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Article

Development and Validation of a Method using SPE and LC-ESI-MS-MS for the Determination of Multiple Classes of Pesticides and Metabolites in Water Samples

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Um método analítico baseado na extração em fase sólida e cromatografia líquida acoplada à espectrometria de massas *tandem* (LC-ESI-MS-MS) foi desenvolvido e validado para a determinação e confirmação de dezoito agrotóxicos (herbicidas, inseticidas e fungicidas) e dois metabólitos em amostras de água. Os limites de detecção variaram de 0,4 a 40,0 ng L^{-1} e os limites de quantificação de 4,0 a 100,0 ng L^{-1}. Foi obtida boa linearidade, com $r^2 > 0,99$ para todos os compostos. As recuperações, para 95% dos compostos, variaram de 70 a 120%, com RSDs menores que 21% para todos. Através do monitoramento de reações múltiplas (MRM), foram selecionadas duas diferentes transições íon precursor-íon produto para cada agrotóxico. A metodologia proposta pode ser usada para a determinação de resíduos de agrotóxicos em águas de superfície e potável, em concordância com a Lei n° 518 do Ministério da Saúde, Brasil, e com os parâmetros da União Européia para água potável (Directive 98/83/EC).

An analytical method using solid-phase extraction and liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) was developed and validated for the determination and confirmation of eighteen polar pesticides (herbicides, insecticides and fungicides) and two metabolites in water samples. The limits of detection varied between 0.4-40.0 ng L^{-1} and the limits of quantification between 4.0-100.0 ng L^{-1}. Good linearity with $r^2 > 0.99$ for all compounds was obtained. The recovery for 91% of the accuracy experiments varied from 70 to 120%, with RSD below 21% for all. Through multiple reaction monitoring (MRM) two different precursor ion-product ion transitions were selected for each pesticide. The proposed methodology can be used for the convenient and effective determination of pesticide residues in surface and drinking waters in accordance with Law No. 518 of the Ministry of Health, Brazil, and the European Union Directive on drinking water quality (98/83/EC).

Keywords: validation, SPE, LC-ESI-MS-MS, pesticides, metabolites, water

Introduction

In the last decades, agriculture has been making a great effort to increase food production because of the population growth. Since the beginning of its development, agriculture has been directly related to pesticide application: it stands out as the main way to control pests, plagues and weeds which attack agricultural products, harming crops and reducing productivity.[1,2] On one hand, pesticides play an important role in protecting crops, but, on the other hand, its indiscriminate use continues to cause many serious problems for the environment and human health.[3-5] Nowadays, pesticide monitoring in waters, both in natural ones and in the ones destined for public supply, is a major issue.

According to the literature, a pesticide is able to contaminate groundwater if its solubility in water is higher than 30 mg L^{-1}, its K_{OC} (organic carbon partition coefficient) is lower than 300 mL g^{-1}, its K_d (distribution adsorption constant) is lower than 5 mL g^{-1} and its soil half-life is longer than 3 weeks. Due to the fact that water springs are sources of drinking water, many environmental agencies have introduced rigorous legislation regarding the quality of those waters. The European Union has established rigid limits for pesticides in water destined for human consumption after treatment, namely 0.1 μg L^{-1}

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for individual pesticides and 0.5 μg L^{-1} for the sum of all pesticides.[6] In the case of surface waters that will be destined for human consumption, the limits are 1.0 μg L^{-1} for individual pesticides and 5.0 μg L^{-1} for the total of pesticides.[7,8] In Brazil, the Ministry of Health has set responsibilities regarding the control and monitoring of water quality and its potability for human consumption. Some of the pesticides selected for this study are regulated by the law which establishes that up to 300 μg L^{-1} are accepted for some pesticides, such as the herbicide bentazone; 30 for 2,4-D; 20 for propanil and 2 μg L^{-1} for simazine and atrazine.[9]

To assess the impact of pesticides on aquatic ecosystems, analytical methods are required for simultaneous determination of such compounds at trace concentrations in water samples.[10] Those compounds are usually analyzed by gas chromatography (GC) and by liquid chromatography (LC), depending on their polarity, volatility and thermal stability.[11,12] In GC, different detection systems are used, such as electron-capture detection (ECD),[13] nitrogen-phosphorus detection (NPD),[14,15] flame ionization detection (FID),[16] flame photometric detection (FPD),[17] and mass spectrometry (MS).[18,19] As most modern pesticides and their degradation products are polar, have low volatility, and/or are thermally labile compounds, the general use of liquid chromatography-based methods for pesticide determination has increased.[20]

