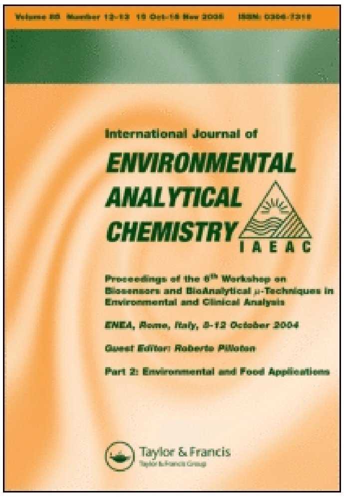

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Development and application of methods using SPE, HPLC-DAD, LC-ESI-MS/MS and GFAAS for the determination of herbicides and metals in surface and drinking water

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Development and application of methods using SPE, HPLC-DAD, LC-ESI-MS/MS and GFAAS for the determination of herbicides and metals in surface and drinking water

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A preliminary study of the pollution in surface and drinking waters caused by herbicides and metals in the Municipal Water Supply System (CORSAN) in Rio Grande city, RS, Brazil, is reported. The occurrence of 5 herbicides and 9 metals was studied in surface and drinking water through the analysis of 2 sampling spots at CORSAN. The analytical determination was performed by solid-phase extraction (SPE), high performance liquid chromatography-photodiode array detection (HPLC-DAD) and liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) for herbicides, and graphite furnace atomic absorption spectrometry (GFAAS) for metals. The concentrations of herbicides in the surface water were very low; however, the herbicide clomazone was detected in more than 50% of the samples analysed in concentration exceeding 1.0 $\mu g\ L^{-1}$. The concentration of metals was below the Maximum Contaminant Level (MCL) set by the Brazilian regulation.

Keywords: SPE; LC-ESI-MS/MS; GFAAS; metals; herbicides; water

1. Introduction

Protecting water quality is a top environmental priority in the twenty-first century [1]. Pesticide analysis in environmental samples has attracted much attention, not only owing to the wide use of such compounds in agriculture and in households, but also because of their environmental impact. Herbicides represent about 50% of the demand in agricultural chemicals; their prolonged use involves the risk of retention in crops and soils. Besides, because of washing and leaching processes, these substances pass to surface and ground waters. The uptake of pesticides into watercourses, their transport by the wind and propagation through biological chains show the necessity to monitor and to know the fate of herbicides and their degradation products, not only in the areas where they are applied, but also in more or less proximal areas [2,3]. Extensive research has been done regarding the occurrence of herbicides in the hydrological system, their fate and their effects on human health and the environment [4,5].

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Herbicides are potential contaminants of natural waters because they are directly applied to the soil and are leached into the surface water or transported into ground water [6–8]. However, a pesticide is said to be able to contaminate ground water if its water solubility is higher than $30\,\mathrm{mg\,L^{-1}}$, if its *K*oc (organic carbon partition coefficient) is lower than $300\,\mathrm{mL\,g^{-1}}$, if its *K*d (distribution adsorption constant) is lower than $5\,\mathrm{mL\,g^{-1}}$ and if its soil half-life is longer than 3 weeks [6–8].

Due to the fact that the springs of water are sources of drinking water, many environmental agencies have passed rigorous legislation regarding the quality of those waters. The European Union sets rigid limits for pesticides in water destined for human consumption, having established $0.1\,\mu\mathrm{g\,L^{-1}}$ for individual pesticides and $0.5\,\mu\mathrm{g\,L^{-1}}$ for the sum of all pesticides. In the case of surface waters that will be destined for human consumption after treatment, the limits are $1\,\mu\mathrm{g\,L^{-1}}$ for individual pesticides and $5\,\mu\mathrm{g\,L^{-1}}$ for the total of pesticides [9–11]. In Brazil, levels up to $300\,\mu\mathrm{g\,L^{-1}}$ are accepted for some pesticides, such as the herbicide bentazone; $30\,\mu\mathrm{g\,L^{-1}}$ for 2,4-D; and $20\,\mu\mathrm{g\,L^{-1}}$ for propanil [12].

To assess the impact of pesticides on aquatic ecosystems, skilled analytical methods are required for the routine simultaneous determination of such compounds at trace concentrations in water samples [1]. The herbicides are usually determined by gas chromatography (GC), liquid chromatography (LC) or capillary electrophoresis (CE), depending on their polarity, volatility, and thermal stability [2,5,13–20]. Liquid chromatography (LC) is the preferred approach to analyse polar thermally labile herbicides. Conventional UV detection and diode array detection (DAD) have been extensively used in LC for the determination of herbicides in environmental samples. Liquid chromatography combined with mass spectrometry (LC-MS) has also been proposed to determine herbicides [2].

Due to the low detection levels imposed by regulatory agencies 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 to be considered when selecting the analytical methods. Sample preparation techniques, such as extraction, concentration and isolation of analytes, have a strong influence on the reliability and accuracy of the results. Solid-phase extraction (SPE) has been widely used for herbicide extraction in water samples prior to analysis, since SPE reduces sample handling, labour, and solvent consumption [5,13].

Metals are as important as herbicides, in connection with water quality. The former have been used for a long time to evaluate environmental quality, since the presence of these elements on natural grounds can result from anthropogenic activity. The understanding of the process that occurs in the environment and the improvement of analytical methods, and subsequent lowering of detection limits, has forced governments to reduce their Maximum Contaminant Levels (MCL). In Brazil, the MCL for metal in water can range from $0.001\,\mathrm{mg\,L^{-1}}$ for mercury to $2\,\mathrm{mg\,L^{-1}}$ for copper, for example.

