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Article

Development of a Multiresidue Method for Pesticide Analysis in Drinking Water by Solid Phase Extraction and Determination by Gas and Liquid Chromatography with Triple Quadrupole Tandem Mass Spectrometry

Filipe F. Donato, Manoel L. Martins, Juliana S. Munaretto, Osmar D. Prestes, Martha B. Adaime and Renato Zanella*

Laboratório de Análises de Resíduos de Pesticidas (LARP), Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria-RS, Brazil

In this work, a method for multiclass determination of 70 pesticides residues with different properties in drinking water using solid phase extraction (SPE) with polymeric sorbent and determination by gas and liquid chromatography coupled to tandem mass spectrometry (GC-MS/MS and LC-MS/MS) was developed and validated. Different sample volumes, sorbents and elution solvents were evaluated. The best results were obtained using the sorbent Oasis® HLB, sample acidified at pH 2.5 and a mixture of dichloromethane/methanol as eluent. The limit of quantification (LOD) of the method was 0.02 μg L^{-1} for aldrin, dieldrin and chlordane and 0.5 μg L^{-1} for the other compounds. Satisfactory accuracy, with recoveries between 70 and 117.3%, and good precision, with relative standard deviation (RSD) values below 19.7% for most of the compounds, were achieved. The validated method was applied to real water samples and results indicated that the proposed method is suitable for the determination of pesticide residues in water samples.

Keywords: pesticides, solid phase extraction, LC-MS/MS, GC-MS/MS, water analysis

Introduction

The application of pesticides in agriculture has been associated with effective control of pests, diseases and weeds in order to increase food production.[1] However, because of the massive and often incorrect use of these compounds, they are related to environmental damage, especially as source of the contamination of surface water and groundwater due to runoff or leaching from agricultural landscapes.[2] Due the pesticides potential risk of toxicity to human health, persistence, and tendency to bioaccumulation, much efforts have been made for the determination of pesticide residues in environmental samples. As a result, different international legislations such as the European Union (EU),[3] United States Environmental Protection Agency (US EPA)[4] and World Health Organization (WHO)[5] established maximum allowed concentrations for pesticides in drinking water. In 2011, the Brazilian Ministry of Health enacted Ordinance 2914, which sets the procedures for control and surveillance the drinking water potability parameters.[6] Among other parameters, this legislation set the maximum limits of pesticide residues permitted in water for human consumption. Considering the importance of the monitoring the presence of pesticide residues at low levels in drinking water, reliable methods with high detectability, selectivity, confidentiality and speed are still required.

The complexity of water samples combined with the low concentration levels requires an efficient sample preparation step before their instrument determination. In this regard, sample preparation techniques such as liquid-liquid extraction and solid phase extraction (SPE) techniques are commonly used. In the past decade, different miniaturized sample preparation and concentration techniques have been developed and successfully applied for the analysis of pesticides from different aqueous samples. These methods have several merits such as easy to perform, fast analysis time, high enrichment factor, low cost of analysis and use of small volume of organic solvents. Otherwise, these techniques are developed for limited number of compounds. SPE is the most applied sample preparation technique for pesticide multiresidue analysis in water. This technique involves different retention mechanisms (adsorption, partition, ionic exchange, etc.) and has been extensively used to remove or concentrate trace organic compounds from liquid samples. The sample matrix can also affect the ability of the sorbent to retain the

*e-mail: rzanella@base.ufsm.br

analyte due the competition for retention. Many sorbents (e.g., C_{18}) are limited in terms of selectivity, being a difficult task due to the different pesticides physicochemical properties. Furthermore, insufficient retention of very polar compounds (e.g., herbicides) can also be a problem.[7] The SPE major advantages are the high percentage of recovery, good robustness, and concentrate analytes for better sensitivity.[8-11]

The quantitative determination of pesticides at trace levels environmental was frequently performed utilizing sensitive analytical methods including liquid chromatography (LC) and gas chromatography (GC) with various detectors. These techniques have shown different advantages as good selectivity, suitable separation of a large number of compounds, etc. GC and LC coupled with mass spectrometry increased selectivity with compound structural information.[12] In the last years, several applications of pesticide multiresidue analysis by SPE and GC-MS/MS or LC-MS/MS have been reported. In 2010, Dujaković *et al.*[13] developed a multiclass method for the determination of 14 pesticides using Oasis HLB® cartridges and LC-MS/MS. Donato *et al.*[14] reported a multiclass method for the determination of 81 pesticides by LC-MS/MS in water using Strata™-X sorbent. Different authors published applications with LC-MS/MS and polymeric sorbents for pesticide multiresidue analysis in water.[15] However, Cahill *et al.*,[16] Caldas *et al.*[17] and Demoliner *et al.*[18] used C_{18} as extraction sorbent for SPE. GC-MS was applied to evaluate the environmental impact of eight pesticides on the surface water and groundwater from agricultural areas.[19]

However, there are few studies using a simple SPE procedure to extract a great number of different chemical classes of pesticides in trace level in water samples and determination with a short run by both GC-MS/MS and LC-MS/MS instruments.[20-22] Therefore, considering the importance of monitoring programs to protect human health, the aim of this study was to develop and validate a sensitive and efficient analytical method for the determination of 70 pesticide residues, with different physicochemical properties, in drinking water. The SPE was applied for sample preparation and the determination was performed by GC-MS/MS and LC-MS/MS in order to attend the legislation for drinking water.