In the LC pesticide analysis, fluorescence and diode-array detections (DAD)[21-25] have been used as detection techniques. However, in the last decades, the use of detection systems, MS or tandem (MS-MS) has increased the sensitivity and specificity of the methods, and for this reason, applications using LC-MS-MS became the most common tool to analyze and monitor contamination by pesticides.[26-39]

Nowadays, mass spectrometry is the best detection technique for chromatography because spectrometry is sensitive to a small amount of analyte. It supplies quantitative and qualitative information about the compounds that are eluted from the column, and it can distinguish different substances with the same retention time.[2,40]

Due to the low detection levels required by regulatory bodies and the complex nature of the matrices in which the target compounds are present, efficient sample preparation and trace-level detection and identification are important aspects of analytical methods. Sample preparation, such as extraction, concentration and isolation of analytes, has great influence on the reliability and the accuracy of the analysis. In recent years, many innovations in the analytical processes, which can be applied to prepare food and environmental samples for extraction and determination of pesticide residues, have been developed.[12] For this purpose, solid-phase extraction (SPE) has been widely used for extraction of water samples prior to analysis. SPE reduces sample handling, labor, and solvent consumption.[12,41]

This study reports a simple, relatively fast, and efficient SPE and LC-ESI-MS-MS method which was developed to determine eighteen pesticides from different classes (herbicides, insecticides and fungicides) and two metabolites in water samples. The selection of the pesticides was based on their extensive use in rice plantations in Brazil and the risk of reaching water bodies, including drinking water resources. To obtain efficient pre-concentration with good precision and recovery, a C18 solid-phase extraction system was applied. The method was validated and the parameters involved in the validation were calibration, linearity and range, limit of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision), and accuracy (recovery).

Experimental

Reagents and chemicals

In this study, high purity standards of eighteen pesticides were selected: clomazone, tebuconazole, diuron, irgarol, atrazine, simazine, metsulfuron-methyl, quinclorac, 2,4-D, pyrazosulfuron-ethyl, bentazone, pronanil, carbofuran and the two metabolites: 3,4-DCA and carbofuran-3-hidroxy, which derive from propanil and carbofuran, respectively, were purchased at Sigma Aldrich (São Paulo, Brazil). Imazethapyr, imazapic, fipronil, bispyribac-sodium and penoxsulam were purchased at Dr. Ehrenstorfer GmbH (Augsburg, Germany). Methanol and acetonitrile of chromatographic grade were supplied by Mallinckrodt (Phillipsburg, NJ, USA). Phosphoric acid (85%) of analytical grade was purchased at Merck (Darmstadt, Germany). Ultra pure water was produced by a Direct-Q UV3® system (Millipore, Bedford, MA, USA). The SPE extraction tubes were Chromabond C18ec, 500 mg *per* 3 mL (Macherey-Nagel, Düran, Germany).

Individual pesticide stock solutions containing 1000 mg $L^{-1}$of the target compounds were prepared in methanol and stored at −18 °C. Intermediate working standard mixtures in methanol, containing 100 mg L^{-1} for each pesticide were prepared and they were employed to make the working standard solution containing 10 mg L^{-1}; the latter was used to spike samples and to prepare the calibration curve. Working standard solutions were prepared monthly, while the dilutions used for the calibration curves were prepared daily. Calibration standards of 20 compounds mixture were prepared by diluting it with methanol.

Liquid chromatography separation

Analyses were performed on a Waters Alliance 2695 Separations Module HPLC, equipped with a quaternary pump, an automatic injector and a thermostatted column compartment (Waters, Milford, MA, USA). The chromatographic separation was performed with an XTerra® MS C18 (3.0 mm × 50 mm i.d., 3.5 µm film thickness) column Waters (Milford, MA, Ireland). The mobile phase components are (A) ultra-pure water +0.01% formic acid, (B) acetonitrile +0.01% formic acid, and (C) pure methanol in the proportion 46:24:30 (v/v/v), respectively, with elution in isocratic mode at a flow rate 0.5 mL min^{-1} resulting in a 10 min run time. The temperature of the column compartment was set to 20 ºC. The injection volume was 20 µL for samples after the pre-concentration step by SPE.

