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Determination of 48 pesticides and their main metabolites in water samples by employing sonication and liquid chromatography–tandem mass spectrometry

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ABSTRACT

In this work, a rapid and sensitive analytical multiresidue method has been developed for the simultaneous determination of 48 pesticides and 19 metabolites in waters (tap, leaching and sewage), using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with triple quadrupole in selected reaction monitoring (SRM) mode. The procedure involves initial single phase extraction of samples with acetonitrile by sonication, followed by liquid–liquid partition aided by "salting out" process using NaCl. Matrix influence on recoveries was evaluated for the three waters. More than 50% of the compound presented very low signal suppression. The method presents good linearity over the range assayed 10–500 $\mu g\,L^{-1}$ and the most frequent detection limits was 0.05 $ng\,mL^{-1}$. The average recovery by the LC–MS/MS method obtained for these compounds varied from 74.6 to 111.2% with a relative standard deviation between 2.5 and 8.9%. The proposed method was used to determine pesticides levels in leaching water samples from 5 lysimeters from an experimental greenhouse located in Murcia.

1. Introduction

Pesticides are chemicals, synthetic or natural substances that are widely used in agriculture to control of insects, fungi, bacteria, weeds, nematodes, rodents and other pests that damage fruits and vegetables. Thus, the use of pesticides during the cultivation plays an important role in harvest quality and food protection [1]. These compounds and the products of their degradation or metabolism may spread through the environment and frequently contaminate different environmental compartments, including groundwater and surface water [2]. The maximum admissible pesticides concentration in drinking water established by the European Union (EU) is 0.1 $\mu g\,L^{-1}$ for individual pesticides and 0.5 $\mu g\,L^{-1}$ for total pesticides concentration [3]. Because of the pesticides toxicity and their harmful effects to the environment, especially in water, plenty of analytical procedures have been developed to determine and control pesticides in surface and ground waters [4,5]. The interest in the field of pesticides analysis is focusing on improving faster and more selective analytical methodologies, with higher cost-benefit ratios, that are less harmful to the environment and more sensitive to trace levels of pesticide residues in natural and drinking waters [6]. Multi-residue analytical methods have been proposed for the simultaneous determination of pesticides in water. However, these methods poses special problems since the pesticides present different degrees of polarity, solubility, volatility, and acidic characteristics, their concentration in environmental samples is very low and the matrix is complex [7].

Sample preparation is among the most important steps in any analytical process. In this sense, a wide variety of techniques have been used to extract and to purify pesticides from waters, including liquid–liquid extraction (LLE) [8], solid-phase extraction (SPE) [9], solid-phase microextraction (SPME) [10], dispersive liquid–liquid microextraction (DLLME) [11], liquid-phase microextraction (LPME) [12], single-drop microextraction (SDME) [13], ultrasound assisted emulsification-microextraction (USAEME) [14], polymer-coated hollow fiber microextraction (PC-HFME) [15], and stir bar sorptive extraction (SBSE) [16].

Most pesticides are easily analyzed by gas chromatographic techniques, coupled with detectors such as electron capture detector (ECD), nitrogen–phosphorous detector (NPD), flame photometric detector (FPD), and mass spectrometry (MS) [17–20]. However, in the case of non-volatile and/or thermally instable and/or polar pesticides and metabolites, it is necessary the application of alternative analytical techniques. In this sense, liquid chromatography with MS or tandem MS (MS/MS) detection provides an improved sensitivity and selectivity for the analysis of these pesticides [21,22].

In the present study, we have developed a new, simple and rapid method for the determination of various classes of pesticides and metabolites in water samples by LC–MS/MS, with triple quadrupole

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in selected reaction monitoring (SRM) mode, using electrospray ionization (ESI). The method presents advantages compared with other conventional methods given the use of a low volume of organic solvent in the sample extraction and the fact that a cleanup step is not required.

2. Experimental

2.1. Apparatus

The separation of the selected pesticides was carried out using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C8 analytical column of 150 mm × 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25 °C. The injected sample volume was 5 μL. Mobile phases A and B were acetonitrile and 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A after 35 min. The flow-rate used was 0.6 mL min^{-1}. A 10 min post-run time was used after each analysis. The mass spectral analysis was performed on an G6410A triple quadrupole mass spectrometer from Agilent equipped with an ESI interface operating in positive ion mode, using the following operation parameters: capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas, 9 L min^{-1}; drying gas temperature, 350 °C. Mass spectra were recorded across the range 50–1000 *m/z*.

A preliminary study of the optimal SRM transitions for every compound was carried out by injecting individual analytes at a concentration level of 10 μg mL^{-1}. Various fragmentor voltages and collision energies were applied to the compounds under study. Table 1 lists the pesticides along with their retention times and their optimized SRM transitions with a dwell time of 15 ms. Three different time segments were recorded in the chromatographic run, each one containing a third of the compounds studied. Agilent Mass Hunter Data Acquisition, Qualitative Analysis and Quantitative Analysis software were used for method development and data acquisition.

For the extraction of samples, a sonic dismembrator 200 W generator equipped with standard titanium probe (Dr Hielscher GmbH. Stahnsdorf, Germany) was used.