Experimental

Chemicals and reagents

All standards, including triphenylphosphate (internal standard, IS) were purchased from Dr. Ehrenstorfer (Augsburg, Germany), with the highest available purity. The surrogate standards (SS) trifluralin-d14 and linuron-d6 were purchased from CND Isotopes (Quebec, Canada). Solvents as acetonitrile (MeCN), methanol (MeOH), acetone (ACE) and dichloromethane (DCM), HPLC grade, were purchased from J. T. Baker (Xalostoc, Mexico). Ultrapure water was obtained with a Milli-Q Direct UV3® system from Millipore (Molsheim, France). Vortex mixer model QL-901 was acquired from Microtécnica (Curitiba, Brazil). The polymeric SPE sorbent cartridges Strata™ C_{18} (500 mg; 3 mL) and Strata™-X (200 mg; 3 mL) were purchased from Phenomenex (Torrance, USA) and Oasis® HLB (60 mg; 3 mL) was from Waters (Wexford, Ireland).

Instrumentation

Samples were analyzed using GC-MS/MS system with a gas chromatograph model CP 3800 coupled to a triple quadrupole mass spectrometer MS 1200 equipped with the autosampler CP8400 and injector 1079 with programmable temperature vaporizer (PTV). The LC-MS/MS system was equipped with a liquid chromatograph, binary pump system 212 LC, coupled to triple quadrupole mass spectrometer 320MS with autosampler ProStar 410 and column oven. Both systems are from Varian (Walnut Creek, USA) with data acquisition Workstation 6.6 software. A nitrogen generator system LC/MS 12/2 from Domnick Hunter (Gateshead, England) was used.

GC-MS/MS and LC-MS/MS conditions

GC-MS/MS system was operated with a capillary column VF-5-MS (5% phenyl and 95% dimethylpolysiloxane), with 30 m × 0.25 mm (i.d.) and 0.25 μm of film thickness. The temperature program of the column oven was initially 45 °C (1.0 min) and then was increased at 30 °C min^{-1} to 280 °C and maintained for 4.1 min. Finally, the temperature was increased at 20 °C min^{-1} to 300 °C resulting in a total run time of 15 min. Transfer line temperature was set at 250 °C, ion source at 210 °C with electron ionization (EI) at 70 eV. Helium was used as carrier gas at 1 mL min^{-1} and argon as collision gas (2 mTorr). Injection volume was 2 μL (splitless mode) with the injector at 280 °C. For identification of possible interferences in the extract, which could affect or not the analysis or even increase the maintenance of the instrument it was used full scan analysis in a *m/z* range from 50 to 500.

The LC-MS/MS system was composed by analytical column UPS Pursuit C_{18} with 50 × 3.0 mm (i.d.) and 2.4 μm of particle size. The mobile phase was 5 mmol L^{-1} ammonium formate aqueous solution (solvent A) and methanol (solvent B) at a flow rate of 150 μL min^{-1}, resulting

in a total run time of 15 min. The gradient was performed from 0 to 3 min 90% (solvent A) decreasing to 50% (solvent A) at 4 min; to 5% (solvent A) at 8 min and to 2% (solvent A) at 11 min until 13 min, returning to the initial condition until 15 min. The injection volume was 10 μL and the source was operated on electrospray ionization (ESI) mode. Infusions of each standard solution at 1.0 mg L^{-1} were performed in order to optimize the conditions of the mass spectrometer. The mass spectrometer source operate at 50 °C with desolvation gas temperature (N_2, 40 psi) at 250 °C and N_2 as drying gas (20 psi). Argon was used as collision gas at 1.8 mTorr.

Preparation of standard solutions

Initially, 10.0 mL of a stock of each pesticide solution at 1000 mg L^{-1} were prepared in MeOH or MeCN according to their solubility and considering the purity of the standards. Carbendazim is not completely soluble in pure solvents, so it was dissolved in acetonitrile containing 8% (v/v) aqueous HCl 0.1 mol L^{-1}. These stock solutions were stored in amber flask at −18 °C. From these, two working solutions at 10 mg L^{-1} were prepared, including the surrogate standards. One was prepared in MeOH for compounds analyzed by LC-MS/MS and the other in MeCN for the compounds analyzed by GC-MS/MS. These mixtures were used to spike the blank samples during the method development. The linearity of the analytical curves was evaluated from analytical solutions containing all the selected pesticides at the levels 10, 25, 50, 75, 100, 200 and 250 μg L^{-1} for both systems.

Sample preparation

The SPE method was validated using tap water. Samples (500 mL) were filtered with polytetrafluoroethylene (PTFE) membrane (47 mm and 0.45 μm porosity, from Agilent Technologies, Santa Clara, USA) and then the selected volume was transferred to the SPE cartridges through PTFE tubes in a manifold. The adopted percolation flow rate ranged from 2 to 5 mL min^{-1}. After the sample percolation, 3 mL of purified water was passed through the cartridge.