Tandem mass spectrometry detection

A Quattro micro API (triple quadrupole) mass spectrometer, equipped with a Z-spray electrospray (ESI) ionization source, from Micromass (Waters, Milford, MA, USA) was used. Drying gas, as well as nebulizing gas, was nitrogen generated from pressurized air in a NG-7 nitrogen generator (Aquilo, Etten-Leur, NL). The nebuliser gas flow was set to 50 L h^{-1} and the gas flow desolvation, to 350-550 L h^{-1}.

For operation in the MS-MS mode, collision gas was Argon (White Martins, Rio Grande do Sul, Brazil) with a pressure of 3.5×10^{-3} mbar in the collision cell. The optimized values were: capillary voltages, 4 kV; extractor voltage, 2 V; source temperature, 100 ºC; desolvation temperature, 350 ºC; multiplier, 600 V; and the scan range, 50-500 *m/z*.

Optimization of the MS-MS conditions, choice of the ionization mode, identification of the precursor/parent and product ions, and selection of the cone and collision voltages, most favorable for the analysis of the target analytes, were performed with injection of their individual standard solutions by recording in both negative and positive modes of ionization, full scan and product ion mass spectra at different values of cone and collision energies, respectively. Direct infusion of every individual standard pesticide solutions in the concentration of 1 µg mL^{-1} in methanol was performed at a flow rate of 10 µL min^{-1} using a Model 11 single syringe pump (Harvard Instruments, Holliston, MA, USA). Analytical instrument control, data acquisition and treatment were performed by software MassLynx, version 4.1 (Micromass, Manchester, UK).

After the optimization of the collision cell energy of the triple quadrupole, two different precursor ion-product ion transitions were selected for each pesticide, one for quantification and one for qualification, and these ions were monitored under time-scheduled multiple reaction monitoring (MRM) conditions.

Method validation

The analytical curves and the linearity of the detector response for the test compounds were evaluated by injecting in triplicate from six to nine concentration values (ranging from 1.0-500.0 µg L^{-1}) of the standard solutions prepared in methanol and analyzed by using a least-square regression. Satisfactory linearity was assumed when the determination coefficient (r^2) was higher than 0.99 for all compounds.

Accuracy (recovery) and precision (RSD, %) were evaluated by analyzing surface water samples spiked at six concentration levels (0.004, 0.02, 0.05, 0.1, 0.5 and 2.0 µg L^{-1}), including the LOQ and the last point of the analytical curve. All experiments were performed in triplicate ($n = 9$).

The LOD was defined as the lowest concentration that the analytical process could reliably differentiate for a signal-to-noise ratio value from 2.5 to 5.0.[42] The LOQ was established as the lowest concentration level that was fully validated (based on a solution which contains the mix of pesticide standards) and the lowest concentration in each compound was evaluated and could be detected with reliability.

Sample preparation

The samples were pre-concentrated and extracted by SPE tubes containing 500 mg of octadecylsilane (Chromabond C18ec) with an average particle size of 45 µm. A 250 mL volume of surface water samples was fortified by adding an established volume of stock solution (1.0 mg L^{-1}) of mixture of 18 pesticides and 2 metabolites, resulting in six levels of fortification, 0.004, 0.02, 0.05, 0.1, 0.5 and 2.0 µg L^{-1}. Before sample application, the SPE column was conditioned by passing consecutively 3 mL methanol, 3 mL purified water, and 3 mL of purified water acidified (pH 3.0) with phosphoric acid 1:1 (v/v). After adjusting the pH to 3.0 by adding phosphoric acid, the samples were well mixed and passed through the SPE tubes at 10 mL min^{-1}. After that, the tubes were eluted with 1 mL (500 + 500 µL) of methanol. The final organic extracts were directly analyzed by LC-ESI-MS-MS with injection volume of 20 µL.

Results and Discussion

MS-MS optimization parameters

Considering 20 compounds under study, thirteen showed preferential ionization in the positive mode $[M+H]^+$, namely, clomazone, diuron, propanil, 3,4-DCA, atrazine, carbofuran, simazine, carbofuran-3-hidroxy, tebuconazole, imazapic, imazethapyr, irgarol and bispyribac-sodium, as shown in Table 1 whereas the remaining seven compounds showed more efficient ionization in the negative mode $[M-H]^-$, namely, metsulfuron-methyl, quinclorac, fipronil, penoxsulam, 2,4-D, pyrazosulfuron-ethyl and bentazone. Results are shown in Table 2.