This paper presents a new HPLC-DAD and LC-ESI-MS/MS method for the simultaneous detection of herbicides in water samples. To achieve lower detection limits, an SPE step using C18 was used. It enabled simultaneous extraction and pre-concentration of herbicides with previous acidification of the sample and subsequent elution of herbicides. The methods we have proposed are used to investigate the levels of the herbicides quinclorac, bentazone, 2,4-D, clomazone and propanil in drinking water and surface waters in the Municipal Water Supply System (CORSAN) in Rio Grande city, RS. The diagnosis of this environment was completed with the analysis of the metals Cu, Cd, Pb, Cr, Fe, Al, Zn, Mn and Ni.

2. Experimental

2.1 *Chemicals and reagents*

Analytical standards of quinclorac (99.8%), bentazone (99.1%), clomazone (97.4%), 2,4-D (99.6%) and propanil (99.7%) were supplied by Sigma-Aldrich (Sao Paulo-SP, Brazil). Methanol and acetonitrile of chromatographic grade came from Mallinckrodt (Phillipsburg, NJ, USA). Phosphoric acid of analytical grade was provided by Merck (Darmstadt, Germany). Water was purified with a Direct-Q UV3® (resistivicty 18.2 MΩ cm) water purification system (Millipore, Bedford, MA, USA). The SPE extraction tubes were Phenomenex (Strata C18-E, 200 mg/3 mL), manufactured by Phenomenex (Torrance, CA, USA). Individual pesticide stock solutions containing $1000\,\mathrm{mg\,L^{-1}}$ of the target compounds were prepared in methanol and stored at −18°C. Intermediate working standard mixtures in methanol, containing $100\,\mathrm{mg\,L^{-1}}$ of each pesticide were prepared and used to prepare the working standard solutions containing 10.0, 8.0, 5.0, 2.0, 1.0, 0.5 and $0.05\,\mathrm{mg\,L^{-1}}$ for the HPLC-DAD method. For the LC-ESI-MS/MS method standard solutions containing 500.0, 250.0, 100.0, 50.0 and $10.0\,\mu\mathrm{g\,L^{-1}}$ were prepared from the working standard mixture $1.0\,\mathrm{mg\,L^{-1}}$. Then, they were used to spike samples and to prepare the analytical curves. Working standard solutions were prepared monthly while the dilutions used for the analytical curves were prepared daily.

Accuracy in metal determination was assessed by using the certified reference material SLRS-4 (River Water Reference Material for Trace Metals from the Nation Research Council, Canada) and quantitative recoveries. Ninety five per cent accuracy was obtained for all metals under study. The metal standard solutions were prepared by the dissolution of an ICP standard solution (MERCK).

2.2 *Determination of herbicides in water samples*

2.2.1 *Sample extraction*

The isolation of the target organic compounds from water samples was performed using a solid-phase extraction (SPE) procedure, following a method developed by Zanella *et al.* [5]. SPE cartridges (Strata C18-E, 200 mg/3 mL) were conditioned by passing 3 mL methanol, 3 mL purified water and 3 mL purified water pH 3.0 adjusted with phosphoric acid 1 : 1 (v/v), consecutively. The extraction was performed at a flow rate of $5\,\mathrm{mL\,min^{-1}}$. Following extraction, the cartridges were eluted with 1 mL (500 + 500 μL) methanol. The eluents were evaporated to dryness under a gentle stream of nitrogen (25°C) and re-dissolved in 500 μL of mobile phase and injected in HPLC-DAD and LC-ESI-MS/MS.

2.2.2 *Analysis by HPLC-DAD*

Sample extracts of five herbicides were analysed by HPLC-DAD using the system Waters (Milford, USA), with Waters 600 pump model coupled with a Waters 2996 PDA and Empower software for data acquisition. The operating conditions were as follows: the analytical column Hyperclone BDS C18 column (4.6 mm × 250 mm × 5 μm) Phenomenex (Torrance, CA, USA) was operated at ambient temperature; isocratic solvent system composed by 24% methanol, 36% acetonitrile and 40% purified water adjusted to pH 3.0 with phosphoric acid 1 : 1 (v/v); the flow rate was set to $0.8\,\mathrm{mL\,min^{-1}}$; injection volume of 20 μL. The identification of the five pesticides in the samples was accomplished on the basis of their retention times and by comparison between the DAD spectrum of the compounds in the standard solutions and the DAD spectrum of the detected peak in the sample. Quantification was carried out with DAD detection at 220 nm.

2.2.3 *Analysis by LC-ESI-MS/MS*

Sample extracts were analysed using the system Waters Alliance 2695 Separations Module HPLC, equipped with a quaternary pump, an automatic injector and a thermostated column compartment. A Quattro micro API triple quadrupole mass spectrometer, equipped with a Z-spray electrospray (ESI) ionisation source, Micromass, (Waters, Milford, MA, USA) was used. The chromatographic separation was performed with an XTerra® MS C18 (3.0 mm × 50 mm, 3.5 µm) column manufactured by Waters. The mobile phase isocratic solvent system was composed by 46% purified water with 0.01% (v/v) formic acid, 24% acetonitrile and 30% methanol; flow rate was set to 0.5 mL min^{-1}, resulting in a 10-minute run time. The injection volume was 20 µL for samples after the pre-concentration step and the temperature of the column compartment was set to 20°C. Drying gas as well as nebulising gas was nitrogen generated from pressurised air in a Peak Scientifics nitrogen generator (Instruments, Scotland). The nebuliser gas flow was set to 50 L h^{-1} and the desolvation gas flow, to 550 L h^{-1}. The optimisation of the collision energy (CE) for each individual pesticide was done by infusion of the pesticide directly into the LC effluent using syringe pump (Switzerland, CH). For operation in MS/MS mode, collision gas was Argon (White Martins) with a pressure of 3.5 10^{-6} bar in the collision cell. The optimised values were: capillary voltages, 3–4 kV; extractor voltage, 2 V; source temperature, 100°C; desolvation temperature, 350°C; multiplier, 600 V; dwell times, 0.05 s scan^{-1}, and scan range, 50–500 *m*/*z*.