An Eppendorf model 5810R centrifuge (Hamburg, Germany) and a Büchi model R-205 rotavapor (Flawil, Switzerland) were used in the centrifugation and evaporation to dryness of samples, respectively.

2.2. Materials and standards

Acetonitrile, acetone and ethyl acetate residue analysis grade, were purchased from Scharlau (Barcelona, Spain).

Pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) with purity ranging from 95 to 100%. Stocks solutions (1000 μg mL^{-1}) of each pesticide standard were prepared by dissolving 0.025 g of the pesticide in 25 mL of various solvents. A pesticide intermediate standard solution (10 μg mL^{-1}) was prepared by transferring 1 mL from each pesticide solution to a 100 mL volumetric flask and diluting to volume with acetonitrile to obtain a concentration of 10 μg mL^{-1}. Several standard solutions, with concentrations of 0.01–0.5 μg mL^{-1}, were injected to obtain the linearity of detector response. Simazine-2-hydroxy and atrazine-desethyl–desisopropyl were deleted from the list of pesticides studied because of this low sensitivity meaning it was not possible to get reliable information for further studies.

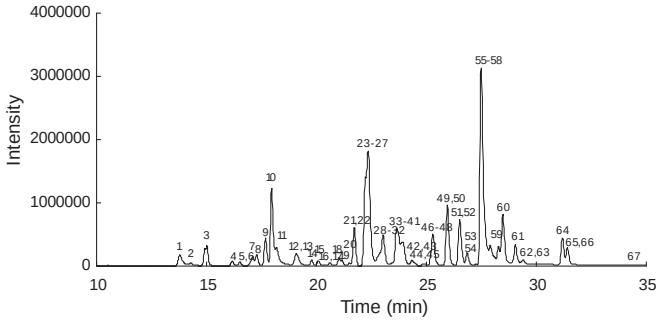

Fig. 1. LC–MS/MS total ion chromatograms in selected reaction monitoring (SRM) mode obtained from 48 pesticides and 19 metabolites at the 250 ng mL^{-1} concentration level. For peak numbers see Table 1.

2.3. Sample preparation

Water samples (10 mL) were added into a centrifuge tube and then extracted with 10 mL of acetonitrile by sonication (15 min at 0.5 cycles and 60% amplitude), followed by a salting-out step with 2 g NaCl. The tube was shaken and centrifuged for 10 min at 3000 × *g*. Extract was filtered quantitatively through glass funnel containing a filter separation phase paper DP302, 150 mm diameter (Albet, Barcelona, Spain). The organic phase was concentrated to dryness using rotary vacuum evaporation. The residue was redissolved in 1 mL of acetonitrile, filtered through 0.45 μm filter and analyzed by LC–MS/MS under conditions described above.

For the matrix-matched standard calibration, "blank" extracts were used. The water samples were taken in different sites. Water samples were passed through 2 mm sieve. The characteristics of the water were as follows: Water A (tap water): pH = 8.22, EC = 0.93 dS m^{-1}, TOC = 1.42 mg L^{-1}, NO_3^- = 6.4 mg L^{-1}, and NO_2^- <LOD. Water B (leaching water): pH = 8.41, EC = 4.32 dS m^{-1}, TOC = 130 mg L^{-1}, NO_3^- = 547 mg L^{-1}, and NO_2^- = 0.12 mg L^{-1}. Water C (sewage water): pH = 7.32, EC = 2.53 dS m^{-1}, TOC = 53.3 mg L^{-1}, NO_3^- = 1.6 mg L^{-1}, and NO_2^- = 0.07 mg L^{-1}. Real samples were obtained from 5 lysimeters (3.5 m × 4 m × 1 m) from an experimental greenhouse located in Campo de Cartagena, Murcia (SE Spain). A clay loam soil (pH = 8.7 and OM = 0.22%) was used and spiked with commercial product at the doses recommended by the manufacturers: terbuthylazine [Gardoprim 50% (w/v) SC (Syngenta)], atrazine [Beltrazina 47.5% (w/v) SC (Probelte S.A)], tolclofos-methyl [Rizolex 50% WP (Kenogard)], propyzamide [Kerb Flo 40% (w/v) SC (Dow)]. In each lysimeters, different treatments were carried out with a sprayer (Matabi) with an adjustable nozzle size of 1 mm. The soil was irrigated every four days by three dripperlines (45 min per day and 50 mL min^{-1} per emitter). About 150 L were collected from each lysimeter. The preparation procedure was the same as the mentioned above.

3. Results and discussion

3.1. Liquid chromatographic determination

Fig. 1 shows the total ion chromatogram of 48 pesticides and 19 metabolites (all of them at 250 ng/mL concentrations). Under the described chromatography conditions, the pesticides eluted from 10 to 35 min. Three solvents (acetonitrile, acetone and ethyl acetate) were tested as extractants. Acetonitrile was the most advantageous overall for all compounds due to: (i) most of the studied compounds, extraction with acetone resulted in higher suppressive matrix effect than the observed when acetonitrile or ethyl acetate were used, probably due to the highest co-extractives

Table 1
Analytical conditions of the studied pesticides.