Table 1. Results of the optimized parameters for the compounds analyzed by LC-ESI-MS-MS in the positive mode

Compounds	Molar mass	Transition (*m/z*) Precursor ion → Product ion	Collision energy (eV)	Cone voltage (V)	t_R (min)	Function	time window (min)
Imazapic	275	276 → 231	20	40	0.80	1	0.5-7.0
		276 → 185	30	40			
Carbofuran-3-hidroxy	237	238 → 163	15	25	0.72	1	
		238 → 135	20	25			
Irgarol	253	254 → 198	19	30	1.66	1	
		254 → 125	26	30			
Bispyribac-sodium	452	453 → 297	25	35	3.20	1	
		453 → 275	22	35			
Tebuconazole	308	308 → 70	20	40	5.96	1	
		308 → 88	50	33			
Imazethapyr	289	290 → 230	20	40	0.92	1	
		290 → 177	20	40			
Simazine	201	202 → 132	18	35	1.5	2	1.0 -3.0
		202 → 124	18	35			
Carbofuran	221	222 → 165	20	25	1.15	2	
		222 → 123	20	25			
Atrazine	215	216 → 174	20	33	1.66	2	
		216 → 146	22	35			
3,4-DCA	162	162 → 127	15	35	1.71	2	
		162 → 109	25	25			
Propanil	218	218 → 127	28	25	2.61	2	
		218 → 162	14	30			
Diuron	233	233 → 72	20	28	1.71	2	
		233 → 160	25	28			
Clomazone	240	240 → 125	20	25	2.14	2	
		240 → 100	15	30			

Dwell time: 0.05 s.

Table 2. Results of the optimized parameters for the compounds analyzed by LC-ESI-MS-MS in the negative mode

Compounds	Molar mass	Transition (*m/z*) Precursor ion → Product ion	Collision energy (eV)	Cone voltage (V)	t_R (mim)	Function	time window (min)
Metsulfuron-methyl	381	380 → 139	15	30	1.12	3	1.0-10.0
		380 → 214	10	30			
Quinclorac	242	240 → 196	6	15	1.28	3	
Bentazone	240	239 → 132	25	35	1.45	3	
		239 → 197	20	35			
Penoxsulam	483	482 → 109	40	35	1.53	3	
		482 → 179	25	35			
2,4-D	221	219 → 161	20	15	2.12	3	
		219 → 89	30	15			
Pyrazosulfuron-ethyl	414	413 → 232	15	35	3.97	3	
		413 → 154	26	35			
Fipronil	437	435 → 330	15	30	8.93	3	
		435 → 250	26	25			