Considering that, in general, the concentration of pesticides in different types of water (river, groundwater and drinking water) is low, a concentration step to quantify these compounds is necessary. Due to it, volume of the samples were studied (100 mL and 250 mL). In an initial evaluation of the SPE procedure, three types of cartridges available commercially were selected: Strata™-X 200 mg, Strata™ C_{18} 500 mg and Oasis® HLB 60 mg. Blank samples were spiked in triplicate at a concentration of 1 μg L^{-1}. The best sorbent was selected based on factors such as percentage of recovery, precision, need of pH adjustment and drying time. The elution solvent should allow an efficient elution of the analytes from the cartridge, keeping the interferences retained in it. The suitability of the eluent is directly related to the polarity of the compounds to be extracted.[23] Acetonitrile, acetone and dichloromethane, as well as the mixture of these solvents were tested.

Method validation

The proposed method was validated by evaluating different parameters as linearity, matrix effect, limit of detection (LOD), limit of quantification (LOQ), accuracy (in terms of recovery) and precision (in terms of repeatability and intermediate precision). All validation parameters evaluated were in accordance with international regulations for pesticide residue analysis by chromatographic analysis.[24]

The linearity was evaluated through the coefficient of determination (r^2) of the analytical curves at the concentration levels 10, 25, 50, 75, 100, 200 and 250 μg L^{-1}. These concentration levels were used in both techniques, GC-MS/MS and LC-MS/MS. The solutions for the analytical curves were prepared with dichloromethane:methanol (1:1, v/v) for GC-MS/MS and with mobile phase 5 mmol L^{-1} ammonium formate aqueous solution:methanol (1:1, v/v) for LC-MS/MS. Matrix effect was calculated comparing the slope of curves prepared in solvent and in the blank extract.[25]

Accuracy was evaluated through recovery assays at three different concentration levels (0.5, 1.5 and 4.0 μg L^{-1}). Precision was evaluated regarding repeatability and intermediate precision by estimating the relative standard deviation (RSD) of the recovery percentage for each spiked level. For compounds dieldrin, aldrin and chlordane, besides these three spike levels, it was performed an extra level at 0.02 μg L^{-1} in consideration to the lower limits for these compounds established by the Brazilian legislation for drinking water. Six replicates of each concentration level were extracted and injected once in the chromatographic system.

The LOD and LOQ were estimated using the method of signal-to-noise ratio, and the LOD was defined as the lowest concentration at which the analytical signal could be reliably differentiated with a signal-to-noise ratio of 3:1. The LOQ was established as the lowest spiked level concentration, which produced a signal-to-noise ratio of 10:1 with acceptable recovery and precision according to legislation.[24]

Results and Discussion

Chromatographic determination by LC-MS/MS and GC-MS/MS

The optimized conditions for LC-MS/MS and GC-MS/MS instruments are available in the Supplementary Information. For the select reaction monitoring (SRM), the two most intense transitions were selected for each compound. The most intense transition used for quantification and the other one for confirmation of the analyte. The compounds 2,4-D, pendimethalin and tetraconazole shown only one transition and, therefore, these compounds were analyzed only for screening purpose. Collision energy in LC-MS/MS was optimized in order to increase the peak signal and improve the limits of detection and quantification. Both instruments allowed a fast analysis with total run time of 15 min each. The selected reaction monitoring (SRM) chromatograms obtained by GC-MS/MS and LC-MS/MS of the pesticides mixed standard solutions are presented in Figure 1.

Optimization of the SPE method

The optimization of the sample preparation is an important process to achieve greater efficiency in the

