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Multiresidue method for the determination of 13 pesticides in three environmental matrices: water, sediments and fish muscle

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ABSTRACT

Pesticides residues in aquatic ecosystems are an environmental concern which requires efficient analytical methods. In this study, we proposed a generic method for the quantification of 13 pesticides (azoxystrobin, clomazone, diflufenican, dimethachlor, carbendazim, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, napropamid, quizalofop and thifensulfuron-methyl) in three environmental matrices. Pesticides from water were extracted using a solid phase extraction system and a single solid–liquid extraction method was optimized for sediment and fish muscle, followed by a unique analysis by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Limits of quantification were below 5 ng L^{-1} for water (except for fluroxypyr and iprodion) and ranged between 0.1 ng g^{-1} and 57.7 ng g^{-1} for sediments and regarding fish, were below 1 ng g^{-1} for 8 molecules and were determined between 5 and 49 ng g^{-1} for the 5 other compounds. This method was finally used as a new routine practice for environmental research.

1. Introduction

The widespread use of pesticides for agricultural activities represents thousands of molecules with a large range of physicochemical properties. It results in the contamination of numerous aquatic ecosystems, including water, sediments and biota [1–3]. A wide spectrum of pesticides can be found in environmental matrices. Some of them, often combined in a mixture, can impact fish health [4,5] or accumulate in edible tissue of fish, with a potential risk for humans [6,7]. However, a lot of residues of pesticides are not investigated in the different environmental matrices (water, sediment and fish) and, so, their levels and behavior in the environment are poorly understood.

Indeed, if numerous analytical methods deal with pesticides analysis in water [8], few of them report the simultaneous analysis of traces of pesticides in sediment and biota [9,10]. Moreover, when bioaccumulation of organic contaminants in fish is documented, the studies focus on organohalogens [11–14], but there is still a lack of data concerning more recent pesticides.

The analysis of traces of organic pollutants in such complex matrices should include an effective sample preparation allowing high recoveries of the analytes while minimizing the presence of interferences. This preparation step should be followed by an analytical method that is sufficiently sensitive and selective to quantify compounds in trace amounts.

Gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) are the most used techniques for monitoring pesticides residues in food and environmental matrices [15,16]. Due to low volatility of many compounds, GC–MS requires a derivatization before analysis [17]. Consequently, LC–MS is the most frequent choice to explore a large range of low volatile and medium-high polar compounds as pesticides [18]. The choice of the extraction method depends on the type of matrix. Finding a simple and easy method to extract compounds with very different physicochemical properties remains an analytical challenge. Pesticides from water are generally extracted by a solid-phase extraction (SPE), offering good recoveries for a large range of compounds [2,19]. For solid matrices, various methods are used. SPE is also applied as purification step but requires a long sample preparation [20]. Consequently quicker methods, such as solid–liquid extraction and the "QuEChERS" (Quick Easy Cheap Effective Rugged and Safe) methods are preferred to extract compounds from fish muscle [21,22]. Considering sediment or soil matrices, accelerated solvent extraction [23–25] and micro-wave assisted extraction [26] systems are usually performed.

In this context, the aim of this work is the development of a multi-residue method for the simultaneous analysis of a list of 13 pesticides, concerning a large range of physicochemical

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Table 1
Use and properties of the 13 target pesticides.

Compounds	Action[a]	Crop[b]	Molar mass[c] ($g\,mol^{-1}$)	Solubility in water[c] ($mg\,L^{-1}$)	Vapor pressure[c] (Pa, 20 °C)	Log P^c
Azoxystrobin	F	Wheat/barley	403.39	6.7	1.1×10^{-10}	2.50
Carbendazim	F	Rape seed	191.19	8	9.0×10^{-5}	1.56
Clomazone	H	Rape seed	239.70	1100	1.9×10^{-2}	2.54
Diflufenican	H	Wheat/barley	394.29	0.05	3.1×10^{-5}	4.90
Dimethachlor	H	Rape seed	255.74	2300	1.5×10^{-3}	2.25
Fluroxypyr	H	Wheat/barley	255.03	6500	3.8×10^{-9}	2.00
Iprodion	F	Rape seed	330.17	13	5.0×10^{-7}	2.99
Isoproturon	H	Wheat/barley	206.28	70	3.3×10^{-6}	2.50
Mesosulfuron-methyl	H	Wheat	503.51	483	1.1×10^{-11}	−0.48
Metazachlor	H	Rape seed	277.75	450	4.7×10^{-5}	2.13
Napropamid	H	Rape seed	271.35	70	5.3×10^{-4}	3.36
Quizalofop	H	Rape seed	344.8	0.31	1.1×10^{-7}	4.66
Thifensulfuron-methyl	H	Maize	387.39	2240	7.5×10^{-9}	−1.70

[a] Action is symbolized by H for herbicide and F for fungicide action.
[b] Examples of crop are presented for illustrate pesticide use [27].
[c] Data come from pesticide databases (data confirmed by 4 web databases: SIRIS, Footprint, Agritox and HSDB).