	Pesticide[a]	Metabolite (letter)	Retention time (min)		Transition (m/z)	Fragmentor voltage (V)	Collision energy (V)
Segment 1							
	Simazine-2-hydroxy		4.12	Quantifier	184 → 114	110	20
				Qualifier	184 → 97	110	20
	Atrazine-desethyl–desisopropyl		4.73	Quantifier	146 → 104	110	20
				Qualifier	146 → 110	110	10
1		4-Chloroaniline (a)	13.79	Quantifier	128 → 93	110	20
				Qualifier	128 → 111	110	20
2		Atrazine-desisopropyl (b)	14.28	Quantifier	174 → 104	110	20
				Qualifier	174 → 132	90	10
3		Terbuthylazine-2-hydroxy (c)	15.02	Quantifier	212 → 156	110	10
				Qualifier	212 → 114	110	20
4	Metamitron		16.51	Quantifier	203 → 175	110	15
				Qualifier	203 → 104	110	25
5		Carbofuran-3-hydroxy (d)	16.98	Quantifier	238 → 163	70	10
				Qualifier	238 → 181	70	5
6	Fenuron		17.09	Quantifier	165 → 72	90	20
				Qualifier	165 → 120	110	10
7	Chloridazon		17.22	Quantifier	222 → 77	130	35
				Qualifier	222 → 104	130	25
8		Atrazine-desethyl (e)	17.29	Quantifier	188 → 146	110	10
				Qualifier	188 → 104	110	30
9	Simetryn		17.68	Quantifier	214 → 96	130	20
				Qualifier	214 → 124	130	20
10	Prometon		17.96	Quantifier	226 → 142	130	20
				Qualifier	226 → 184	130	20
11		3-Chloro-4-methylaniline (f)	18.20	Quantifier	142 → 107	110	20
				Qualifier	142 → 125	110	20
12		2-Ethyl-6-methylaniline (g)	19.09	Quantifier	136 → 91	110	20
				Qualifier	136 → 117	110	20
13		Fenamiphos sulfoxide (h)	19.17	Quantifier	320 → 233	135	15
				Qualifier	320 → 171	135	15
14	Nicosulfuron		19.77	Quantifier	411 → 182	110	20
				Qualifier	411 → 213	90	10
15	Metoxuron		20.08	Quantifier	229 → 72	110	20
				Qualifier	229 → 156	110	20
16		Carbofuran-3-keto (i)	20.58	Quantifier	236 → 161	70	10
				Qualifier	236 → 179	70	10
17		1-(3-Chloro-4-methylphenyl)urea (j)	20.61	Quantifier	185 → 107	110	30
				Qualifier	185 → 142	110	10
18	Simazine[b]		21.02	Quantifier	202 → 132	110	20
				Qualifier	202 → 104	110	30
19	Monuron[a]		21.16	Quantifier	199 → 72	110	20
				Qualifier	199 → 126	110	30
20		1-(3,4-Dichlorophenyl)urea (k)	21.49	Quantifier	205 → 127	110	30
				Qualifier	205 → 162	110	10
21		Terbuthylazine-desethyl (l)	21.71	Quantifier	202 → 110	110	20
				Qualifier	202 → 146	90	10
22		Fenamiphos sulfone (m)	21.80	Quantifier	336 → 266	135	15
				Qualifier	336 → 308	135	15
Segment 2							
23	Metribuzin		22.19	Quantifier	215 → 187	110	20
				Qualifier	215 → 84	110	20
24	Prometryn		22.21	Quantifier	242 → 158	110	20
				Qualifier	242 → 200	130	20
25	Methabenzthiazuron		22.30	Quantifier	222 → 165	90	10
				Qualifier	222 → 150	90	40
26	Terbutryn[c]		22.38	Quantifier	242 → 186	110	20
				Qualifier	242 → 91	110	30
27		Fluometuron-desmethyl (n)	22.44	Quantifier	219 → 142	110	10
				Qualifier	219 → 162	110	30
28	Triasulfuron		22.77	Quantifier	402 → 141	90	20
				Qualifier	402 → 167	130	10
29	Chlorsulfuron		22.85	Quantifier	358 → 141	110	20
				Qualifier	358 → 167	110	20
30		1-(3,4-Dichlorophenyl)3-methylurea (o)	22.92	Quantifier	219 → 127	110	30
				Qualifier	219 → 162	110	10
31	Carbofuran[d,i]		23.01	Quantifier	222 → 123	70	15
				Qualifier	222 → 137	70	20
32	Chlortoluron[f,j]		23.08	Quantifier	213 → 72	110	20
				Qualifier	213 → 140	110	20
33		2,6-Diethylaniline (p)	23.47	Quantifier	150 → 91	110	20
				Qualifier	150 → 105	90	40

Table 1 (*Continued*)