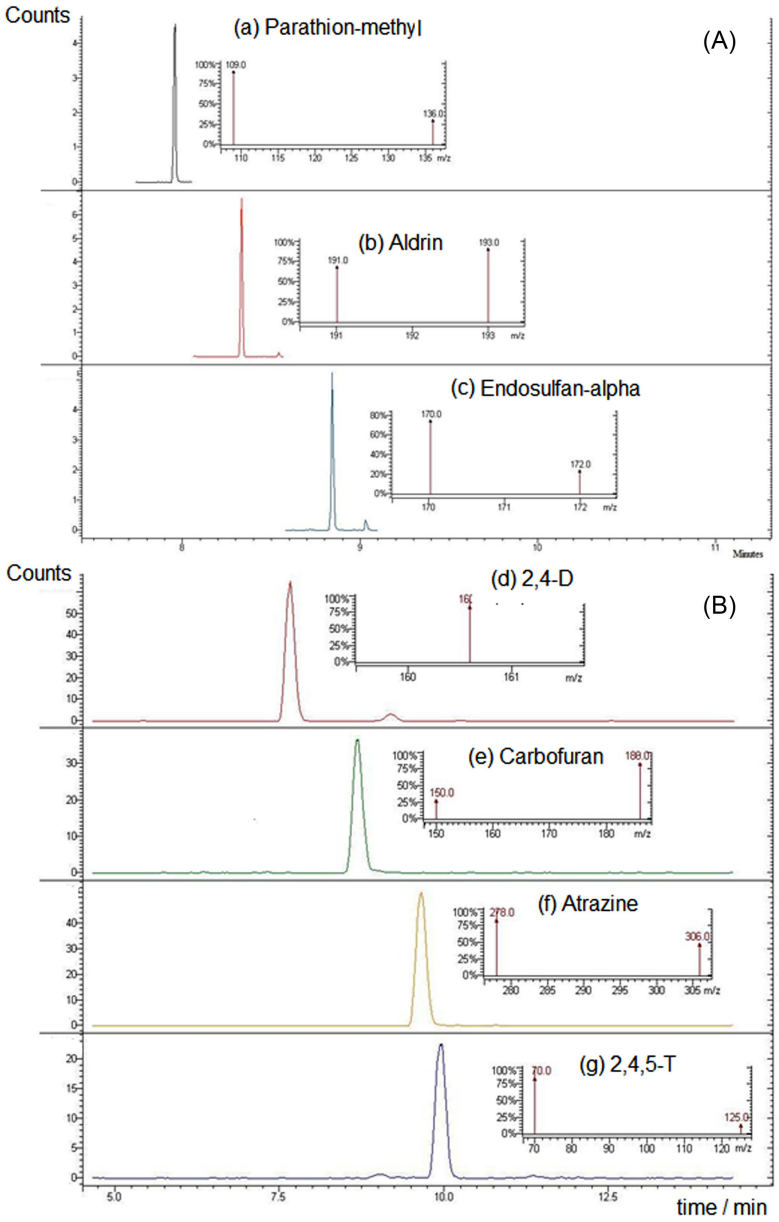

Figure 1. Selected reaction monitoring (SRM) chromatograms and selected ions obtained from a pesticide mixture standard at 100 μg L^{-1} by GC-MS/MS in (A). Legend: (a) parathion-methyl; (b) aldrin; (c) endosulfan-alpha and by LC-MS/MS in (B). Legend: (d) 2,4-D; (e) carbofuran; (f) atrazine and (g) 2,4,5-T.

extraction of pesticides from water samples in order to obtaining better recovery. To choose the best conditions for the SPE, it was attempted to choose a unified SPE procedure, where pesticides with different properties, such as polar and nonpolar compounds could be extracted at the same time. Sample preparation was optimized in terms of sorbent type; combination with different elution solvents; sample pH adjustment prior to extraction and sample volumes.

Figure 2 shows the difference in recovery between the cartridges Strata™-X (200 mg), Strata™ C_{18} (500 mg) and Oasis® HLB (60 mg), using a sample volume of 250 mL with and without acidification. The elution was performed using 5 mL of a 1:1 (v/v) methanol:acetonitrile acidified with 1% (v/v) acetic acid solution according to Donato *et al.*[14]

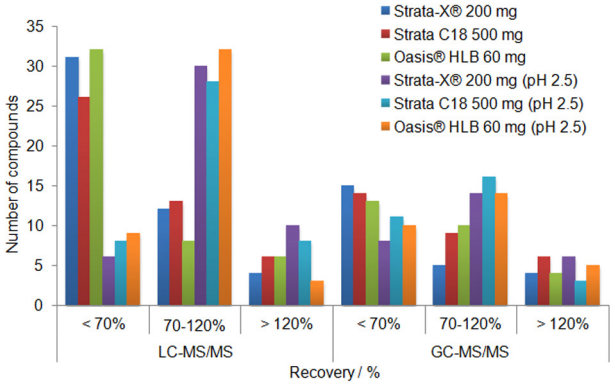

Figure 2. Evaluation based on pesticide recoveries obtained with different SPE sorbents by LC-MS/MS and GC-MS/MS analysis and the influence of the addition of acetic acid in elution solvent.

The results show that a great number of pesticides were better extracted when the sample is acidified at pH 2.5. The need of acidification can be attributed to the low values for the acid dissociation constant (pKa) of several pesticides selected for this study.

When comparing the different types of sorbents evaluated it was observed that the polymeric sorbents (Strata™-X and Oasis® HLB) performed better recovery results. The polymeric sorbents have a modified surface with divinylbenzene (non-polar) and an *N*-vinylpyrrolidone (polar), which facilitates extraction of pesticides of different polarities in a single extraction step. These sorbents are recommended for extraction of acidic, basic and neutral compounds with medium to high polarity.[26]

The best results during evaluation of the polymeric cartridges were obtained with 100 mL of sample (pH 2.5) and 5 mL (2 × 2.5 mL) of dichloromethane:methanol (1:1) as elution solvent. Oasis® HLB 60 mg cartridge presented better results (Figure 3) with a large number of compounds extracted with good recovery and, therefore, this sorbent was chosen for the optimization.

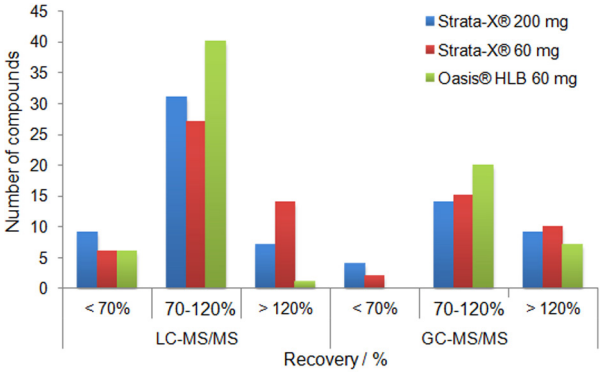

Figure 3. Comparison of different polymeric SPE cartridges based on pesticide recoveries obtained by LC-MS/MS and GC-MS/MS analysis.

It was observed that a reduction in the volume of sample used for extraction could be performed without compromising the LOD. Thus, the loss of polar compounds, adsorbed on the extraction, could be avoided by percolation of a large volume of sample through the cartridge, besides, the possible saturation of the cartridge.[27] For the injections in the LC, 1 mL of the eluate was evaporated and redissolved with 1 mL of mobile phase, only to make the exchange of solvents, due to the incompatibility of dichloromethane with the reversed-phase LC.