properties (Table 1), among the most commonly applied on cereals or oilseeds crop [27]—*i.e.* azoxystrobin, clomazone, diflufenican, dimethachlor, carbendazim, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, napropamid, quizalofop, and thifensulfuron-methyl. The physicochemical characteristics of the selected pesticides are presented in Table 1. Among the 13 pesticides, three of them present a fungicide action (azoxystrobin, carbendazim, and iprodion) and the other ones an herbicide action. Their physicochemical properties vary significantly. For example, the water solubility of fluroxypyr is $6.5\,g\,L^{-1}$ at 20 °C, whereas the diflufenican is practically insoluble in water ($0.05\,mg\,L^{-1}$). On the other hand, the log P of molecules vary from −1.7 for thifensulfuron-methyl to 4.9 for diflufenican. Consequently, developing one analytical method for all of them was a real challenge.

The method was developed for 3 environmental matrices: water, sediment and biota (muscle of 2 fish species). Extraction consisted in a SPE for freshwater and a unique solid–liquid extraction for sediments and fish muscle, all followed by a LC–MS/MS analysis (Fig. 1). Finally, the method was successfully applied to 10 samples of sediments and muscles of 50 carp and 43 perch. To our best knowledge, this is one of the few publications presenting multi-residue methods with $ng\,L^{-1}$ and $ng\,g^{-1}$ reported limits for the analysis of pesticides belonging to a wide variety of chemical families in three different complex matrices (Fig. 2).

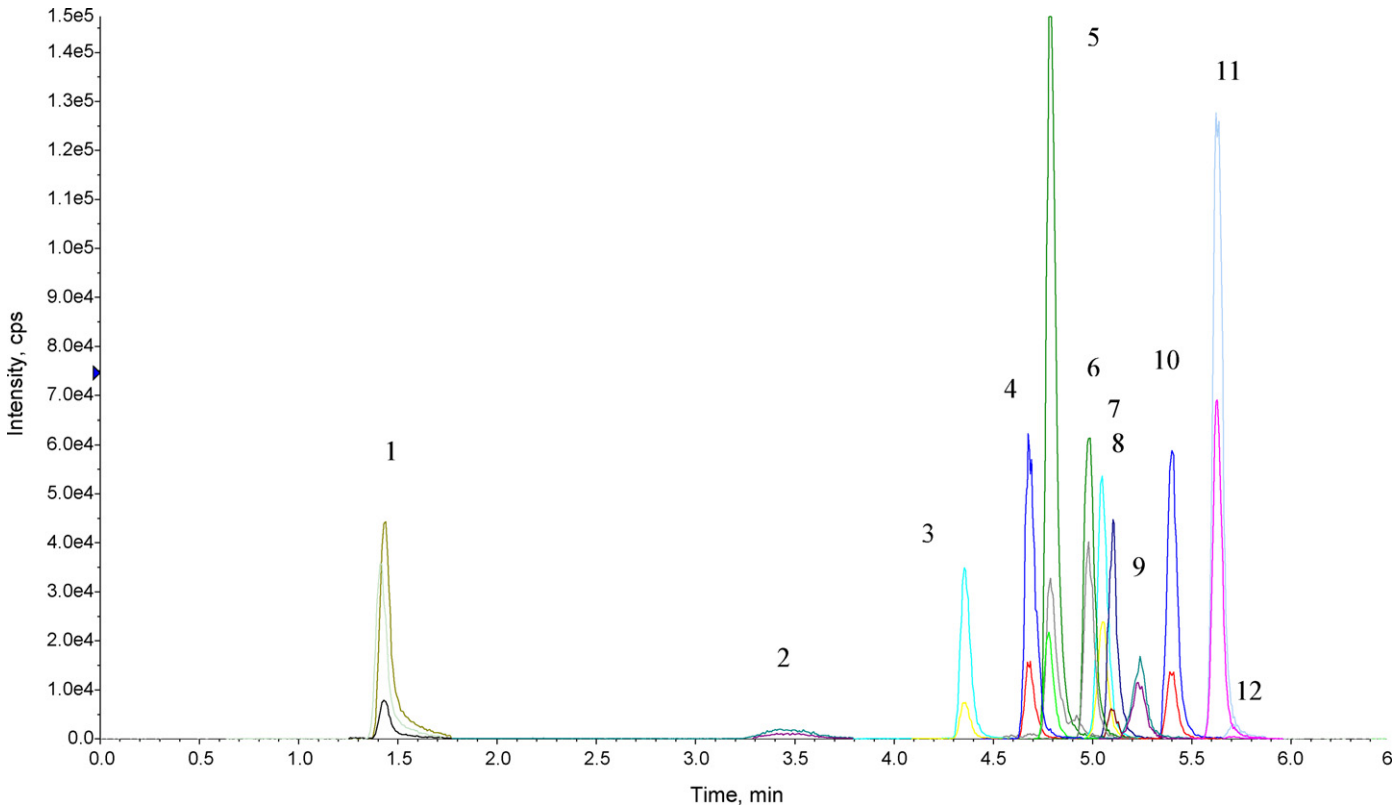

Fig. 1. Chromatogram of spiked fish with the 13 pesticides ($300\,ng\,g^{-1}$): 1 – carbendazim, 2 – fluroxypyr, 3 – thifensulfuron-methyl, 4 – mesosulfuron-methyl, 5 – isoproturon, 6 – metazachlor, 7 – dimethachlor, 8 – clomazone, 9 – quizalofop, 10 – azoxystrobin, 11 – napropamid, 12 – iprodion, and 13 – diflufenican.