	Pesticide[a]	Metabolite (number)	Retention time (min)		Transition (m/z)	Fragmentor voltage (V)	Collision energy (V)
34	Fluometuron[n,r]		23.63	Quantifier	233 → 72	110	20
				Qualifier	233 → 160	110	30
35	Atrazine[b,e]		23.63	Quantifier	216 → 174	110	10
				Qualifier	216 → 96	110	20
36	Metalaxyl		23.73	Quantifier	280 → 220	70	10
				Qualifier	280 → 192	70	10
37		2-Ethyl-6-methyl-2-chloroacetanilide (q)	23.76	Quantifier	212 → 184	110	10
				Qualifier	212 → 108	110	30
38	Isoproturon		23.88	Quantifier	207 → 72	110	20
				Qualifier	207 → 165	70	10
39	Difenoxuron		23.95	Quantifier	287 → 72	130	20
				Qualifier	287 → 123	130	20
40	Diuron[k,o,s]		24.04	Quantifier	233 → 72	110	30
				Qualifier	233 → 160	110	20
41	Sulfosulfuron		24.23	Quantifier	471 → 211	110	10
				Qualifier	471 → 261	110	10
42	Monolinuron[a]		24.34	Quantifier	215 → 126	90	10
				Qualifier	215 → 148	90	10
43		3-Trifluoromethylaniline (r)	24.44	Quantifier	162 → 93	130	20
				Qualifier	162 → 142	130	20
Segment 3							
44	Imazosulfuron		24.82	Quantifier	413 → 156	110	20
				Qualifier	413 → 258	110	20
45	Metobromuron		24.95	Quantifier	259 → 148	90	10
				Qualifier	259 → 170	110	20
46		3,4-Dichloroaniline (s)	25.23	Quantifier	162 → 127	110	20
				Qualifier	162 → 109	110	30
47	Flazasulfuron		25.28	Quantifier	408 → 182	130	20
				Qualifier	408 → 227	110	20
48	Propachlor		25.32	Quantifier	212 → 170	90	10
				Qualifier	212 → 94	90	30
49	Sebuthylazine[b]		25.95	Quantifier	230 → 174	130	20
				Qualifier	230 → 132	130	20
50	Propanil[s]		26.03	Quantifier	218 → 162	70	15
				Qualifier	218 → 127	70	15
51	Propazine[e]		26.51	Quantifier	230 → 146	110	20
				Qualifier	230 → 188	130	20
52	Chloroxuron		26.61	Quantifier	291 → 72	130	20
				Qualifier	291 → 218	130	20
53	Fenamiphos[h,m]		26.85	Quantifier	304 → 217	130	25
				Qualifier	304 → 202	130	40
54	Linuron[k,o,s]		26.91	Quantifier	249 → 182	90	10
				Qualifier	249 → 160	110	20
55	Chlorbromuron		27.29	Quantifier	295 → 182	110	10
				Qualifier	295 → 206	110	20
56	Isoxaflutole		27.76	Quantifier	360 → 251	70	10
				Qualifier	360 → 220	70	10
57	Ethoprophos		27.93	Quantifier	243 → 131	90	20
				Qualifier	243 → 97	90	25
58	Propyzamide		28.09	Quantifier	256 → 190	70	10
				Qualifier	256 → 173	70	10
59	Isoxaben		28.28	Quantifier	333 → 165	90	20
				Qualifier	333 → 85	90	10
60	Terbutylazine[b,c,l]		28.49	Quantifier	230 → 132	130	20
				Qualifier	230 → 174	130	20
61	S-Metolachlor[g,q]		29.05	Quantifier	284 → 252	90	10
				Qualifier	284 → 176	110	30
62	Alachlor[p]		29.21	Quantifier	270 → 238	90	10
				Qualifier	270 → 162	90	10
63	Neburon[k,o,s]		29.40	Quantifier	275 → 88	130	10
				Qualifier	275 → 114	110	10
64	Cadusafos		31.19	Quantifier	271 → 159	90	10
				Qualifier	271 → 131	90	20
65	Pencycuron		31.42	Quantifier	329 → 125	110	30
				Qualifier	329 → 218	110	15
66	Tolclofos-methyl		31.67	Quantifier	301 → 175	110	25
				Qualifier	301 → 269	110	15
67	Pendimethalin		34.27	Quantifier	282 → 212	70	10
				Qualifier	282 → 194	70	20

[a] Superscripts in each pesticide indicate their metabolites.

achieved with acetone; (ii) with ethyl acetate, the recoveries achieved for some of the most polar pesticides, such as some metabolites and sulfonylurea herbicides, were very low, probably due to the fact that these compounds did not readily partition into the ethyl acetate [23].

The identification procedure for pesticide residues in water was based on the following factors: the use of the retention time, two transitions and the selected reaction monitoring (SRM) ratio of the transitions. Three pairs of pesticides presented common transitions: 1-(3,4-dichlorophenyl)3-methylurea-fluometuron-desmethyl, therbuthylazine-sebuthylazine and diuron-fluometuron. These pairs have one or two transitions in common, although they can be identified by using the retention times. The values of the SRM ratios for all of the two transitions selected are between 5% and 100%. More than 90% of the compounds presented SRM variability lower than 25% in concentration ranged studied. This is in accordance with the DG SANCO/2007/3131 of the European Quality Control Guidelines, based on ion-ratio statistics for the transitions monitored. For isoxaben, 2,6-diethylaniline and isoxaflutole the calculation of the SRM ratios was not possible because the intensity of the qualifier transition was very low.