The full-scan spectra and the MS/MS spectra were obtained from infusion of solutions containing 1.0 µg mL^{-1} of each compound at a flow rate of 10 µL min^{-1}. MS/MS detection was performed with an electrospray interface operating in the positive ionisation mode for clomazone and propanil herbicides and in the negative ionisation mode for quinclorac, bentazone and 2,4-D herbicides. Monitoring conditions were optimised for each herbicide. After the optimisation 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 another one for qualification. These ions were monitored under time-scheduled multiple reaction monitoring (MRM) conditions. Analytical instrument control, data acquisition and treatment were performed by the software MassLynx, version 4.1 (Micromass).

2.3 *Metals*

For the metal analysis, an aliquot of sample was digested in a microwave device in nitric/hydrochloric acid medium, according to the EPA method number 3015 [21]. The determination was performed in graphite furnace atomic absorption spectrophotometer with deuterium correction Zeiss AAS-5 (Jena, Germany). SLRS-4 was used as the certified reference material to verify the accuracy and all results were in accordance with nominal values. The material that we used was cleaned by soaking it in 5 mol L^{-1} HNO_3 for 5 days, followed by rinsing in purified water. After drying in a laminar flux bench, the material was individually packed in polyethylene bags [22]. All procedures were done in a laminar flux bench.

2.4 *Validation studies*

The validation for the HPLC-DAD procedure followed a method that was developed by Zanella *et al.* [5].

The performance characteristics of the method for LC-ESI-MS/MS were established by a validation procedure and the use of standard solutions and spiked samples. Linearity, precision, accuracy (recovery), detection limits and quantification limits were evaluated. The linearity in the response was investigated by using calibration solutions at five concentration levels, ranging from 10 to 500 $\mu g\,L^{-1}$. The integrated area data were used to construct the curves. The accuracy of the method was investigated through mean recoveries obtained for the three replicates of spiked blanks samples at four different concentration levels (0.05; 0.1; 0.5 and 2.0 $\mu g\,L^{-1}$). Water samples spiked with all the herbicides were extracted by applying the SPE method described above. The analytical signal was compared with the signal of a blank water extract after solid-phase extraction spiked with the target compounds. In this study, LOD and LOQ were determined considering the LOD 3 times higher than the baseline noise and the LOQ was the concentration that produced a signal 10 times higher than the baseline noise, at a period close to the retention time of the analyte.

Precision was evaluated in terms of repeatability (RSDr) and intermediate precision (RSDip) and expressed as relative standard deviation (RSD). The precision in terms of repeatability was obtained by carrying out the extraction and analysis of fortified samples. Each spike level was extracted in three replicates and each extract was injected three times. The precision of the method was measured by comparing the standard deviations of the responses for nine injections of the four different calibration standard solutions (10.0, 25.0 and 500.0 $\mu g\,L^{-1}$), evaluated in terms of repeatability (RSDr) and intermediate precision (RSDip).

2.5 *Description of the study area and sample collection*

Rio Grande, a city of 185,000 inhabitants, is located in the southeast of Brazil. The local water supply system gets surface water for treatment from the São Gonçalo Channel (Figure 1) which extends for 62 km. There is intense industrial and agricultural activity on its banks, mainly irrigated rice. The herbicides we have used in this study were selected due to their extensive use in rice plantations in Brazil, thus posing a risk of reaching water bodies, including drinking water resources.

Rio Grande city lies on the east bank of the São Gonçalo Channel and consumes 2000 $L\,s^{-1}$ for industrial activity; it represents 90% of the total consumption. The water for public supply (2070 $L\,s^{-1}$) is driven from the São Gonçalo Channel to CORSAN through an artificial channel [23].

The water samples were collected from March 2006 to February 2007 in two spots: (1) after water filtration at the entrance of CORSAN, named *surface water*; and (2) just before distribution to the population. The surface water samples were collected at CORSAN, thus following the recommendations described in Chapter V of the Governmental Decree No. 518 of 03/25/2004, issued by the Ministry of Health, to control physical and chemical water and radioactivity parameters in the water supplied to the population and in springs [12].