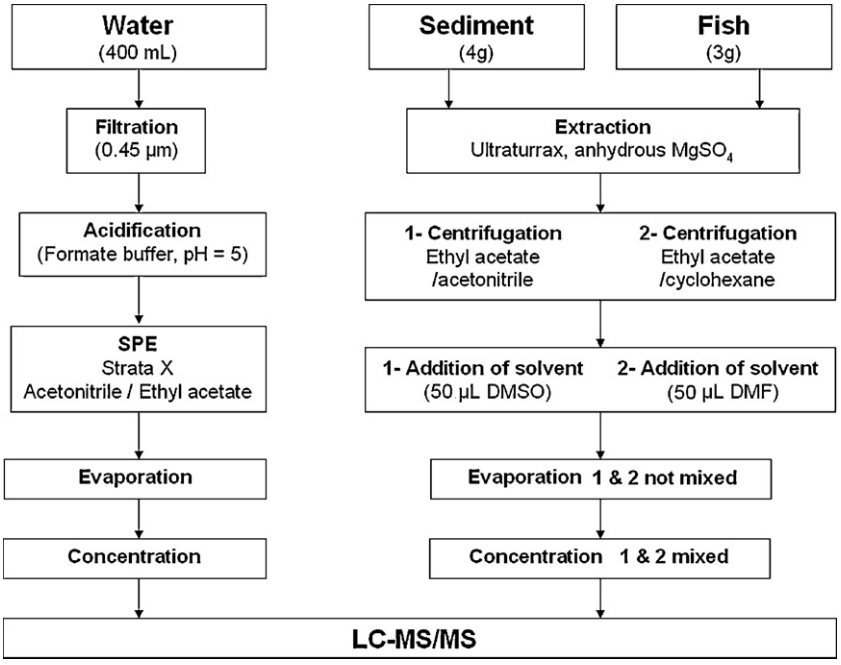

Fig. 2. Scheme of analytical strategy for pesticides from water, sediment and fish muscle samples.

2. Materials and methods

2.1. Chemicals and reagents

Dimethyl-sulfoxyde (DMSO), dimethyl-formamide (DMF) and acetone were of high purity (99%) and purchased from Riedel-de haen (Seelze, Germany). HPLC-grade solvents (methanol, cyclohexane, and ethyl acetate) used for sample preparation were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France), Fisher (Strasbourg, France) and VWR (Fontenay-sous-Bois, France) respectively. For LC–MS/MS analysis, LC–MS-grade solvent, acetonitrile was obtained from Sigma–Aldrich like anhydrous $MgSO_4$. Azoxystrobin, clomazone, diflufenican, dimethachlor, carbendazim, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, napropamid, thifensulfuron-methyl (Sigma–Aldrich), quizalofop (Supelco) and carbendazim-d_4, isoproturon-d_3 (Cluzeau) were powder conditioned with a purity upper than 97%. Ultra pure (UP) water was produced from a Direct-Q-system (Millipore, Molsheim, France). Stock solutions were prepared in brown glass bottles and frozen at −20 °C. Concentration of each compound was 1.0 mg mL^{-1} (in acetonitrile) except for carbendazim (0.1 mg mL^{-1}, in methanol, because of limited solubility).

2.2. Sample collection

Water, sediment and fish were collected in five dam ponds in the East of France (Lorraine region), between 2007 and 2009. These ponds, established on clay soils, dammed a stream and were strongly connected to their agricultural watersheds. Five sites had 0, 25, 45, 75 and 85% of cultivated parcels on the watershed area, respectively.

Water samples were collected (10–15 cm depth) during spraying periods (autumn and spring) at the level of the dam. Water parameters were measured at each sample time: temperature was ranged from 8 to 21 °C, dissolved oxygen was ranged from 5 to 15 mg L^{-1}, pH was ranged from 7.5 to 9.5. Sediments (0–4 cm depth, pH was 8 ± 1) were sampled during the emptying period of pond in five sedimentation areas of each pond. They were homogenized to have a single sample per site and per period (two emptying period were considered per site). Sediments or surface water samples were dark conditioned in glass bottles and frozen at −20 °C. Sediments samples were not dried to a better taking account of environmental expectations evaluating pesticides also in accessible part of sediments (*i.e.* pore water).