3.2. Linearity and matrix effects

Under the chromatographic conditions described above, the calibration graphs were constructed by plotting peak area vs. concentrations in the range 10–500 $\mu g\,L^{-1}$. The correlation coefficients derived from linear regressions were in all cases higher than 0.998, with significant correlation between concentration and area for all pesticides. The calibration curves were constructed and compared in solvent and in the three different waters (tap, leaching and sewage). The study of the ratio of the slopes, in solvent and in matrix, provided information about the matrix effect. The results of the present study showed that 100% of the pesticides presented correlation coefficients (R) higher than 0.98 in all calibration curves. These results are included in Table 2.

The effect of co-eluting matrix components, which can cause ion suppression or enhancement of the pesticide, is especially relevant when using HPLC tandem mass spectrometry methodology [24]. The ratio of slopes for the solvent and matrix (S_s/S_m) and the matrix effect (calculated as the percentage of the ratio of slopes) were determined to provide information about the enhancement or suppression of the signal in the different matrices (Table 2). In the three matrices, more than 50% of the compound presented very low signal suppression (−25% to +25%). In general, sulfonylureas, chloroacetamides, ethoprophos, isoxaflutole, cadusafos and most of the metabolites presented the highest matrix effects. The three types of waters matrices had similar values with a slightly lower percentage.

3.3. Detection limits

LODs were evaluated by injecting standard solution into blank-matrix at the different concentration levels. The limits of detection (LOD) of the proposed method was estimated as the value where the intensities for both transitions were significantly higher than the background, and where the signal-to-noise ratio was higher than 3. Fig. 2 shows the distribution of the LODs obtained for the individual pesticides in three different waters. More than 60% of the cases were below or equal to 0.1 $ng\,mL^{-1}$ and the most frequent LOD was 0.05 $ng\,mL^{-1}$. The highest limit of detection obtained in water was 5 $ng\,mL^{-1}$ for isoxaflutole and tolclofos-methyl. These comply with the maximum admissible concentration of pesticides and related products for drinking water established by the European Union (EU). For most of the pesticides, the detection limits were not affected, or were only slightly affected, by the studied matrices.

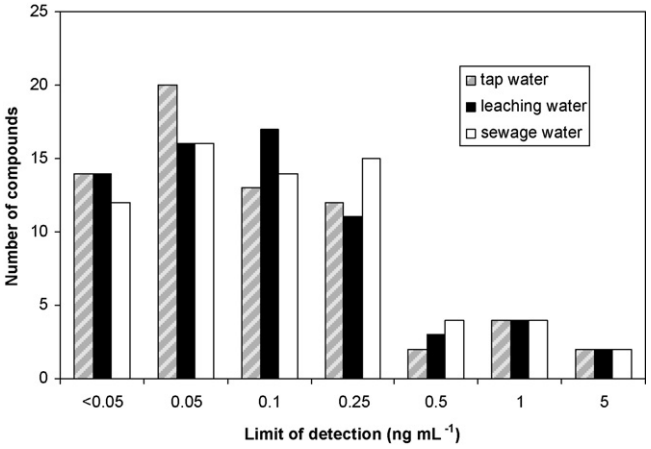

Fig. 2. Distribution of the limits of detection.

Overall, the LODs obtained in the present study were similar or even lower than those obtained by other authors that analyzed these pesticides in water by using SPE-HPLC-DAD [25], SPME-GC–MS [26] or LPME-MS/MS [27]. In these previous works, sample extraction involved a cleanup step and presented higher time-consuming (from 40 min to 4 h) than the proposed method (approximately 25 min).

3.4. Recovery

A study of recoveries for each pesticide at two different fortification levels was carried out in order to assess the extraction efficiency of the proposed method. For that, three uncontaminated water samples were spiked with 10 and 50 $\mu g\,L^{-1}$ of pesticide and processed as described. The data evaluation was carried out by comparing the peaks areas of the spiked samples to those obtained by matrix-matched standard calibration. The distribution of the recoveries is showed in Fig. 3. More than 94% of the pesticides under study presented recoveries between 80 and 110%. The recoveries obtained for all pesticides ranged from 74.6 to 107.5% for water A, from 76.3 to 110.2% for water B and from 76.0 to 111.2% for water C. These recoveries were in the acceptance range of the DG SANCO/2007/3131 of the European Quality Control Guidelines: 70–120% in all cases. The relative standard deviation (RSD) was <8.9% in the most unfavourable case.