The mixture of dichloromethane:methanol was more effective due to the different characteristics of the analyzed compounds. As dichloromethane is a nonpolar solvent, the extraction of compounds with similar polarity to this solvent was favored, while the methanol favored the extraction of compounds with medium to high polarity. The use of this solvent combination was essential for the extraction of a large number of compounds from SPE cartridge. Therefore, the optimized SPE extraction procedure using the polymeric cartridge Oasis® HLB was established as shown in Figure 4.

Samples were pre-filtered through a 0.45 µm nylon membrane. The cartridge was conditioned in sequence with 3 mL of methanol, 3 mL of ultrapure water and 3 mL of ultrapure water with pH adjusted at 2.5. The sample volume used was 100 mL, acidified to pH 2.5 with aqueous phosphoric acid (1:1, v/v). For the elution of the pesticides from the cartridge, aliquots of 2 × 500 µL of dichloromethane followed by 2 × 500 µL of methanol was used to provide a higher concentration factor than using 5 mL of the mixture 1:1 (v/v). An aliquot of 1 mL of the eluate was evaporated under gentle stream of nitrogen at room temperature and redissolved with 1 mL of mobile

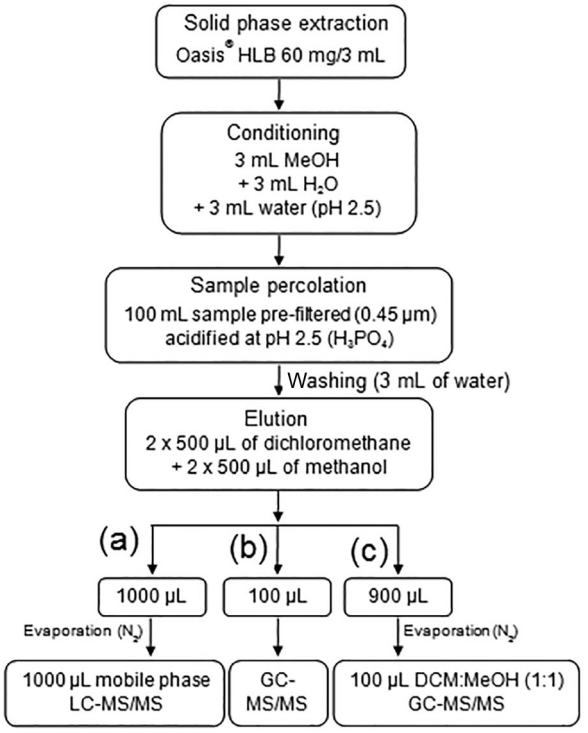

Figure 4. Schematic representation of the solid phase extraction (SPE) approach applied for pesticide multiresidue determination in water by LC-MS/MS in (a) and GC-MS/MS in (b). The procedure (c) was used for determination of aldrin, dieldrin and chlordane.

phase for the determination by LC-MS/MS, according to procedure of the Figure 4a. From the remained eluate, 100 µL were transferred to insert and analyzed by GC-MS/MS, as shown in the procedure Figure 4b. The remained 900 µL was evaporated under a gentle stream of nitrogen at room temperature and redissolved in 100 µL of dichloromethane:methanol (1:1, v/v), according to procedure described in Figure 4c for the determination of aldrin, chlordane and dieldrin by GC-MS/MS. A concentration factor of 450 times was reached with the procedure in Figure 4c, in order to achieve the low limits required by the legislation, which are 0.03 µg L^{-1} for aldrin and dieldrin and 0.2 µg L^{-1} for chlordane. The procedures of Figure 4a and Figure 4b provided a concentration factor of 50 times, adequate for most of the pesticides analyzed.

Method validation

The selectivity of the method was ensured in both GC and LC analyses, as no interference peaks were detected on blank samples. The analytical curves presented good linearity with r^2 higher than 0.99 for all the compounds studied. The instrumental limit of detection (LOD) and limit of quantification (LOQ) were 7.5 and 25 µg L^{-1}, respectively.

Compounds extracted with the procedures a and b (Figure 4) showed limit of detection (LOD) and limit of quantification (LOQ) of 0.15 and 0.5 µg L^{-1}, respectively. The compounds aldrin, dieldrin and chlordane (procedure c, Figure 4) presented LOD and LOQ values of 0.006 and 0.02 µg L^{-1}, respectively. These method limits were satisfactory since they are lower than those established by the Brazilian legislation (Ordinance 2914).[6]

The results of accuracy and precision were evaluated through recovery tests and are showed in Table 1. It can be observed that 89% of the compounds showed recovery between 70 and 117.3% with satisfactory precision since the RSD values were lower than 19.7%. The recovery and RSD results obtained for aldrin, dieldrin and chlordane at the extra concentration level (0.02 µg L^{-1}) were 52.5 and 9.6%, 88.5 and 1.4%, 118.3 and 19.5%, respectively. It is noticed that only aldrin presented recovery below the acceptable range (70 to 120%), however, considering the very low concentration level and the good RSD (< 10%), these recovery values are acceptable and the LOQ for aldrin was established also as 0.02 µg L^{-1}.

Among the pesticides that have not presented recovery between 70 and 120% six compounds are determined by LC and two by GC. The compounds 2,4-DDD and 2,4-DDE analyzed by GC showed recoveries between 53 and 61% in the three spike levels evaluated. However, they exhibited satisfactory precision (< 10%) and so were included in the proposed method.