Fish species were carp (*Cyprinus carpio*) and perch (*Perca fluviatilis*), collected during emptying periods of pond. Lipid contents in muscle were 0.5 ± 0.2% and 4 ± 3% for perch and carp, respectively. A three months depuration in clean water was performed for some of them in laboratory, for recovery studies and validation (three months corresponded to three times the maximum half-life observed in fish muscle for these compounds). Others (without depuration) were environmental samples. Fish were killed by a blow to the head, following anaesthesia in ice bath. Then, muscles were quickly removed and frozen (−20 °C). All fish treatments used to obtain fish collection were in accordance with the general guidelines of the Council of European Communities (1986, No. 86/609/CEE) [28] and the French Animal Care Guidelines.

2.3. Extraction method

2.3.1. Water

Water samples were thawed out at +4 °C. Solid-phase extraction (SPE) was performed with Strata X column (200 mg, 33 µm, 3 mL; Phenomenex, Torrance, California) conditioned successively with acetonitrile (3 mL), methanol (3 mL) and ultrapure water (3 mL). 400 mL of water sample was used. Column was rinsed with water (5 mL) and dried during 20 min. Pesticides were extracted with acetonitrile (3 mL) and then ethyl acetate (3 mL). 50 µL of DMSO and 50 µL of DMF were added (Fig. 2). After evaporation (nitrogen flux, 40 °C), residue was dissolved in 2900 µL of acetonitrile/water (10:90, v/v) and dark stored (−20 °C).

2.3.2. Sediments and fish

Sediments and fish samples were thawed out at +4 °C and grinded. 4 g of sediments or 3 g of muscle were sampled in 50 mL tubes (734-0492, VWR). A single extraction method was applied for the two matrices. A high-performance disperser, Ultra-turrax (IKA, Lille, France), was used to grind the sample in tubes maintained

Table 2
LC–MS/MS conditions: retention time, MRM transitions selected, declustering potential (DP), collision energy (CE), applied in positive ESI mode.

Compounds	Formula	RT (min)	MRM transitions	DP (V)	CE (V)
Azoxystrobin	$C_{22}H_{17}N_3O_5$	5.4	404 → 372	16	19
		5.4	404 → 344	16	27
Carbendazim	$C_9H_9N_3O_2$	1.5	192 → 160	26	31
		1.5	192 → 132	26	43
Carbendazim-d_4		1.5	196 → 164	26	25
Clomazone	$C_{12}H_{14}ClNO_2$	5.3	240 → 125	21	23
		5.3	240 → 89	21	59
Diflufenican	$C_{19}H_{11}F_5N_2O_2$	6.6	395 → 266	41	39
		6.6	395 → 246	41	41
Dimethachlor	$C_{13}H_{18}ClNO_2$	5.1	256 → 224	16	17
		5.1	256 → 148	16	33
Fluroxypyr	$C_7H_5Cl_2FNO_3$	3.5	255 → 209	21	21
		3.5	255 → 181	21	33
Iprodion	$C_{13}H_{13}C_{l2}N_3O_3$	5.7	330 → 245	21	19
		5.7	330 → 288	21	17
Isoproturon	$C_{12}H_{18}N_2O$	4.8	207 → 72	26	35
		4.8	207 → 165	26	16
Isoproturon-d_3		4.8	210 → 75	26	21
Mesosulfuron-methyl	$C_{17}H_{21}S_2N_5O_9$	4.7	504 → 182	36	31
		4.7	504 → 139	36	69
Metazachlor	$C_{14}H_{16}ClN_3O$	5.0	278 → 134	16	27
		5.0	278 → 210	16	15
Napropamid	$C_{17}H_{21}NO_2$	5.6	272 → 129	21	19
		5.6	272 → 171	21	21
Quizalofop	$C_{17}H_{13}ClN_2O_4$	5.2	345 → 299	36	23
		5.2	345 → 273	36	25
Thifensulfuron-methyl	$C_{12}H_{13}S_2N_5O_6$	4.3	388 → 167	16	21
		4.3	388 → 141	16	21

in water at 20 °C with 10 mL of acetonitrile/water (50:50, v/v) and 4 g of $MgSO_4$ anhydrous. Surrogate standards, carbendazim-d_4 and isoproturon-d_3, were added to check extraction recovery. The tube was shaken 10 min and then centrifuged (12,000 × *g*, 10 min, 25 °C). The upper phase was put in a glass tube with 100 μL DMSO. A second solid–liquid extraction was performed with ethyl acetate/cyclohexane (75:25, v/v), 10 min agitation and centrifugation (12,000 × *g*, 10 min, 25 °C). The upper phase was remote in a glass tube with 100 μL DMF. After evaporation (nitrogen flux, 40 °C) of each tube, residue was dissolved in 900 μL of acetonitrile/water (10:90, v/v). Residues of the two tubes (first and second centrifugation) were mixed in one and dark stored at −20 °C (Fig. 2).