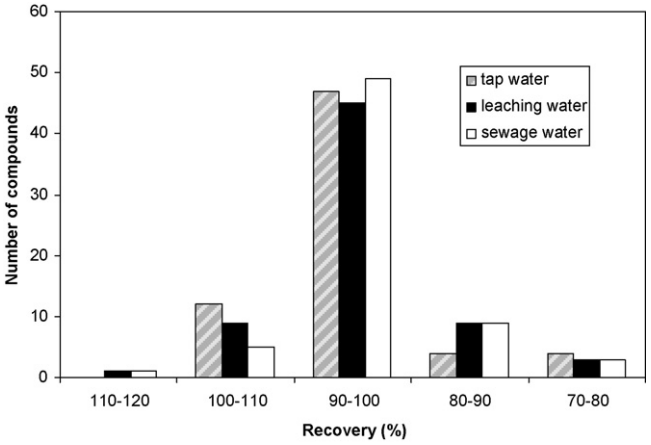

Fig. 3. Data distribution of recoveries.

Table 2
Linearity and matrix effects.

Compound	Solvent		Water A (tap water)		Water B (leaching water)		Water C (sewage water)		Slope matrix/slope solvent			Matrix effect (%)[a]		
	Slope	*R*	Slope	*R*	Slope	*R*	Slope	*R*	Water A	Water B	Water C	Water A	Water B	Water C
4-Chloroaniline	4561.4	0.999	782.3	0.993	675.2	0.989	883.5	0.990	0.172	0.148	0.194	82.8	85.2	80.6
Atrazine-desisopropyl	445.8	0.999	386.2	0.995	338.3	0.997	282.2	0.999	0.866	0.759	0.633	13.4	24.1	36.7
Terbuthylazine-2-hydroxy	6946.7	0.999	3256.3	0.997	3129.4	0.998	2478	1.000	0.469	0.450	0.357	53.1	55.0	64.3
Metamitron	625.7	1.000	489.6	0.996	464.5	0.997	439.1	0.997	0.782	0.742	0.702	21.8	25.8	29.8
Carbofuran-3-hydroxy	451.3	0.999	368.1	0.994	338.0	0.992	316.6	0.998	0.816	0.749	0.702	18.4	25.1	29.8
Fenuron	2341.0	0.999	2256	0.998	1904.5	0.997	1722.5	0.996	0.964	0.814	0.736	3.6	18.6	26.4
Chloridazon	344.6	0.999	254.6	0.993	263.8	0.995	232.8	0.999	0.739	0.766	0.676	26.1	23.4	32.4
Atrazine-desethyl	2111.6	0.999	1788.6	0.996	1711.5	0.995	1580.6	0.998	0.847	0.811	0.749	15.3	18.9	25.1
Simetryn	3910.7	1.000	3325.6	0.997	3810.8	0.994	3805.2	0.998	0.850	0.974	0.973	15.0	2.6	2.7
Prometon	14338.3	1.000	14395.2	0.994	11678.8	0.994	14469.2	0.998	1.004	0.815	1.009	−0.4	18.5	−0.9
3-Chloro-4-methylaniline	4960.6	1.000	1032.6	0.994	403.6	0.998	957.7	0.996	0.208	0.081	0.193	79.2	91.9	80.7
2-Ethyl-6-methylaniline	3321.2	1.000	165.2	0.992	193.2	0.991	251.5	0.996	0.050	0.058	0.076	95.0	94.2	92.4
Fenamiphos sulfoxide	2872.3	0.998	2645.9	0.987	2349.7	0.992	2900.1	0.996	0.921	0.818	1.010	7.9	18.2	−1.0
Nicosulfuron	876.4	0.999	76.4	0.991	73.6	0.989	102.1	0.998	0.087	0.084	0.116	91.3	91.6	88.4
Metoxuron	1166.1	1.000	1301.6	0.994	940.9	0.996	839.0	0.998	1.116	0.807	0.719	−11.6	19.3	28.1
Carbofuran-3-keto	620.2	0.999	538.1	0.993	510.2	0.992	514.7	0.993	0.868	0.823	0.830	13.2	17.7	17.0
1-(3-Chloro-4-methylphenyl)urea	398.7	0.999	330.2	0.995	297.6	0.997	280.2	0.998	0.828	0.746	0.703	17.2	25.4	29.7
Simazine	1088.6	1.000	1075.3	0.999	885.3	0.997	1126.0	0.999	0.988	0.813	1.034	1.2	18.7	−3.4
Monuron	1026.8	0.999	834.1	0.997	999.2	1.000	790.3	0.998	0.812	0.973	0.770	18.8	2.7	23.0
1-(3,4-Dichlorophenyl)urea	411.9	1.000	365.6	0.996	326.5	0.998	322.4	0.997	0.888	0.793	0.783	11.2	20.7	21.7
Terbuthylazine-desethyl	6450.2	1.000	5376.2	0.997	4706.3	0.998	6125.3	0.996	0.833	0.730	0.950	16.7	27.0	5.0
Fenamiphos sulfone	7183.4	0.999	7325.5	0.997	7452.2	0.991	6832.8	0.993	1.020	1.037	0.951	−2.0	−3.7	4.9
Metribuzin	976.7	0.999	826.1	0.993	1003.1	0.996	837.1	0.994	0.846	1.027	0.857	15.4	−2.7	14.3
Prometryn	18514.2	1.000	15030.1	0.990	13828.4	0.999	16998.3	0.991	0.812	0.747	0.918	18.8	25.3	8.2
Methabenzthiazuron	5297.8	0.999	4452.3	0.994	4897.0	0.990	4152.3	0.998	0.840	0.924	0.784	16.0	7.6	21.6
Terbutryn	21577.9	1.000	17157.3	0.992	20654.2	0.993	21658.6	1.000	0.795	0.957	1.004	20.5	4.3	−0.4
Fluometuron-desmethyl	789.3	0.999	793.2	0.993	765.2	0.993	810.3	0.998	1.005	0.969	1.027	−0.5	3.1	−2.7
Triasulfuron	710.6	0.999	432.2	0.994	421.9	0.989	463.7	0.999	0.608	0.594	0.653	39.2	40.6	34.7
Chlorsulfuron	931.7	0.999	537.7	0.997	625.3	0.995	540.4	0.999	0.577	0.671	0.580	42.3	32.9	42.0
1-(3,4-Dichlorophenyl)3-methylurea	918.8	0.999	899.3	0.999	762.9	0.998	711.3	0.999	0.979	0.830	0.774	2.1	17.0	22.6
Carbofuran	3494.2	1.000	2558.2	0.995	2775.3	0.997	3125.2	0.996	0.732	0.794	0.894	26.8	20.6	10.6
Chlortoluron	2044.4	0.999	2098.3	0.992	2139.2	0.993	1644.5	0.999	1.026	1.046	0.804	−2.6	−4.6	19.6
2,6-Diethylaniline	721.7	1.000	61.3	0.999	55.5	0.995	75.9	0.992	0.085	0.077	0.105	91.5	92.3	89.5
Fluometuron	2110.2	1.000	1699.1	0.996	1975.9	0.996	2231.2	1.000	0.805	0.936	1.057	19.5	6.4	−5.7
Atrazine	5089.1	1.000	4459.3	0.991	4107.1	0.995	3791.1	0.998	0.876	0.807	0.745	12.4	19.3	25.5
Metalaxyl	2572.7	0.999	2675.3	0.994	1924.9	0.994	2364.3	0.998	1.040	0.748	0.919	−4.0	25.2	8.1
2-Ethyl-6-methyl-2-chloroacetanilide	657.1	0.999	447.9	0.999	587.3	0.993	431.3	0.993	0.682	0.894	0.656	31.8	10.6	34.4