The compounds determined by LC, methamidophos, thiophanate-methyl, benomyl, terbufos, aldicarb and benfuracarb, showed recovery problems, so they could not be quantified by this method due to the unsatisfactory accuracy and precision results. The inconsistent results for methamidophos can be explained by it high polar characteristic, presenting high solubility in water (log K_{ow}: −0.8), making the extraction from aqueous matrices extremely difficult. According to Geib and Gebert,[28] the extraction efficiency using materials derived from silica (using SPE) is strongly dependent on the log K_{ow}. Thus, only compounds with log K_{ow} up to zero may be efficiently extracted. By the evaluation of twenty-one different SPE sorbents for the extraction of highly polar substances, including methamidophos, authors have found that sorbents containing octadecylsilane (C_{18}) were not adequate to extract methamidophos. Polymeric sorbents showed maximum recoveries of 60%. In 2006, Liu *et al.*[29] conducted a study with some polar pesticides, including methamidophos, in order to compare the efficiency of liquid-liquid extraction and solid phase extraction with Oasis HLB and Chromabond HR-P sorbents and 500 mL of spiked sample at 0.5 µg L^{-1}. Recovery results were 4.6% for

Table 1. Recovery and relative standard deviation (RSD) results for the repeatability of the proposed method for the determination of 70 pesticides in water samples

Compound	Method	Maximum permitted limit / (μg L^{-1})	Spike level / (μg L^{-1})			Intermediate precision, n = 6 Recovery ± RSD_r^k / %	Matrix effect / %
			0.5	1.5	4.0		
			Recovery ± RSD_r^k / %	Recovery ± RSD_r^k / %	Recovery ± RSD_r^k / %		
2,4-D	LC[a]	30[d]	95 ± 6	90 ± 2	81 ± 6	82 ± 6	18.3
2,4-DDD	GC[b]	1[e]	59 ± 9	61 ± 5	60 ± 5	63 ± 7	15.2
2,4-DDE	GC[b]	1[e]	55 ± 10	53 ± 3	58 ± 4	65 ± 6	4.0
2,4,5-T	LC[a]	30[d]	108 ± 14	94 ± 5	81 ± 5	88 ± 9	20.7
Alachlor	GC[b]	20	87 ± 11	82 ± 3	79 ± 4	90 ± 3	19.2
Aldicarb sulfone	LC[a]	10[f]	95 ± 6	93 ± 8	89 ± 6	70 ± 8	14.0
Aldicarb sulfoxide	LC[a]	10[f]	88 ± 1	73 ± 2	97 ± 5	94 ± 5	3.5
Aldicarb	LC[a]	10[f]	–	–	–	–	–8.1
Aldrin	GC[b]	0.03[g]	58.0 ± 6	75 ± 5	86 ± 2	76 ± 7	3.6
Atrazine	LC[a]	2	97 ± 12	104 ± 3	82 ± 7	86 ± 7	9.5
Azinssulfuron	LC[a]	–	84 ± 15	87 ± 11	82 ± 5	90 ± 6	17.5
Azoxystrobin	LC[a]	–	87 ± 9	95 ± 3	84 ± 8	87 ± 4	7.8
Benfuracarb	LC[a]	–	–	–	–	–	45.2
Benomyl	LC[a]	120[h]	–	–	–	–	–34.5
Bentazone	LC[a]	–	73 ± 6	111 ± 1	81 ± 6	88 ± 4	13.3
Bifenthrin	GC[b]	–	73 ± 10	85 ± 2	103 ± 1	82 ± 8	2.4
Bispyribac sodium	LC[a]	–	112 ± 13	89 ± 16	90 ± 10	89 ± 13	25.6
Carbaryl	LC[a]	–	86 ± 9	104 ± 5	84 ± 10	89 ± 3	18.1
Carbendazin	LC[a]	120[h]	85 ± 5	70 ± 3	79 ± 10	77 ± 10	7.1
Carbofuran	LC[a]	7	90 ± 10	103 ± 3	92 ± 8	87 ± 7	7.4
Cyhalofop-butyl	LC[a]	–	101 ± 19	70 ± 19	73 ± 20	81 ± 13	27.2
Cyhalothrin-lambda	GC[b]	–	77 ± 9	86 ± 2	98 ± 4	96 ± 7	60.9
Cyfluthrin	GC[b]	–	75 ± 6	86 ± 5	117 ± 3	97 ± 10	54.8
Cypermethrin	GC[b]	–	74 ± 5	83 ± 5	115 ± 3	95 ± 10	52.7
Clomazone	LC[a]	–	97 ± 8	99 ± 7	87 ± 9	81 ± 8	–1.0
Chlordane	GC[b]	0.2	75 ± 19	82 ± 8	88 ± 4	74 ± 9	26.8
Chlorpyrifos-ethyl	LC[a]	30[i]	82 ± 10	88 ± 4	72 ± 8	72 ± 5	31.1
Chlorpyrifos-methyl	LC[a]	–	107 ± 13	85 ± 7	79 ± 6	95 ± 15	–0.5
Chlorpyrifos oxon	LC[a]	30[i]	97 ± 6	98 ± 9	83 ± 18	91 ± 3	4.1
DDT	GC[b]	1[e]	85 ± 10	92 ± 3	98 ± 4	81 ± 8	50.5
Deltamethrin	GC[b]	–	92 ± 6	74 ± 5	86 ± 6	102 ± 9	63.3
Dieldrin	GC[b]	0.03[g]	72 ± 12	90 ± 5	91 ± 6	76 ± 7	4.7
Difenoconazole	LC[a]	–	92 ± 3	88 ± 6	75 ± 7	86 ± 6	19.5
Diuron	LC[a]	90	88 ± 7	99 ± 9	79 ± 10	82 ± 5	2.6
Endosulfan-alpha	GC[b]	20[j]	76 ± 12	81 ± 5	84 ± 5	83 ± 11	17.2
Endosulfan-beta	GC[b]	20[j]	72 ± 12	85 ± 6	86 ± 5	78 ± 8	18.4
Endosulfan-sulfate	GC[b]	20[j]	91 ± 9	77 ± 2	87 ± 4	106 ± 9	39.4
Endrin	GC[b]	0.6	89 ± 12	96 ± 5	83 ± 4	92 ± 10	32.6
Etoxissulfuron	LC[a]	–	95 ± 6	92 ± 3	94 ± 11	92 ± 1	4.8
Fenitrothion	GC[b]	–	85 ± 3	93 ± 4	107 ± 5	96 ± 6	43.9
Fipronil	LC[a]	–	78 ± 6	103 ± 5	79 ± 6	87 ± 3	19.3
Imazapic	LC[a]	–	87 ± 8	100 ± 5	86 ± 10	92 ± 6	16.3
Imazapyr	LC[a]	–	76 ± 4	105 ± 9	84 ± 14	97 ± 6	16.4
Imazethapyr	LC[a]	–	104 ± 14	96 ± 5	82 ± 6	96 ± 9	7.1
Imidacloprid	LC[a]	–	92 ± 7	97 ± 7	87 ± 7	87 ± 6	4.9
Lindane (gama HCH)	GC[b]	2	80 ±8	84 ± 9	89 ± 10	82 ± 8	16.5
Linuron d-6[c]	LC[a]	–	98 ± 7	96 ± 10	93 ± 10	97 ± 6	5.7
Malathion	LC[a]	–	102 ± 10	101 ± 7	80 ± 10	87 ± 4	15.2