2.4. LC–MS/MS analysis

Analyses were performed on Agilent 1100 series HPLC (Agilent Technologies, Heilbronn, Germany) equipped with a reversed-phase Zorbax XDB-C_{18} (Agilent, 50 mm × 2.1 mm, 1.8 μm). Mobile phases were 0.0125% (v/v, pH = 4.04) acetic acid (A) and 100% acetonitrile (B). Pesticides were separated following gradient program: gradient was from 15% B to 60% B in 1 min and secondly, from 60% B to 100% B in 6 min. Solvents were maintained to 100% B for 5 min and then, returned to initial conditions with an equilibration of 7 min. Column temperature was 60 °C, flow rate was 0.3 mL min^{-1}. The sample injection volume was set at 10 μL.

The mass spectrometer used was a 3200 Q-Trap system (Applied Biosystems, Foster City, CA, USA) equipped with an ESI (TurboV) source operated in positive ionization mode. The operating parameters were: ionspray voltage 5500 V, curtain gas 20 (arbitrary units), nebulizer gas 50 (arbitrary units), auxiliary gas 60 (arbitrary units), probe temperature 600 °C. For each compound, declustering potential (DP) and collision energy (CE) of the main transitions were optimized from a continuous flow of a standard injection (1 mg L^{-1} solution at 10 μL min^{-1}). Two multiple reaction monitoring (MRM) transitions were acquired for each analyte, using the *Q*/*q* intensity ratio as confirmatory parameter. LC–MS/MS conditions are presented in Table 2.

2.5. Quantification and validation

2.5.1. Instrument performance

Intra-day and inter-day precision were determined by five repeated injections of standards at 5 μg L^{-1} in the same day and different days [24]. Eight calibration standards from 0.01 to 100 μg L^{-1} were injected to evaluate the linearity of the instrumental method and the instrumental detection limit (ILOD). The latter corresponds to the analyte concentration that produced a chromatographic peak signal of three times the background noise (Table 3).

2.5.2. Method performance

To determine recoveries, freshwater, sediment and fish samples were spiked before and after extraction in triplicate. Recoveries were evaluated at 3 concentrations, 1, 2.5 and 5 ng L^{-1} for water and 1.5, 15 and 30 ng g^{-1} for solid and biotic matrices except diflufenican, iprodion, quizalofop and fluroxypyr (2.5, 5 and 50 ng L^{-1} and 15, 30 and 300 ng g^{-1}). Recoveries were calculated by the ratio of the areas of each analyte in the sample spiked before extraction and in the sample spiked after extraction. Repeatability is associated with the intra-day precision. To estimate it, the samples need to be spiked, extracted and analyzed in the same conditions, by the same manipulator and on the same day. Therefore, each level of concentration was repeated three times. The intra-day precision

Table 3
Extraction parameters (recovery rate and coefficient of variation CV, $n = 3$) and limits of detection for instrument (ILOD) and limit of 24 quantification of the method (LOQ) in each matrix.

Compounds	Repet (RSD %) ($n = 5$)	Repro (RSD %) ($n = 5$)	ILOD (pg)	LOQ (ng L^{-1})	Water			Sediment			Fish muscle	
					Extraction recovery (%)	RSD (%)	LOQ (ng g^{-1})	Extraction recovery (%)	RSD (%)	LOQ (ng g^{-1})	Extraction recovery (%)	RSD (%)
Azoxystrobin	6	8	0.1	0.2	83	9	0.2	102	18	0.2	71	4
Carbendazim	7	10	0.1	0.2	81	3	0.1	68	10	0.1	82	6
Clomazone	4	12	0.2	0.3	58	10	0.7	95	19	0.6	85	2
Diflufenican	18	24	1.8	3.4	60	4	47.6	35	25	49.0	36	21
Dimethachlor	3	13	0.1	0.3	58	11	0.2	80	28	0.2	80	6
Fluroxypyr	8	12	15.4	41.0	42	6	10.4	40	20	29.9	115	5
Iprodion	17	27	82.6	74.6	125	23	57.7	125	21	24.0	52	22
Isoproturon	2	18	0.2	0.4	63	8	0.2	61	10	0.3	90	4
Mesosulfuron-methyl	2	22	0.1	0.2	94	6	0.3	83	3	0.4	89	3
Metazachlor	1	10	0.1	0.3	60	9	0.2	118	23	0.1	82	4
Napropamid	7	8	0.1	0.2	70	7	0.3	115	18	0.2	67	3
Quizalofop	7	9	1.6	4.2	43	16	3.7	50	15	5.0	68	0.5
Thifensulfuron-methyl	2	7	0.1	0.3	59	13	11.7	61	7	17.9	93	5

is expressed as the relative standard deviation (RSD) of a series of measurements [29].

Limits of quantification (LOQ) were determined as the analyte concentration that produced a peak signal of three and 10 times the background noise from the chromatogram respectively.