Dwell time: 0.05 s

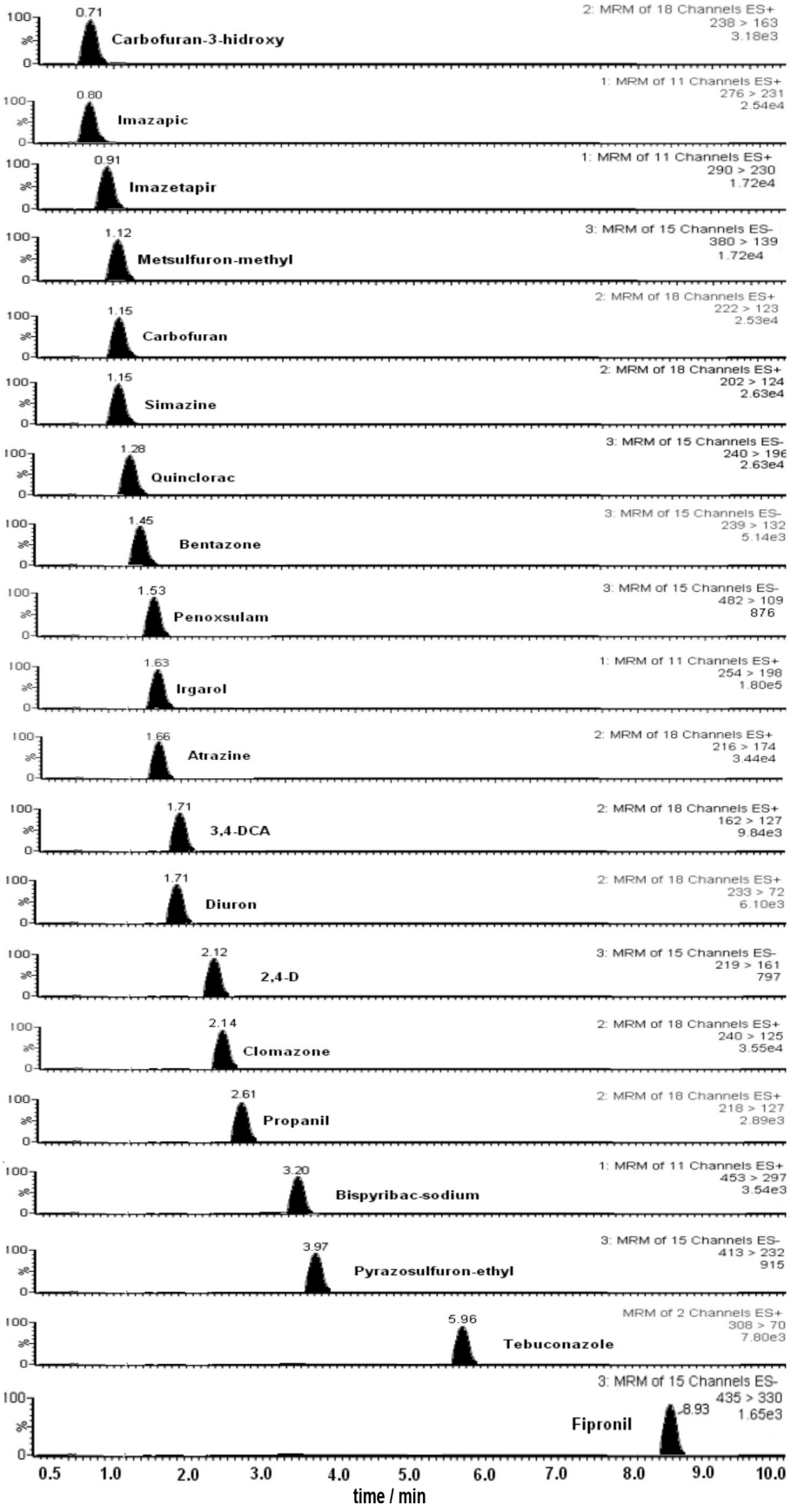

Figure 1. LC-MS-MS chromatogram obtained for pesticides.

To carry out the analysis of all compounds in a single run, the electrospray interface was programmed to change the mode of operation, between $[M-H]^-$ and $[M+H]^+$, along the chromatographic analysis.

The development of confirmative method made necessary to acquire two specific transitions for each compound, at least. The exception was quinclorac due to the difficulty the molecule has to fragment and generate stable ions. So, for quinclorac, only one fragment was found: 240.0 → 196.0 *m/z* in the negative mode.

An MRM chromatogram obtained from the analysis of a standard mixture of twenty compounds can be observed in Figure 1, which shows the transitions of the compounds used for quantification. A similar chromatogram can be obtained for the transitions employed for confirmation.

The chromatogram shows that some analytes have the same retention time, such as the pairs carbofuran/simazine and 3,4-DCA/diuron. However, the technique using LC-MS-MS can solve these problems regarding chromatographic resolution by using the higher selectivity of the mass spectrometer when operating in the MRM mode.

Analytical method validation

For the linear dynamic range, from six to nine point calibration curves were constructed using a least-square regression analysis in the range 1.0-500.0 μg L^{-1} for irgarol, tebuconazole, fipronil, simazine and atrazine; 5.0-500.0 μg L^{-1} for carbofuran and clomazone; 10.0-500.0 μg L^{-1} for imazapic, imazethapyr, propanil, diuron, 3,4-DCA, carbofuran-3-hidroxy, 2,4-D, bentazone, metsulfuron-methyl and pyrazosulfuron-ethyl and 25.0-500.0 μg L^{-1} for bispyribac-sodium, quinclorac and penoxsulam. For all compounds determination coefficients were higher than 0.99, showing good results (Table 3).

Limits of detection (LOD) were calculated through the injection of 20 μL standard solutions with an analyte concentration that leads to a signal-to-noise ratio 2.5 to 5.0, following USEPA's recommendation.[46] Instrumental LOD were determined in 20 compound mix and obtained a range from 0.1 to 10.0 μg L^{-1}. Limits of instrument quantification (LOQ), varied from 1.0 to 25.0 μg L^{-1}. By using SPE, the method pre-concentration factor was 250 times, allowing the LODs and LOQs levels of the compounds investigated in water samples to reach ng L^{-1}accuracy levels.

However, taking into consideration the pre-concentration factor, the LODs for the method varied from 0.4 to 40.0 ng L^{-1} whereas the intervals of the LOQs changed from 4.0 to 100.0 ng L^{-1} (Table 4).

