulfate were added. The sample was extracted for 1 h using the mechanical horizontal shaker at 250 rpm and centrifuged at 2,500 rpm for 10 min. Upper organic layer was then treated in the same way as in the QuEChERS method.

Method using basic conditions (AM): A sample (4 g) and 10 mL of the extracting mixture of acetonitrile, water and 25 % aqueous ammonia (80:20:1, v:v:v) in the 50 mL PE centrifuge tube were shaked for 1 h using the mechanical horizontal shaker at 250 rpm and centrifuged at 2,500 rpm for 10 min. Upper organic layer was treated in the same way as in the methods above.

The LC/MS/MS analyses were carried out in the system consisting of an Agilent High Performance LC 1200 RR chromatograph equipped with thermostated autosampler, a degasser, binary pump and thermostated column connected with a 4000QTrap hybrid triple quadrupole-linear ion mass spectrometer equipped with an ESI Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA). Chromatographic separation was achieved using a reverse-phase Betasil C18 endcapped column (100 × 2.1 mm, particle size 5 μm, Thermo Fisher Scientific) at a flow rate 600 μL/min. The mobile phase consisted of solutions A (5 mM CH_3COONH_4 in water) and B (5 mM CH_3COONH_4 in methanol). The gradient was 98 % A at 0 min., 5 % A at 12 min., 5 % A at 15 min., 98 % A at 15.10 min., 98 % A at 20 min. Thermostat temperature was 35°C, injection volume was 10 μL. Individual analytes were detected by the comparison of the retention time with the retention time of standards and with the ratio of the integrated peak area of the signal of the two transitions of single studied compound with appropriate standard in calibration solution.

Results and Discussion

The optimization of MS signal parameters was performed by the on-column off-line injection of the standard solution of the individual target analytes or their mixtures. Optimized ionization mode, ionization conditions and the values of declustering potential (DP) were found for selected parent ions in a full-scan mode. Among the nine pesticides investigated, fluroxypyr showed higher response in the negative ionization mode (NI), while the remaining eight pesticides (8) were more sensitive in the positive ionization mode (PI). The instrument was hence operated first in the negative mode and after the detection of fluroxypyr was switched to the positive mode. The optimized LC/MS/MS conditions are summarized in Table 3.

The model samples were extracted using the methods described in Table 2 and the contents of studied pesticides were determined using LC/MS/MS. The LC/MS/MS results were compared based on the recovery of individual pesticides in Table 4. Obtained data show that to achieve acceptable recovery (70 %–130 %) for all selected compounds, the use of the combination of two extraction

Table 3 Optimized multiple reaction monitoring (MRM) conditions used for the LC/MS/MS analysis of selected pesticides in sediments, applied in negative (fluroxypyr) and positive ESI mode

Target compound	Retention time (min)	MRM transitions (m/z)		DP[a] (V)	CE[b] (V)	ESI
		Precursor ion	Product ion			
Fluroxypyr	5.46	255.0	197.0	−40	−18	NI
		253.0	233.0	−40	−10	
Chloridazon	6.84	222.1	104.1	86	33	PI
			92.0	86	37	
Carbendazim	7.34	192.2	160.1	36	25	PI
			132.1	36	43	
Carboxin	9.08	236.1	143.1	56	21	PI
			87.1	56	35	
Phenmedipham	10.34	301.2	168.1	66	13	PI
			136.0	66	29	
Clomazon	10.36	240.2	125.1	56	25	PI
			89.1	56	67	
Fenpropidin	11.49	274.4	147.2	81	41	PI
			117.1	81	77	
Spiroxamine	12.59	298.4	144.2	66	29	PI
			100.1	66	47	
Cypermethrin	13.66	433.3	191.0	46	21	PI
		435.3	193.0	51	21	

[a] Declustering potential

[b] Collision energy

procedures is essential. For the work-up of natural samples, the combinations of Q2B (for chloridazon, cypermethrin, fluroxypyr and phenmedipham) with Q2D (for carbendazim, chloridazon, clomazon, fenpropidin and spiroxamine, the values marked by italics in Table 4) or of L2 (for chloridazon, clomazon and phenmedipham) with AM (for carbendazim, chloridazon, cypermethrin, fenpropidin, fluroxypyr and spiroxamine, the underlined values) were chosen. Very low recoveries of carboxin confirmed its rapid decomposition in solid matrices (half-live in the soil ranges between 0.5 and 3 days, Hustert et al. 1999).