Table 1. Recovery and relative standard deviation (RSD) results for the repeatability of the proposed method for the determination of 70 pesticides in water samples (cont.)

Compound	Method	Maximum permitted limit / (μg L^{-1})	Spike level / (μg L^{-1})			Intermediate precision, n = 6 Recovery ± RSD_r^k / %	Matrix effect / %
			0.5	1.5	4.0		
			Recovery ± RSD_r^k / %	Recovery ± RSD_r^k / %	Recovery ± RSD_r^k / %		
Methamidophos	LC[a]	12	35 ± 65	12 ± 49	26 ± 35	25 ± 80	11.3
Metolachlor	GC[b]	10	113 ± 6	114 ± 7	94 ± 7	119 ± 14	20.0
Metsulfurom-methyl	LC[a]	–	89 ± 12	91 ± 9	95 ± 11	86 ± 6	–2.7
Molinate	LC[a]	6	77 ± 18	72 ± 9	79 ± 7	78 ± 14	–2.8
Oxifluorfen	GC[b]	–	82 ± 9	89 ± 6	103 ± 5	79 ± 6	36.6
Parathion-methyl	GC[b]	9	74 ± 8	79 ± 3	115 ± 4	96 ± 4	38.1
Pendimethalin	LC[a]	20	79 ± 12	101 ± 10	70 ± 8	94 ± 18	26.0
Permethrin	GC[b]	20	80 ± 7	76 ± 5	80 ± 4	91 ± 6	49.8
Pyrazossulfuron	LC[a]	–	95 ± 17	93 ± 8	81 ± 9	94 ± 10	16.7
Profenofos	LC[a]	60	83 ± 17	88 ± 5	104 ± 8	83 ± 7	–13.2
Propanil	LC[a]	–	102 ± 8	112 ± 1	102 ± 12	92 ± 11	–9.4
Propiconazole	LC[a]	–	78 ± 8	96 ± 4	84 ± 7	92 ± 2	4.5
Quincloraque	LC[a]	–	83 ± 10	100 ± 7	83 ± 8	93 ± 1	2.4
Simazine	LC[a]	2	94 ± 4	99 ± 2	86 ± 6	89 ± 6	12.8
Tebuconazole	LC[a]	180	91 ± 9	106 ± 6	83 ± 5	92 ± 2	17.3
Terbufos	LC[a]	1.2	05 ± 31	03 ± 38	04 ± 40	03 ± 32	13.8
Tetraconazol	LC[a]	–	100 ± 13	93 ± 11	82±17	93 ± 7	2.3
Thiabendazole	LC[a]	–	86 ± 11	93 ± 6	92 ± 6	90 ± 5	1.0
Thiamethoxam	LC[a]	–	85 ± 7	108 ± 8	85 ± 5	91 ± 1	9.5
Thiophanate-methyl	LC[a]	–	–	–	–	–	–34.6
Tricyclazole	LC[a]	–	98 ± 7	87 ± 11	80 ± 7	86 ± 3	26.5
Trifloxystrobin	LC[a]	–	77 ± 13	87 ± 7	73 ± 6	82 ± 5	13.4
Trifluralin	GC[b]	20	84 ± 8	74 ± 4	75 ± 5	73 ± 11	22.5
Trifluralin d-14[c]	GC[b]	–	86 ± 11	74 ± 3	88 ± 4	74 ± 11	32.8