Table 3. Results for calibration curves

Compounds	Linearity range (μg L^{-1})	Determination coefficient (r^2)	Number of standards (*n*)
Irgarol	1.0-500.0	0.9948	9
Imazapic	10.0-500.0	0.9939	7
Imazethapyr	10.0-500.0	0.9967	7
Tebuconazole	1.0-500.0	0.9972	9
Bispyribac-sodium	25.0-500.0	0.9930	6
3,4-DCA	10.0-500.0	0.9933	7
Simazine	1.0-500.0	0.9972	9
Atrazine	1.0-500.0	0.9963	9
Propanil	10.0-500.0	0.9905	7
Carbofuran	5.0-500.0	0.9957	8
Diuron	10.0-500.0	0.9952	7
Carbofuran-3-hidroxy	10.0-500 .0	0.9949	7
Clomazone	10.0-500.0	0.9931	7
2,4-D	10.0-500.0	0.9955	7
Bentazone	10.0-500.0	0.9917	7
Quinclorac	25.0-500.0	0.9910	6
Metsulfuron-methyl	10.0-500.0	0.9930	7
Pyrazosulfuron-ethyl	10.0-500.0	0.9990	7
Fipronil	5.0-500.0	0.9967	8
Penoxsulam	25.0-500.0	0.9909	6

Recovery results

The recovery results obtained for six levels of pesticide concentration under study are shown in Table 5. Extractions in triplicate at each studied concentration level and injections in triplicate ($n = 9$) were made.

The results showed recovery percentages from 70 to 120% for 95% of the compounds in six fortification levels. Only a few exceptions showed recoveries under 70%, such as the compounds 3,4-DCA with 65.3% at spike level 0.5 μg L^{-1}; bentazone with 60.2% at spike level 0.1 μg L^{-1} and pyrazosulfuron-ethyl with 65% at spike level 2.0 μg L^{-1}. For recoveries higher than 120%, imazapic showed 136.4% at spike level 2.0 μg L^{-1}, imazethapyr 136.3% and 123.1% at spike levels 2.0 and 0.05 μg L^{-1}, respectively. For bispyribac-sodium, recoveries were 122.5 at spike levels 0.5 μg L^{-1}, and for simazine and penoxsulam, recoveries were 134.7 and 127.7% at spike levels 2.0 μg L^{-1}, respectively. Accuracy values were also satisfactory since RSD values were lower than 20% for all compounds.

Method applicability in real samples

Environmental sample analysis

After the optimization and validation, the method was applied to real samples, to evaluate its applicability. The

Table 4. Study of LOD and LOQ values obtained by LC-ESI-MS-MS

Compounds	LOD[a] (μg L^{-1})	LOQ[a] (μg L^{-1})	LOD[b] (ng L^{-1})	LOQ[b] (ng L^{-1})
Irgarol	0.1	1.0	0.4	4.0
Imazapic	0.1	10.0	0.4	40.0
Imazethapyr	1.0	10.0	4.0	40.0
Tebuconazole	0.5	1.0	2.0	4.0
Bispyribac-sodium	5.0	25.0	20.0	100.0
3,4-DCA	0.5	10.0	2.0	40.0
Simazine	0.1	1.0	0.4	4.0
Atrazine	0.5	5.0	2.0	20.0
Propanil	0.5	10.0	2.0	40.0
Carbofuran	0.5	5.0	2.0	20.0
Diuron	5.0	10.0	20.0	40.0
Carbofuran-3-hidroxy	5.0	10.0	20.0	40.0
Clomazone	0.5	5.0	2.0	20.0
2,4-D	5.0	10.0	20.0	40.0
Bentazone	0.1	10.0	0.4	40.0
Quinchlorac	10.0	25.0	40.0	100.0
Metsulfuron-methyl	5.0	10.0	20.0	40.0
Pyrazosulfuron-ethyl	5.0	10.0	20.0	40.0
Fipronil	0.5	1.0	10.0	4.0
Penoxsulam	10.0	25.0	40.0	100.0

[a]Limits of detection/quantification of the instruments for the standard solution mix of pesticides. [b]Limits of detection/quantification of the method for the standard solution mix of pesticides.

sampling was carried out monthly at CORSAN, the water treatment station in Rio Grande, from January, 2008 to April, 2009. Two different samples were collected: a surface water sample, collected at the entrance of the water channel (São Gonçalo channel), and a drinking water sample, collected after the water treatment, in the output of the station.