Quantification, based on peak areas, was performed by external calibration concerning water samples and matrix-matched calibration regarding solid matrices (sediment and fish muscle). Eight-point calibration curves (0.01–100 μg L^{-1}) were generated using linear regression analysis. The linearity was qualified by linear correlation coefficient, r^2.

3. Results and discussion

3.1. LC–MS/MS method

In order to obtain good separation of the target analytes prior to mass detection, an optimization of the liquid chromatography conditions was performed by the injection of standard solutions of the mixture of all analytes. The column chosen was a Zorbax Eclipse C18 (Agilent, 50 mm × 2.1 mm, 1.8 μm) for its good retention of hydrophobic and hydrophilic compounds, even with a short size. The short length of the column is advantageous since the required equilibration time is only 6 min, which reduces the total time of run. The 13 pesticides were separated within 7 min (Table 2). Even if 0.3 mL min^{-1} is not the optimum flow with this kind of column, a significative gain in sensitivity was observed keeping a bearable pressure for a chromatographic system like Agilent 1100.

The detection was performed using the electrospray interface in the positive mode, as it represents a good compromise in the detection of the 13 pesticides. The optimization of ionisation was performed by a serial of preliminary experiments, testing for example different modifiers in the mobile phase such as acetic acid and ammonium acetate at various concentrations. Finally, the addition of 0.0125% acetic acid gave the best sensitivity in positive mode. Two different transitions were monitored per compound when the fragmentation was appropriate: the first and the more abundant ion was used for quantification and the second for confirmation (ratio of quantity of signal of these two transitions in range of ±20% of a standard). Besides, the sensitivity of the mass spectrometer was further improved using the scheduled MRM mode. Resulting instrumental limits of detection (ILOD) are reported in Table 3.

3.2. Extraction method

3.2.1. Water

The extraction of the target analytes was performed by SPE. First experiments allowed the selection of the Strata X column (200 mg, 33 μm, 3 mL) for the extraction of the 13 compounds. The best recoveries were obtained using acetonitrile and ethyl acetate as solvent elution. In order to improve the sensitivity of the method, the sample volume was progressively increased. The recoveries obtained with spiked water did not show any significant breakthrough before 500 mL. Nevertheless the volume was finally set at 400 mL to reduce the analysis time and matrix effects.

3.2.2. Sediments and fish muscle

In order to develop a generic method, solid liquid extraction was investigated. Three parameters were particularly investigated in order to obtain optimum extraction conditions for analysis of 13 pesticides in sediment and fish muscle: (i) the grinding step, (ii) choice of solvents, and (iii) the centrifugation parameters.

The grinding step was investigated. A high-performance disperser, Ultra-turrax, was tested to increase accessibility of solvents to pesticides trapped in matrices, cutting cells or particles. Speed of use was important: low speed did not crush finely matrices and

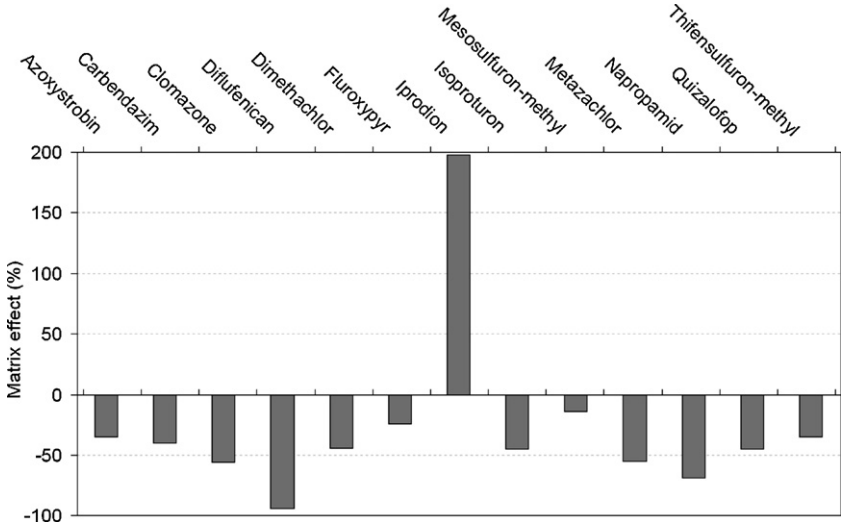

Fig. 3. Evaluation of matrix effect on fish muscle.

high speed caused a too much increase of heat that could induce degradation of pesticides. Middle speed (3/5) was a better compromise if sample tube was maintained at 20 °C in a water bath. Water in solid matrices was trapped by anhydrous $MgSO_4$. $NaSO_4$ was also tested but produced a strong aggregation with sediments and did not enable centrifugation.