Isoproturon	4072.6	0.999	4125.3	0.999	3385.7	0.993	3838.2	0.999	1.013	0.831	0.942	−1.3	16.9	5.8
Difenoxuron	2050.6	0.999	2126.6	0.999	2070.3	0.998	2098.4	0.999	1.037	1.010	1.023	−3.7	−1.0	−2.3
Diuron	878.7	0.999	711.5	0.996	661.8	0.998	843.2	0.996	0.810	0.753	0.960	19.0	24.7	4.0
Sulfosulfuron	70.6	0.999	46.2	0.995	53.6	0.993	48.1	0.998	0.654	0.759	0.681	34.6	24.1	31.9
Monolinuron	608.0	1.000	443.7	0.999	587.6	0.992	549.1	0.990	0.730	0.966	0.903	27.0	3.4	9.7
3-Trifluoromethylaniline	231.5	0.999	36.3	0.994	40.2	0.989	27.9	0.993	0.157	0.174	0.121	84.3	82.6	87.9
Imazosulfuron	448.2	0.999	486.2	0.999	477.6	0.997	452.3	0.996	1.085	1.066	1.009	−8.5	−6.6	−0.9
Metobromuron	236.4	0.998	236.8	0.998	199.7	0.996	203.3	0.995	1.002	0.845	0.860	−0.2	15.5	14.0
3,4-Dichloroaniline	3546.7	0.999	1108.9	1.000	1378.6	0.994	1265.4	0.999	0.313	0.389	0.357	68.7	61.1	64.3
Flazasulfuron	2196.9	0.999	1454.0	0.995	1457.2	0.999	1627.1	0.998	0.662	0.663	0.741	33.8	33.7	25.9
Propachlor	4176.0	0.999	1656.5	0.992	1793.8	0.991	2001.0	0.990	0.397	0.430	0.479	60.3	57.0	52.1
Sebuthylazine	11609.9	1.000	9620.1	0.995	8874.8	0.997	10256.7	0.993	0.829	0.764	0.883	17.1	23.6	11.7
Propanil	255.9	0.999	215.3	0.997	263.1	0.995	189.2	0.998	0.841	1.028	0.739	15.9	−2.8	26.1
Propazine	9604.3	1.000	8999.2	0.999	7738.3	0.994	7129.8	0.997	0.937	0.806	0.742	6.3	19.4	25.8
Chloroxuron	2019.4	1.000	2036.3	0.995	1607.6	0.994	1516.3	0.997	1.008	0.796	0.751	−0.8	20.4	24.9
Fenamiphos	2003.9	0.999	1679.2	0.994	1556.1	0.999	1896.6	0.997	0.838	0.777	0.946	16.2	22.3	5.4
Linuron	237.6	0.999	241.3	0.999	202.9	0.998	236.4	0.997	1.016	0.854	0.995	−1.6	14.6	0.5
Chlorbromuron	124.5	0.999	97.8	0.993	117.5	0.998	105.9	0.997	0.786	0.944	0.851	21.4	5.6	14.9
Isoxaflutole	433.4	0.999	14.5	0.987	15.6	0.986	14.9	0.986	0.033	0.036	0.034	96.7	96.4	96.6
Ethoprophos	1991.3	0.999	809.7	0.995	917.7	0.991	936.2	0.990	0.407	0.461	0.470	59.3	53.9	53.0
Propyzamide	650.0	0.999	518.8	0.996	491.3	0.994	625.3	0.995	0.798	0.756	0.962	20.2	24.4	3.8
Isoxaben	3997.1	1.000	4069.5	0.996	3373.1	0.994	4023.3	0.999	1.018	0.844	1.007	−1.8	15.6	−0.7
Terbutylazine	6493.4	1.000	4794.9	0.997	4958.2	0.993	4609.1	0.993	0.738	0.764	0.710	26.2	23.6	29.0
S-Metolachlor	4465.3	1.000	3131.1	0.994	2960.5	0.997	3256.3	0.997	0.701	0.663	0.729	29.9	33.7	27.1
Alachlor	268.4	0.999	158.2	0.991	156.2	0.999	169.8	0.991	0.589	0.582	0.633	41.1	41.8	36.7
Neburon	783.3	1.000	654.3	0.994	587.1	0.994	736.2	0.995	0.835	0.750	0.940	16.5	25.0	6.0
Cadusafos	4400.7	0.999	1962.3	0.998	2356.4	0.994	2159.3	0.989	0.446	0.535	0.491	55.4	46.5	50.9
Pencycuron	4328.4	1.000	4156.3	0.998	3220.4	0.990	4023.6	0.994	0.960	0.744	0.930	4.0	25.6	7.0
Tolclofos-methyl	27.8	0.999	20.3	0.999	20.1	0.989	23.6	0.992	0.651	0.644	0.849	27.0	27.7	15.1
Pendimethalin	141.6	1.000	103.5	0.994	101.0	0.995	99.5	0.993	0.590	0.586	0.681	26.9	28.7	29.7