In Figure 2, the sum of pesticide concentrations of the monthly results, from January, 2008 to April, 2009, of monitoring analysis of the surface water from the São Gonçalo channel and of the drinking water in Rio Grande are presented. Taking into account the 18 pesticides and 2 metabolites under analysis, it was detectable contamination of diuron, irgarol, imazethapyr, imazapic, fipronil, clomazone, tebuconazole, atrazine, pyrazosulfuron-ethyl, simazine, carbofuran-3-hydroxi and 3,4-DCA. It is noticed that, except to in February, 2009, in drinking water the sum of concentrations of pesticides was always lower than 0.5 μg L^{-1}, the value established by European legislation.[6]

The presence of diuron and irgarol during all the monitoring period indicates that the region of São Gonçalo channel, concerning the contamination by pesticides, is influenced not only by the agricultural process but also by other sources. The occurrence of

Table 5. Average recovery (%), $n = 9$ and RSD (%)

Pesticide	Spike level 2.0 μg L^{-1}		Spike level 0.5 μg L^{-1}		Spike level 0.1 μg L^{-1}		Spike level 0.05 μg L^{-1}		Spike level 0.02 μg L^{-1}		Spike level 0.004 μg L^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Irgarol	110.4	4.9	107.8	8.7	101.9	9.3	105.4	5.1	111.9	11.4	121.3	4.9
Imazapic	136.4	1.9	111.9	15.4	92.3	9.7	96.8	9.7	n.d.	n.d.	n.d.	n.d.
Imazethapyr	136.3	7.4	109.2	0.7	117.8	9.3	123.1	3.0	n.d.	n.d.	n.d.	n.d.
Tebuconazole	96.5	2.6	102.5	9.9	92.2	6.9	91.2	5.5	109.2	11.9	95.3	12.5
Bispyribac-sodium	98.5	8.1	122.5	7.2	89.5	5.1	93.3	4.2	n.d.	n.d.	n.d.	n.d.
3,4-DCA	103.1	3.2	65.3	8.2	104.2	7.0	93.2	5.6	n.d.	n.d.	n.d.	n.d.
Simazine	134.7	16.2	95.1	10.2	89.6	11.1	93.2	3.2	97.4	14.1	101.8	17.7
Atrazine	82.3	9.8	119.1	14.9	75.7	6.3	115.9	15.4	96.1	7.8	n.d.	n.d.
Propanil	86.3	7.2	92.8	5.5	101.2	4.0	108.4	4.9	n.d.	n.d.	n.d.	n.d.
Carbofuran	95.2	6.3	111.9	9.9	84.5	4.2	76.7	4.8	95.7	11.9	n.d.	n.d.
Diuron	100.4	7.1	109.5	1.7	75.3	15.1	92.7	6.2	n.d.	n.d.	n.d.	n.d.
Carbofuran-3-hidroxy	100.1	15.3	94.8	7.5	93.3	15.7	103.6	10.1	n.d.	n.d.	n.d.	n.d.
Clomazone	110.5	5.2	91.6	7.7	82.7	7.8	74.5	2.1	n.d.	n.d.	n.d.	n.d.
2,4-D	110.3	7.8	128.4	20.3	108.0	3.4	100.1	11.6	n.d.	n.d.	n.d.	n.d.
Bentazone	75.6	2.7	81.6	8.5	60.2	7.8	72.5	2.9	n.d.	n.d.	n.d.	n.d.
Quinclorac	84.1	12.7	87.4	11.5	94.2	3.8	98.1	3.1	n.d.	n.d.	n.d.	n.d.
Metsulfuron-methyl	71.9	2.8	80.7	10.2	118.6	5.7	96.3	13.6	n.d.	n.d.	n.d.	n.d.
Pyrazosulfuron-ethyl	64.9	7.8	70.0	4.1	91.3	1.9	82.3	10.2	n.d.	n.d.	n.d.	n.d.
Fipronil	103.1	4.5	99.8	5.7	84.0	4.7	103.1	11.2	93.0	14.0	102.2	10.2
Penoxsulam	127.7	2.4	53.1	12.8	120.2	4.5	93.5	19.9	n.d..	n.d.	n.d.	n.d.