Samples for herbicide analysis were collected in 1 L glass bottles and, immediately after collection, they were acidified to pH 3.0 with phosphoric acid and filtered through

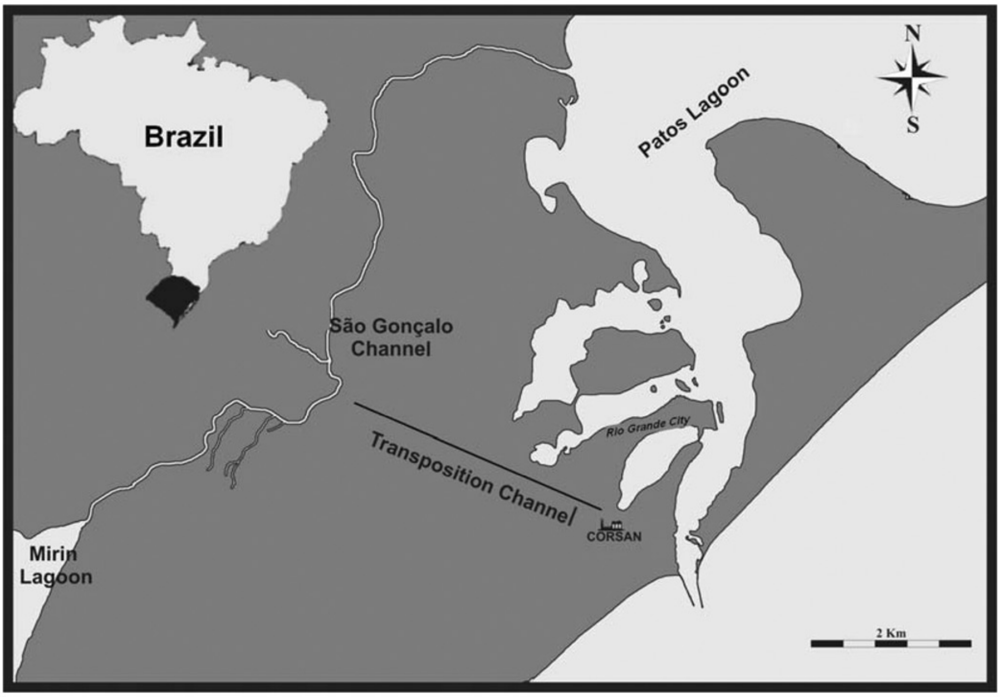

Figure 1. Study area, São Gonçalo Channel and Rio Grande city.

a 0.45 μm PTFE filter to remove particulate matter. The pre-treated samples were stored at 4°C before extraction, which is normally performed within 48 hours by the method describe above.

Metal analysis, sampling and analytical techniques followed the Standard Method [24]. Water samples were collected in a 500 mL clean HDPE bottle and acidified to pH 2.0 using Ultrapure^{R} nitric acid. The samples were then kept refrigerated at 4°C until digestion, being analysed immediately afterwards.

3. Results and discussion

3.1 *Herbicides*

3.1.1 *Method using HPLC-DAD*

For the five herbicides under study, the linearity extended from 0.05 to less than 10 mg L^{-1}. After the 500-fold SPE pre-concentration step, the effective Limit of Quantification (LOQ) in the surface water was 0.1 μg L^{-1} for 2,4-D, clomazone and propanil and 0.5 μg L^{-1}, for quinclorac and bentazone. The method remained repeatable ($n = 9$) and reproducible ($n = 9$) with Relative Standard Deviation (RSD) <15%. The recovery of the two compounds at three levels (0.5, 1.0 and 10.0 μg L^{-1}) ranged between 88.7 and 109.8% for quinclorac and from 86.0 to 95.1%, for bentazone. Recoveries of the three compounds at four levels (0.1, 0.5, 1.0 and 10.0 μg L^{-1}) ranged between 86.4 and 100.5% for 2,4-D, from 86.0 and 105.0%, for clomazone and from 91.7 and 98.4%, for propanil.