Method validation was performed according to Suchánek et al. (1997). Linear calibration curves were obtained for all pesticides analysed by the LC/MS/MS method over the whole range of 2–20 ng/mL. They were tested at five concentration levels by the injections of the standard solutions prepared in the mobile phase. Calculations were performed of the average peak areas as responses. The limits of detection (LODs), defined as the lowest concentration detectable, were estimated on the base of signal-to-noise ratio (s/n) of 3 and are shown in Table 5, where calculated limits of quantifications (LOQ's) for s/n = 10 are also reported.

Table 4 Recoveries of studied pesticides (%) in single used extraction methods

Method number	Q1A	Q1B	Q1C	Q2A	*Q2B*	Q2C	*Q2D*	L1	L2	AM
Carbendazim	09	46	41	32	35	44	*61*	<1	38	81
Carboxin	<1	<1	<1	<1	<1	<1	<1	5	<1	1
Chloridazon	159	88	78	68	*89*	90	*95*	116	87	102
Clomazon	143	30	16	62	56	62	*81*	65	66	55
Cypermethrin	124	54	55	61	*51*	44	26	30	29	65
Fenpropidin	22	27	23	43	20	18	*55*	<1	6	86
Fluroxypyr	3	40	29	24	*46*	51	14	<1	3	76
Phenmedipham	126	65	60	51	*60*	58	43	53	67	3
Spiroxamine	37	34	29	52	27	23	*52*	2	20	87

Table 5 Detection and quantification limits for the studied pesticides in the sediment samples

	Carbendazim	Carboxin	Chloridazon	Clomazon	Cypermethrin	Fenpropidin	Fluroxypyr	Phenmedipham	Spiroxamine
LOD (ng/g)	0.3	0.3	0.5	0.2	2	0.2	2	0.1	0.1
LOQ (ng/g)	1	1	2	1	6	1	6	1	1

Table 6 Repeatability (% RSD)

Pesticide	Carbendazim	Chloridazon	Clomazon	Cypermethrin	Fenpropidin	Fluroxypyr	Phenmedipham	Spiroxamine
Method	AM	AM	L2	AM	AM	AM	L2	AM
RSD (%)	5.0	9.6	5.9	16.4	4.0	9.2	13.7	3.0
Method	Q2D	Q2D	Q2D	Q2B	Q2D	Q2B	Q2B	Q2D
RSD (%)	9.0	11.6	8.2	22.2	12.9	7.0	19.8	8.5

Table 7 Concentration of studied pesticides in natural sediments (μg/kg)

Sample/date of sampling		Carbendazim		Clomazon		Fenpropidin	
		Q2D	AM	Q2D	L2	Q2D	AM
Bilina—Usti n.L.	2.8.2011	1.3	2.1	*n*	*n*	*n*	*n*
Martinicky potok I.	19.6.2011	2.2	2.5	15.2	n.d.	2.1	1.9
Martinicky potok II.	3.8.2011	1.0	1.5	3.5	3.2	1.5	1.8

n concentration <1 μg/kg; *nd* not determined

In the terms of repeatability, the majority of pesticides gave less than 15 % RSD with n = 6 at spiking level of 10 ng/g with the exception of cypermethrin and phenmedipham. Acquired results are given in Table 6. Carboxin was removed from this comparison due to very low recovery.

Totally 15 naturale river sediment samples were analysed. The concentration of the majority of studied pesticides was under limits of their quantification. Carbendazim, clomazon and fenpropidin were detected in Bilina at Usti n.L. and repeatedly in Zelivka's supply, Martinicky potok. Both methods used for the analysis gave comparable results (Table 7), which correspond well with the recoveries of individual compounds mentioned in Table 4.

Three different methods, originally used for the sample preparation for the multiresidual analysis of pesticides in food, were used for the extraction from river sediments. For the selected group of pesticides, two different extraction procedures had to be employed. Carbendazim, clomazon and fenpropidin, which were determined in natural samples, could be extracted together using the QuEChERS method modified by water and base addition (Q2D). For all extraction methods employed, speed and facility of the sample preparation represents their main advantage.

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