Choice of solvents was based on miscibility of pesticides in order to increase recoveries. Ethyl acetate, cyclohexane, acetone, acetonitrile were tested in two successive centrifugations in different combinations (Table 4). Better recoveries were generally observed with ethyl acetate/acetonitrile (50:50, v/v) conditions, except for diflufenican, clomazone, and quizalofop which had very low recoveries. These three compounds had a better recovery with ethyl acetate/cyclohexane (75:25, v/v). For this reason two successive solvent extractions were chosen to final conditions: firstly with ethyl acetate/acetonitrile (50:50, v/v) conditions and then, ethyl acetate/cyclohexane (75:25, v/v). Recoveries were increased for all compounds.

Centrifugation parameters were also tested to get a good separation solid/liquid for sediments and muscle. Temperature and speed of rotation were tested in order to evaluate a good phase separation (visually) and recuperation of solvents (measured volume). Three temperature levels (5, 20 and 25 °C) and three rotation rates (4000; 10,000 and 12,000 × *g*) were tested. Below 25 °C condition, recuperation of solvents was not enabled because of insufficient phase separation. When a 12,000 × *g*/25 °C centrifugation was performed, volume of solvent recovered was the highest (95% of volume initially added).

3.3. Matrix effect

Matrix effects like enhancement or suppression of signals can severely compromise the quantitative analysis of environmental samples. To evaluate the influence of the matrix on the analysis, samples were spiked after extraction and the signals ($A_{\text{spiked\_matrix}} - A_{\text{blank\_matrix}}$) were compared with those of injected standards (A_{solvent}) at the same concentration. The matrix effect was calculated by means of the following equation:

$$\text{matrix effect}\,(\%) = \left(\frac{A_{\text{spiked\_matrix}} - A_{\text{blank\_matrix}}}{A_{\text{solvent}}} - 1\right) \times 100$$

Results of this study are summarized in Fig. 3. Strong MS signal suppression effects were observed for most of the compounds except iprodion. The percentage varies from −94 to 198% for fish samples. Thus, to compensate matrix effect and avoid any under/over estimation of pesticides, a matrix-matched calibration in eight points was used, for sediment and fish matrices.

3.4. Method performance and validation

Instrument performance evaluation is detailed in Section 2.5.1. Limits of detection for instrument (ILOD) thus determined are reported in Table 3: they are all inferior to 2 pg injected except for fluroxypyr (15.42 pg injected) and iprodion (82.64 pg injected). Reproducibility and repeatability expressed as relative standard deviation, RSD, were lower than 18% for intra-day and 27% for inter-day analysis, respectively.

The performance of the method was evaluated through estimation of recovery, intra-day precision, and sensitivity of the method.

Regarding water samples, optimized extraction using Strata X cartridges gave high recovery values, presented in Table 3. The

Table 4
Comparison between recovery rates obtained with different solvents of extraction in the case of spiked muscle (4 g) of *Cyprinus carpio* (300 ng g^{-1} for each compound except for carbendazim and carbendazim-d_4, 30 ng g^{-1}).

Compounds	Extraction recovery (%) (n = 6)				
	A	B	C	D	E
Azoxystrobin	21	22	11	33	48
Carbendazim	49	40	30	41	53
Carbendazim-d_4	49	40	26	47	65
Clomazone	11	12	27	23	32
Diflufenican	6	8	22	2	25
Dimethachlor	18	20	1	31	44
Fluroxypyr	29	14	35	20	29
Iprodion	7	7	9	23	66
Isoproturon	26	29	41	36	53
Isoproturon-d_3	27	25	6	39	58
Mesosulfuron-methyl	127	94	68	70	86
Metazachlor	14	18	13	23	31
Napropamid	6	7	43	14	21
Quizalofop	10	11	32	15	25
Thifensulfuron-methyl	76	66	39	44	48

Tested solvents are: (A) ethyl acetate/cyclohexane/acetone (1:1:1, v/v/v) for the two centrifugations; (B) ethyl acetate/cyclohexane (50:50, v/v) for the two centrifugations; (C) ethyl acetate/cyclohexane (75:25, v/v) for the two centrifugations; (D) ethyl acetate/acetonitrile (50:50, v/v) for the two centrifugations; (E) ethyl acetate/acetonitrile (50:50, v/v) for the first centrifugation and ethyl acetate/cyclohexane (75:25, v/v) for the second.