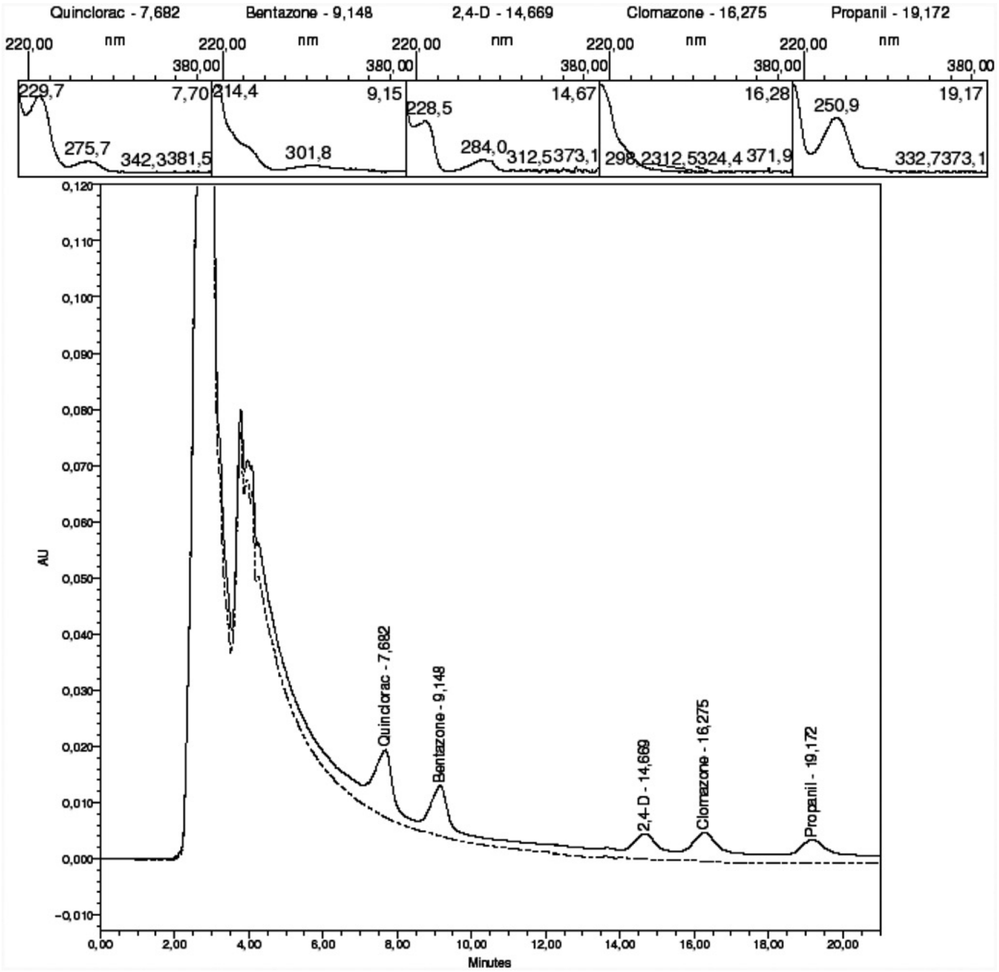

Figure 2. HPLC-DAD chromatograms obtained from (- - -) water blank and (–) final extracts 250 mL surface water spiked with the herbicides at a level of 0.5 μg L^{-1} each.

The main problem in the analysis of water samples using HPLC-DAD is the presence of organic substances, mainly humic and fulvic acids. Samples collected at Spot 1 had large amounts of organic compounds which remained in the SPE cartridges and resulted in light orange/yellow extracts.

A typical chromatogram for this analysis is presented in Figure 2. The presence of a large peak at the beginning of each chromatogram and then other peaks of low intensity did not interfere in the qualitative and quantitative analysis of the herbicides.

3.1.2 *Method using LC-ESI-MS/MS*

The optimal conditions for the method LC-ESI-MS/MS are summarised in Table 1. The efficiency of the extraction method was evaluated using blank matrix spike samples at

Table 1. MS/MS conditions for multiple reaction monitoring.

Herbicide	MW ($g\,mol^{-1}$)	Ionisation mode	Precursor ion (m/z) >Product ion (m/z)	Collision energy (eV)	Cone voltage (V)
Quinclorac	242	ESI−	240 > 196	6	15
Bentazone	240	ESI−	239 > 132	25	35
			239 > 197	20	35
2,4-D	219	ESI−	219 > 161	20	15
			219 > 89	30	15
Clomazone	240	ESI+	240 > 125	20	25
			240 > 100	15	30
Propanil	218	ESI+	218 > 127	28	25
			218 > 162	14	30

Note: MW = molecular weight.

0.05; 0.1; 0.5 and 2.0 $\mu g\,L^{-1}$ (Figure 3). The average recoveries and standard deviations are shown in Table 2. Good recoveries, between 80.2 and 128.4%, were obtained for the target herbicides at the four assayed fortification levels, with RSD varying from 2.1 to 12.7%. The linearity was also good for all compounds with correlation coefficients: they were always higher than 0.99 (above the concentration range 10–500 $\mu g\,L^{-1}$ under study). With instrumental limits of detection were in the range of $ng\,L^{-1}$, as shown in Table 3. Limits of quantification, obtained after a pre-concentration factor of 250, were 40 $ng\,L^{-1}$. In all cases, the method guarantees the determination of the pesticides below the maximum concentration established by the European Union for pesticides in water samples. Table 4 summarises the results of instrumental precision of this method, which presented satisfactory values with all RSD values ranging between 0.9% and 7.3% for RSD_r and 1.5–13.4, for RSD_{ip}.

The advantages supplied by the LC system were strengthened with its coupling to a tandem quadrupole mass spectrometer, which provided enhanced selectivity and sensitivity. Fast mass spectral data acquisition was, however, required to allow a rapid switch among MRM acquisition channels and among positive and negative ionisation modes. Most of the compounds were analysed under the negative ionisation mode (ESI–) with the exception of propanil and clomazone which exhibited an increased response in the positive mode (ESI+). Two acquisition periods were set during the analysis, one (0.5–3 min retention window) for the herbicides propanil and clomazone analysed under ESI+ and the other one (1–3 min) for 2,4-D, bentazone and quinclorac (ESI−). The change of polarity during the acquisition method was performed without any damage in the intensity of the observable peaks. Characteristic source parameters were optimised to get one very intense precursor ion. The most important analyte-dependent parameter in this case is the cone voltage. Under the selected conditions, the quasimolecular ion, $[M+H]^+$ or $[M-H]^-$ was obtained for all herbicides. Suitable transitions from these precursor ions to product ions were automatically optimised by the instrument software. Table 1 shows the ions used for MRM and the optimised collision energy values. Triple quadrupole operating conditions were studied in order to work in the MRM mode. This study was performed by injecting every individual standard pesticide solution using the Daughter scan mode. For each compound, we selected the optimum collision energies