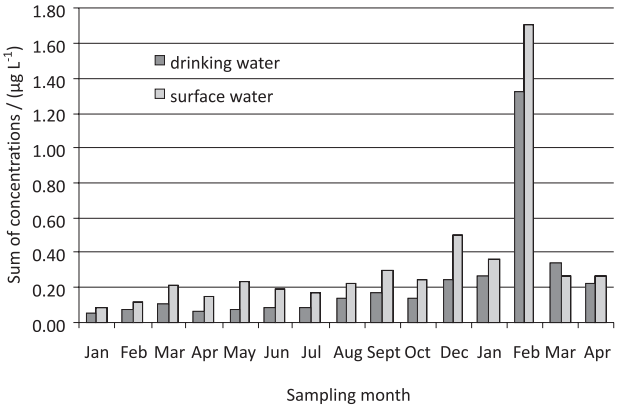

Figure 2. Sum of pesticide concentrations determined from January 2008 to April 2009.

diuron can be due to the fact that this herbicide is largely used in other kinds of cultures such as lettuce, citrus fruits, and onion.[43] Besides, diuron and irgarol are used as antifouling in paints for vessels.[44,45] The association of these uses increase the contamination by diuron in waters, specially in portuary regions such as Rio Grande where there is intensive navigation. The presence of 3,4-DCA, the main metabolite of diuron and of propanil confirm the use of these compounds. Whereas diuron is used in the culture of rice, the irgarol herbicide is not, but both are used as antifouling,[46] indicating that the contamination by these compounds has the same source, the vessels.

The other compounds were probably found in the samples are probably due to irrigated rice farming, because all compounds are recommended for this culture and this is the agricultural practice that dominates the southern region in RS state, besides the plantation of onion and tomato.[43]

The herbicides imidazolinones analysed in this study, imazethapyr and imazapic, were detected during all the sampling period, both in the surface water and in potable water. The fact that they are frequently detected in the samples can be explained, for example, by the system of rice cultivation, which works with a variety of rice that is very resistant to the pesticides of the imidazolinones class; they are effective against red rice, the main pest in the irrigated rice plantation.[45]

The fungicide tebuconazole and the herbicides atrazine and simazine were detected in the sampling since May, and the tebuconazole kept being detected in all samples. Triazines are used in the world as pre and post-emerging selective herbicides to control weeds in many cultures, such as corn, wheat, sugar cane and barley.

The insecticide fipronil was detected in all samples, since it is recommended for several cultures (agriculture and silviculture). The herbicide pyrazosulfuron-ethyl was detected in some samples too, this compound is recommended for rice cultivation.

The detection of the metabolite carbofuran-3-hydroxi from carbosulfan and carbofuran, indicates that one of these compounds had been used on farms near the São Gonçalo channel.

The herbicide clomazone was detected in concentrations lower than the LOQ in all samples, except in July, when it was detected in surface and potable waters.

The method was shown to be sensitive enough to detect these pesticides, even at this sub-μg L^{-1} level.

Conclusions

An analytical methodology for multiclasses, using SPE and LC-ESI-MS-MS, was developed to analyze 18 selected pesticides and 2 metabolites fast and simultaneously in surface and drinking water samples. The main advantage of the method was the use of MS-MS because it provides a high level of certainty to identify the target compounds. The mode MRM in MS-MS enables us to select specific fragments of each compound, with the optimization of cone voltage and collision energy, thus allowing a high level of selectivity in this technique, even if the work is done with a large number of compounds per analysis. In this research, another great advantage of the analytical technique is that LC-ESI-MS-MS showed high sensitivity with detection limits below 40.0 ng L^{-1} for all compounds allowing the determination of pesticide residues in surface and drinking waters in accordance with Law nº 518 of the Ministry of Health, Brazil, and the European Union Directive on drinking water quality (98/83/EC).

Good linearity of the calibration curves was obtained over the range from the LOQ of each compound to 500.0 μg L^{-1}, with $r^2 > 0.99$. Instrument LOD values generally varied from 0.1 to 10.0 μg L^{-1}, however, the pre-concentration factor LOD was from 0.4 to 40.0 ng L^{-1}. For 91% of the accuracy experiments, recoveries were in the range 70-120% as well as good reproducibility with relative standard deviations below 20%. The method was fast (10 min for analysis), thus reducing the solvent consumption. Another important factor determined by the method was the detection and quantification limit that were below the maximum permissible concentration of 0.1 μg L^{-1} established in the EU regulation (Directive 98/83/EC) for drinking water,[8] showing that SPE and LC-ESI-MS-MS is a useful methodology for the analysis of pesticides.

The application of the method in real samples showed excellent performance. The results proved that the method is adequate for the utilization in routine analysis for drinking and surface water.

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