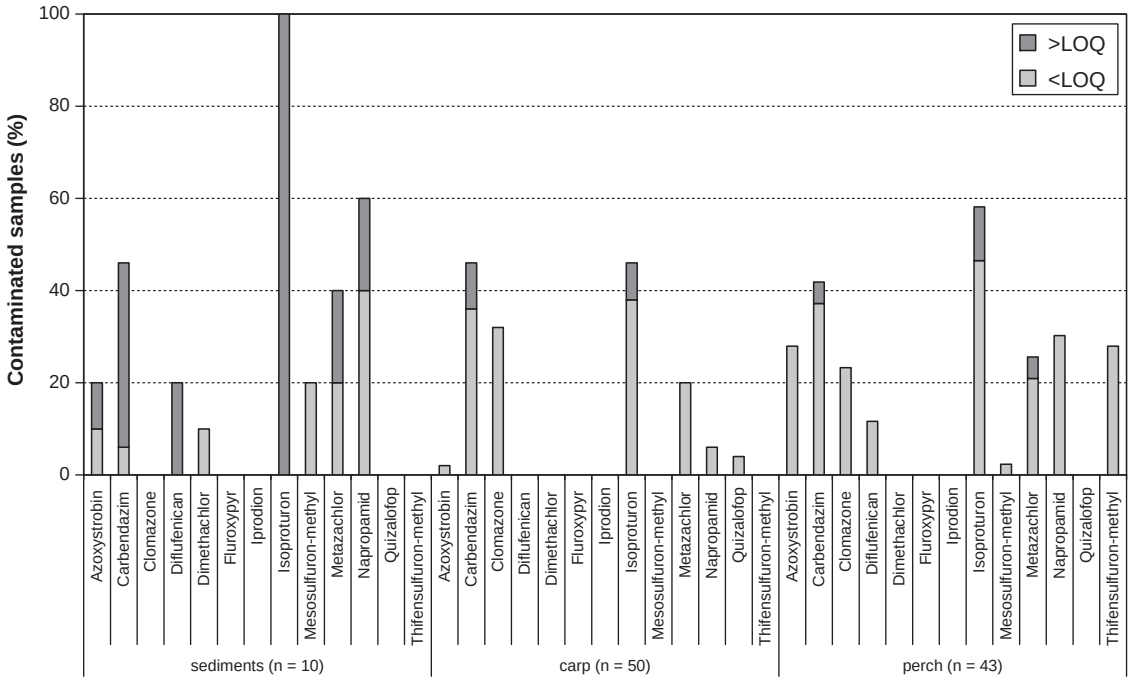

Fig. 4. Frequency of contaminated samples (%) for sediments, carp and perch studied.

obtained values range between 42% (fluroxypyr) and 125% (iprodion) with satisfactory intra-day precision (RSD lower than 23%). Extraction recoveries obtained on 3 g fish muscle and 4 g sediments, with the fully optimized method, are presented in Table 3. The obtained recoveries are satisfactory for such complex matrices between 40 and 125% for sediment and 52 and 115% for fish muscle. Only the diflufenican, the more hydrophobic compound in the list, presented a recovery of 35% in sediment and 36% in fish muscle. The recoveries varied significantly depending on compound and matrix. But, considering fish, RSD were less than 6.2% for all the compounds in both matrices (except for diflufenican (20.8%) and iprodion (22.1%)), which is considered as good method precision.

The limits of quantification in fish and sediment were lower than 5 ng L^{-1} and 1 ng g^{-1} for most of the compounds. The method provides lower sensitivity for diflufenican, fluroxypyr, iprodion, quizalofop and thifensulfuron-methyl (5.03 < LOQ < 49.02).

3.5. Method application

Method was applied to 10 samples of sediments and muscles of 50 carps and 43 perches. Each sample was extracted and analyzed in duplicate. These environmental matrices were few contaminated; molecules were detected at concentration inferior to LOQ in most samples (Fig. 4). Isoproturon and carbendazim were the most frequently detected or quantified in sediments or fish muscle. Maxima concentrations concerned isoproturon with 9.5, 0.50 and 0.85 ng g^{-1} for sediments, carp and perch muscles, respectively. Maximum value in sediments is higher than concentration measured in Garonne river [3]. Carbendazim was measured respectively at 0.92, 0.34 and 0.21 ng g^{-1} in sediments, carp and perch muscles. The other molecules were probably not persistent in these environmental matrices. Comparison with literature is difficult because, to our knowledge, there is a lack of data about levels of these compounds in environmental matrices.

4. Conclusion

This work proposed a generic multiresidue analysis to extract and quantify 13 pesticides (azoxystrobin, clomazone, diflufenican, dimethachlor, carbendazim, iprodion, isoproturon, mesosulfuron-methyl, metazachlor, napropamid, quizalofop and thifensulfuron-methyl) from water, sediments and fish muscle. The developed extraction step is an easy and relatively quick method using few solvent quantities. Two extraction methods were developed: a solid-phase extraction for water and a unique solid–liquid extraction method for solid matrices, all followed by a single quantification method by LC–MS/MS using a matrix matched calibration. LOQ were below 75 ng L^{-1}, 58 ng g^{-1} and 49 ng g^{-1} for water, sediment and fish muscle respectively. But, for most compounds, LOQ were below 1 ng g^{-1} and recoveries were higher than 80%.

To our knowledge, it is the first time that one method is performed to extract these 13 pesticides from freshwater and several types of sediments and fish muscles. It allows a routine practice for environmental research, *i.e.* monitoring and understanding pesticide behavior. This kind of development is of great importance for two reasons. First, it allows a global view of aquatic ecosystems permitting a better understanding of contamination of fishes. Secondly, reliable analytical methods are needed for bioaccumulation studies which we have planned to undertake in another work.

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