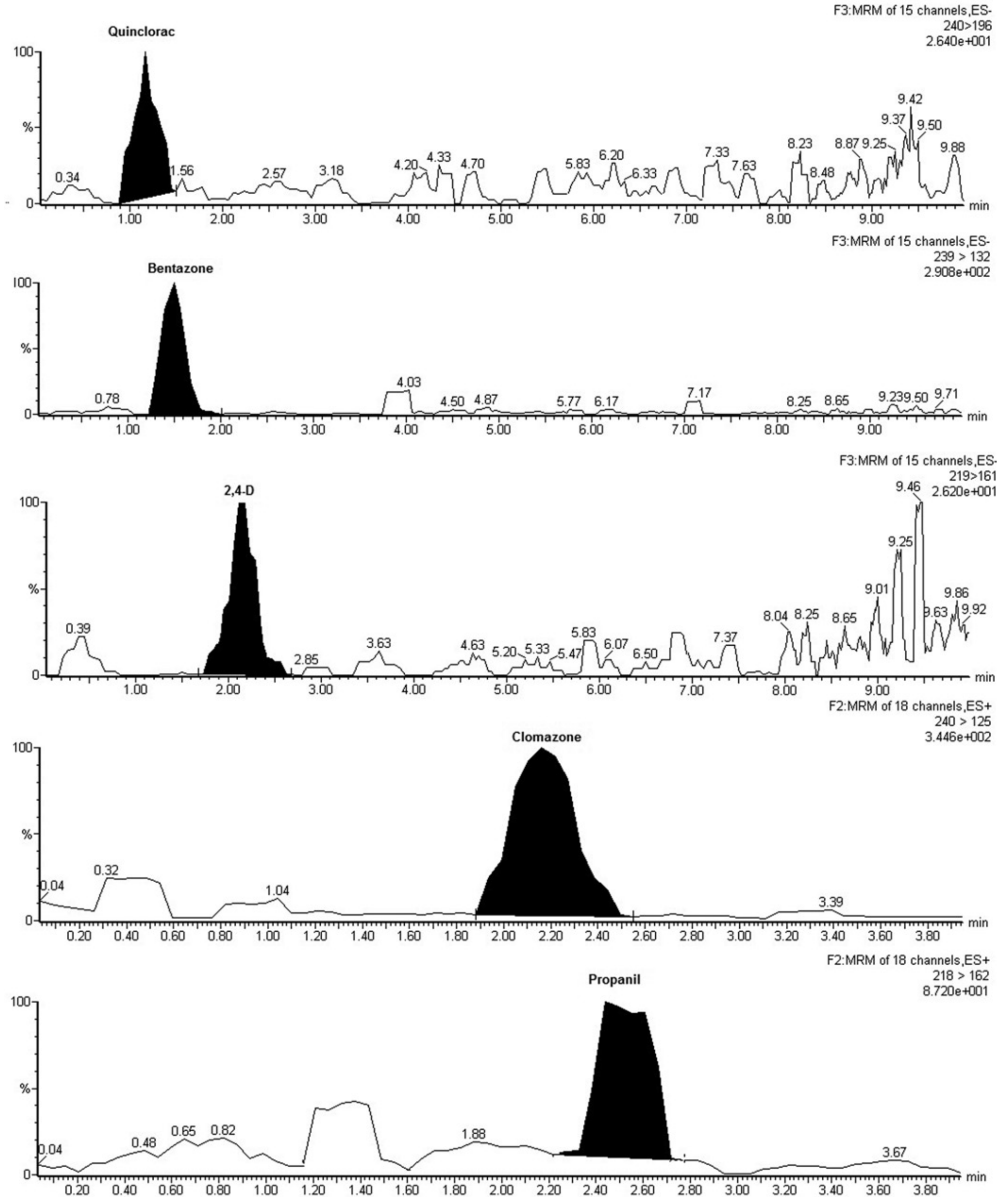

Figure 3. LC-MS/MS chromatogram obtained from surface water spiked with the herbicides at a level of 0.05 μg L^{-1} each.

aiming at getting two characteristic MRM transitions with the best signal intensity. The MRM transition with the highest signal intensity for quantification and the less intense one for the confirmation of the pesticide were chosen. The main advantage of the method was the use of MS/MS because it provides a high level of certainty to identify the target

Table 2. Results of recovery and RSD for LC-ESI-MS/MS method after the SPE step.

Herbicide	% Recovery (RSD%) 2.0 μg L^{-1}	 0.5 μg L^{-1}	 0.1 μg L^{-1}	 0.05 μg L^{-1}
Propanil	86.3 (7.2)	92.8 (5.5)	101.2 (4.0)	108.4 (4.9)
Clomazone	110.5 (5.2)	91.6 (7.7)	82.7 (7.8)	84.5 (2.1)
2,4-D	110.3 (7.8)	128.4 (4.3)	108.0 (3.4)	100.0 (11.6)
Bentazone	85.6 (2.7)	81.6 (8.5)	80.2 (7.8)	82.5 (3.0)
Quinclorac	84.1 (12.7)	87.4 (11.5)	94.2 (3.8)	98.1 (3.1)

Table 3. Validation data of the LC-ESI-MS/MS method.

Herbicides	r^2	t_R (mim)	Analytical curve	LOD (ng L^{-1})	Concentration range (μg L^{-1})
Propanil	0.9949	1.28	$y = 0.1985x + 1.7094$	2.0	10–500
Clomazone	0.9949	1.45	$y = 2.7137x + 14.88$	2.0	10–500
2,4-D	0.9971	2.12	$y = 0.2107x + 0.9742$	40.0	10–500
Bentazone	0.9960	2.14	$y = 3.5867x + 2.7891$	0.4	10–500
Quinclorac	0.9928	2.61	$y = 72.2876x + 0.1745$	40.0	10–500

Table 4. Results of repeatability (RSD_r) and intermediate precision (RSD_{ip}) for the LC-ESI-MS/MS method.