[a] Matrix effect (%) = (1 − (slope matrix/slope solvent)) × 100.

Table 3
Pesticide residues ($\mu g\,L^{-1}$) Found in real water samples.

	Terbutylazine[a]	Atrazine[a]	Terbuthylazine-desethyl[a]	Tolclofos-methyl[a]	Propyzamide[a]
Water 1				53.4 ± 0.5 (54.1 ± 0.6)[b]	
Water 2					17.3 ± 0.4 (16.8 ± 0.5)[b]
Water 3		39.0 ± 0.2 (38.8 ± 0.2)[b]			
Water 4	72.9 ± 0.3 (71.8 ± 0.2)[b]		2.3 ± 0.1		
Water 5				43.9 ± 0.4 (44.5 ± 0.4)[b]	28.4 ± 0.4 (29.1 ± 0.5)[b]

[a] Mean of four determinations ± RSD.
[b] Residue values obtained by a reference method described by Fenoll et al. [28].

3.5. Repeatability

The repeatability study was carried out with 25 and 100 $\mu g\,L^{-1}$ matrix-matched standard calibration points, injected five times, to evaluate the intra-day (within 1 day) and inter-day (between days) precision. For determining inter-day precision, samples were stored at −20 °C. Intra-day and inter-day RSDs were below 12 and 15%. This complies with the RSD accepted by the DG SANCO/2007/3131 of the European Quality Control Guidelines.

3.6. Real samples

Waters from five lysimeters from an experimental greenhouse located in Campo de Cartagena (SE Spain) were sampled and analyzed following the extraction methods described above. Pesticide residues found in these samples included terbutylazine, terbuthylazine-desethyl, atrazine, tolclofos-methyl and propyzamide (Table 3). However, other terbutylazine and atrazine metabolites (atrazine-desethyl, terbuthylazine-2-hydroxy and atrazine-desisopropyl) were not found. Residues values for these pesticides were included in the linear range of the analytical method. In order to justify the extractability of the compounds using the described method, the results obtained for terbutylazine, atrazine, tolclofos-methyl and propyzamide residues in waters were also analyzed by GC according to the methods described previously by Fenoll et al. [28]. Similar results were obtained by both methods (Table 3). Therefore, analysis of real samples showed the validity of method used, which allowed the determination and identification of pesticides present in the samples.

4. Conclusions

The new proposed HPLC–MS/MS method allows the simultaneous determination of pesticide and their metabolites residues in waters. Due to the matrix effect observed, matrix-matched standard calibration was necessary to determine these compounds in the studied matrices. The described method is very simple, rapid and involves little sample preparation. Another advantage of the method is its excellent sensitivity, with LODs lower than 0.1 $ng\,mL^{-1}$, for most of the studied compounds. Linearity, repeatability and recovery were found to be within the range of acceptance. The method was applied for different classes of pesticide and their metabolites residues in leaching waters. Finally, our method is versatile and is capable of allowing the inclusion of new pesticides and metabolites.

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