Herbicide	Standard concentration (μg L^{-1})	(%RSD_r)	(%RSD_{ip})
2,4-D	10.0	7.3	10.3
	25.0	4.2	6.1
	500.0	4.0	9.2
Bentazone	10.0	1.1	2.4
	25.0	1.4	5.9
	500.0	0.9	1.5
Quinclorac	10.0	6.7	13.4
	25.0	5.3	9.2
	500.0	2.5	6.6
Clomazone	10.0	2.5	5.9
	25.0	1.1	6.8
	500.0	1.1	4.1
Propanil	10.0	2.1	8.4
	25.0	1.1	4.5
	500.0	1.0	3.1

compounds. The mode MRM in MS/MS enables us to the select specific fragments of each compound, with the optimisation 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. A dwell time of 50 ms was selected in the method; however, it

Table 5. Results in $\mu g\,L^{-1}$ of the water samples collected in Spot 1 at the water supply system analysed by HPLC-DAD and confirmed by LC-MS/MS.

Period	Quinclorac	Bentazone	2,4-D	Clomazone	Propanil
March/2006	<LOQ	n.d.	n.d.	<LOQ	n.d.
April/2006	<LOQ	n.d.	n.d.	<LOQ	n.d.
May/2006	n.d.	n.d.	<LOQ	5.4	n.d.
June/2006	n.d.	n.d.	n.d.	n.d.	n.d.
July/2006	n.d.	n.d.	n.d.	n.d.	n.d.
August/2006	n.d.	<LOQ	n.d.	13.0	n.d.
September/2006	<LOQ	<LOQ	n.d.	12.3	n.d.
October/2006	<LOQ	n.d.	n.d.	14.2	n.d.
November/2006	<LOQ	<LOQ	n.d.	16.2	n.d.
December/2006	<LOQ	n.d.	n.d.	13.4	n.d.
January/2007	<LOQ	n.d.	n.d.	23.0	n.d.
February/2007	<LOQ	n.d.	n.d.	21.0	n.d.

Note: n.d. = not detected.

would still be possible to reduce this value if a higher number of transitions were included in the analysis.

The very narrow chromatographic peaks generated by LC-ESI-MS/MS, with peak width lower than 2 s, result in an increase of the chromatographic efficiency and sensitivity, with instrumental LODs in the range between 0.4 and $40\,ng\,L^{-1}$. Other performance characteristics of the analytical method that we have developed include good linearity, precision and selectivity. Therefore, this analytical LC-ESI-MS/MS approach can be applied to the analysis of these herbicides in water samples.

3.1.3 *Analysis of surface and drinking water using SPE, HPLC-DAD and LC-ESI-MS/MS*

The results of the analysis for herbicides, using HPLC-DAD with confirmation by LC-ESI-MS/MS, of the water samples from CORSAN shown in Table 5, have confirmed the presence of the herbicides quinclorac, bentazone, 2,4-D and clomazone (Figure 4). The pesticide clomazone was frequently detected in the São Gonçalo Channel water samples (Spot 1) exceeding the EC limit established for pesticides in surface waters that will be destined for human consumption: the limits are $1.0\,\mu g\,L^{-1}$ for individual pesticides. For drinking water for human consumption, the total pesticide levels did not exceed $0.1\,\mu g\,L^{-1}$ for individual pesticides and $0.5\,\mu g\,L^{-1}$ for the sum of all pesticides [10].

The results obtained for the samples collected in Spot 1, all herbicide concentrations are well below the established limit in the Brazilian legislation, and the herbicide clomazone was more present not only because it is used thoroughly in the area but also because of its high stability in water. Samples of Spot 2 showed no occurrence of the herbicides under investigation.

3.2 *Metals*

Table 6 presents the results for the sampling period and the Maximum Contaminant Level set by the Brazilian regulation for water that will be distributed to the population before

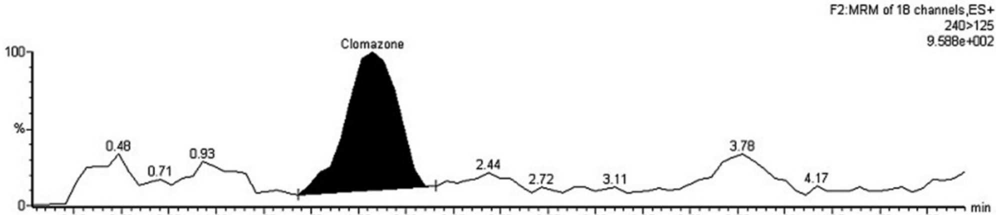

Figure 4. LC-MS/MS chromatogram of a sample containing the herbicide clomazone.

Table 6. Metal concentration ($\mu g\,L^{-1}$) of water samples before treatment.

Period	Fe	Mn	Zn	Cd	Pb	Al	Cr	Cu	Ni
May/2006	1117	43.4	4.8	0.35	5.4	119	0.9	1.0	0.5
Jun/2006	886	10.7	4.4	0.12	1.5	114	1.7	0.5	0.2
Jul/2006	2522	15.4	4.7	0.07	1.6	980	4.2	1.0	0.3
Aug/2006	2985	20.3	3.9	0.11	0.9	1030	5.7	0.6	0.2
Sep/2006	2149	14.6	5.1	0.21	0.9	482	3.5	0.4	0.3
Oct/2006	2537	13.6	4.5	0.08	1.0	918	4.8	0.5	0.2
Nov/2006	1787	13.9	3.8	0.05	1.1	540	3.3	0.6	0.3
MCL	1400	100	180	1	10	100	50	9	25

and after being treated. The results pointed out that the concentrations of iron and aluminum is above these limits in the samples collected before the treatment. The high concentration of these metals is not important because the flocculation and filtration will reduce them to an acceptable value, as shown in Table 7. Besides, the origin of these metals is natural and the concentration of other metals also shows there is no antropic influence.

The plant that treats the water comprises filtration, flocculation, sedimentation, chlorination and fluorination. During all these steps, metals can be separated due to the formation of insoluble hydroxides or flocculation and co-precipitation. Table 7 shows the metal concentration after the treatment and Brazilian Drinking Water Standards. During the sampling period, no concentration higher than the admissible values was observed, with the exception of lead in the three first samples. The high concentration of lead in the treated water samples has no equivalence in raw water samples. Therefore, it can be shown that some material used in the process was rich in lead, since the concentrations are lower and acceptable for the next samples.

The samples can be grouped in three categories; the first is composed by metals whose concentration is highly reduced after the treatment. This group includes iron, chromium, manganese and aluminum with mean reduction of 99.8, 90.4, 66.5 and 90.9%, respectively. The second group comprises zinc whose concentration is not substantially modified by the treatment (9.1%). The third group is composed by metals whose concentrations are higher in treated water than in raw water (cadmium: 176.5%; lead: 389.5%; copper: 215.2%).

The first group mainly contains metals that form insoluble hydroxides. The results pointed out that chromium is present in trivalent state; otherwise, it would not have had its concentration reduced. The manganese ion was probably present as bivalent, being

Table 7. Metal concentration (μg L^{-1}) in treated (drinking) water.

Period	Fe	Mn	Zn	Cd	Pb	Al	Cr	Cu	Ni
May/2006	2.7	4.4	4.7	0.84	17.2		0.2	0.6	0.3
Jun/2006	1.5	2.9	3.8	0.30	13.6	16	0.1	5.1	0.2
Jul/2006	10.2	7.7	4.8	0.14	14.6	72	0.2	0.2	<0.1
Aug/2006	6.5	4.7	2.9	0.09	1.2	68	0.6	0.7	0.1
Sep/2006	6.0	4.3	3.8	0.14	0.8	149	<0.1	0.6	0.2
Oct/2006	3.5	7.0	4.5	0.13	0.4	43	<0.1	0.6	0.2
Nov/2006	1.3	5.8	3.7	0.09	0.5	3	0.2	2.1	0.2
MCL	300	100	5000	5	10	200	50	200	*

*MCL for Ni is not defined in the Brazilian Regulations.

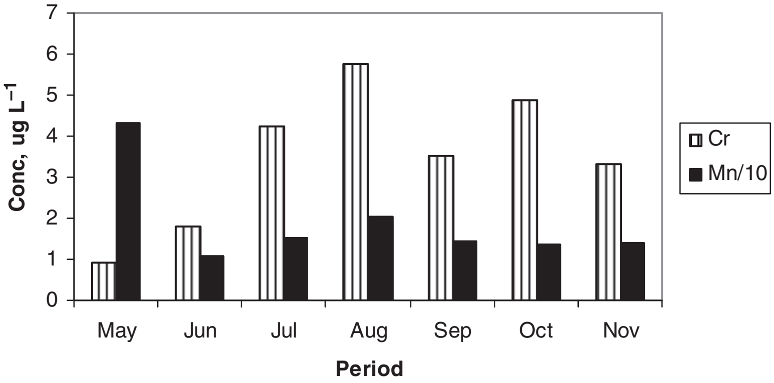

Figure 5. Pattern of metal distribution in untreated water.

oxidised during the treatment process to Mn (IV) and precipitating as MnO_2. The concentration increase observed in the third group is probably related to the chemical reagents used during the treatment.

The content of iron, aluminum and chromium in untreated water follows a normal distribution, with maximum concentration in August when it is winter and when it rains more in Brazil, while cadmium and manganese have similar distribution, but with maximum concentration in May. The distribution of the other metals does not follow a predictable pattern. Figure 5 shows the distribution of chromium and manganese.

4. Conclusion

The methods we propose employ SPE, HPLC-DAD and LC-ESI-MS/MS and are rapid, sensitivite and selective with a simple sample preparation procedure that can be used for the convenient and effective determination of herbicide residues in surface and drinking water samples. The results obtained during the validation step show that this is a rapid and efficient method for the quantification of herbicides in surface water samples. These characteristics make the methods attractive for regular monitoring and water quality control when herbicides may be present, thus assuring the quality of the water for human consumption. Regarding sensitivity, it may be observed that both methods SPE, HPLC-DAD and HPLC-ESI-MS/MS allowed the determination of the herbicides at concentrations below $0.1\,\mu g\,L^{-1}$, the limit established by the European legislation for individual pesticides in drinking water. In the light of the foregoing, we consider that both methods would be suitable for the simultaneous determination of herbicides in natural waters.

The contamination of the water of the São Gonçalo Channel by clomazone exceeded $1\,\mu g\,L^{-1}$ in more than 50% of the samples under analysis. In the case of the other herbicides, the concentration in the samples was below the method LOQ. No significant levels of herbicides were detected in drinking water.

Regarding the metal content, it should be emphasised that the quality of the water distributed in the community is satisfactory and that the treatment reduces the level of the metals naturally present in the water even when the concentration increases during the rainy season. The exceptions are copper and cadmium. No significant distribution pattern of metals was observed in untreated water.

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