[a]LC: liquid chromatography; [b]GC: gas chromatography; [c]surrogate standard; [d]sum of 2,4-D and 2,4,5-T; [e]sum of 2,4-DDD, 2,4-DDE and DDT; [f]sum of aldicarb sulfone, aldicarb sulfoxide and aldicarb; [g]sum of aldrin and dieldrin; [h]sum of benomyl and carbendazim; [i]sum of chlorpyrifos-ethyl and chlorpyrifos oxon; [j]sum of endosulfan alpha, endosulfan beta and endosulfan sulfate, [k]RSD: relative standard deviation.

liquid-liquid extraction and 5.2% for SPE. However, when a smaller amount of sample (5 mL), simultaneously with spiking at higher levels (5 μg L^{-1}) was tested, the recovery was 93.6% for SPE. However, the adoption of multiresidue methods is impractical with these conditions, but confirms the influence of water solubility.

The compound terbufos showed low recovery probably due to its loss in the evaporation step. This compound has a vapor pressure of 34.6 mPa, which characterizes it as a volatile analyte (> 0.1 mPa). The same for aldicarb, which is also classified as volatile by its vapor pressure (3.87 mPa). Donato *et al.*[14] have found a suitable method for the compound terbufos using the polymeric sorbent Strata™-X 200 mg and eluting with acetonitrile/methanol (1:1, v/v) acidified with 1% acetic acid (v/v). Authors reported good recoveries and it can be explained by the fact that no evaporation step was used.

Unsatisfactory recovery of benomyl and thiophanate-methyl were attributed to possible degradation of these compounds to carbendazim. The fungicide benomyl is rapidly converted to carbendazim in the environment, with a half-life of 2 h and 19 h in water and soil, respectively.[30] The transformation of thiophanate-methyl into carbendazim occurs in alkaline medium. This compound has pKa of 7.28 (weak acid), so it is stable in acid medium (pH 2-5), but is unstable in medium alkaline remaining undegraded for about 46 days in aqueous solutions at pH 7 and 22 °C, and 4 min at pH 9 and 65 °C.[31] A suitable result for the pesticide thiophanate-methyl was obtained when using the polymeric adsorbent Strata™-X 200 mg in combination with an eluting solution of methanol: acetonitrile (1:1, v/v) acidified with 1% acetic acid. The fact of the elution solution has acid character may have favored the elution of this compound.

The benfuracarb pesticide has shown unsatisfactory recovery with the proposed method. Good recovery was obtained with 500 mg of C_{18}, without acidification of the sample. The C_{18} sorbent may have favored the extraction of

benfuracarb since this analyte has a partition coefficient of organic carbon (K_{oc}) equals to 9100, characterizing a strong tendency to bond with organic compounds, for example C_{18}. However, with the polymeric sorbents tested in this study the retention was not efficient.

Intermediate precision was evaluated at the intermediate level (1.5 μg L^{-1}). Recovery results for the compounds that presented good results in the repeatability assay ranged from 70.0 to 118.6% with RSD lower than 20.0%. These results are in accordance with international regulations for analysis of pesticides at low concentration levels by chromatographic techniques.

The compounds 2.4-DDD and 2.4-DDE, determined by GC, continued to show low recoveries with RSD values ≤ 6.7%. In the same sense, methamidophos, aldicarb, thiophanate methyl, benfuracarb, benomyl and terbufos repeated the unsatisfactory results obtained in the evaluation of repeatability.

In this study, it was observed that most of the compounds analyzed have shown matrix effect with enhancement of the analytical signal. These results presented in Table 1 demonstrate the need of use analytical curves prepared in blank matrix extract for the quantification of pesticides in drinking water. In order to generate accurate results, all analytical curves were prepared with matrix matched standards. The matrix effect is defined as a significant increasing or suppression (> 10%) of the analytical signal for a given compound present in the matrix extract compared to the analytical signal obtained for the same analyte in organic solvent.[32] The components of the aqueous matrices that can influence the analysis are: organic matter, humic and fulvic acids and salts present in water samples.[33] These substances are a complex mixture of molecules with high molar mass that can be formed by decomposing plants and marine organisms.[34]

Application to real samples

After the validation of the proposed method, it was applied to 12 samples collected in the central region of the Rio Grande do Sul State, Brazil, where the agriculture is the main economic activity. It was collected 12 drinking water samples from different places. The samples were collected in 1 L glass amber flasks, stored protected from light, at temperatures between 4 to 10 °C to prevent possible degradation of the compounds, and analyzed in the same day of sampling. Two of the twelve samples analyzed showed pesticide residues. In one sample, was found the presence of metolachlor, below the LOQ (0.5 μg L^{-1}). This value is lower than the limit established by the Brazilian legislation for this residue in drinking water (10 μg L^{-1}). In another sample, was found the presence of pesticides permethrin and cypermethrin, both below the LOQ, and cyhalothrin-lambda 0.65 μg L^{-1}. SRM chromatogram of cyhalothrin-lambda is available in Supplementary Information. No limit is established in Brazil for this compound in drinking water. The fact that the samples have low concentrations of pesticide residues can be related with the low agricultural activity in the period that samples were collected. The prevailing culture in the region investigated is the rice, which at this time (July) the producers are just preparing the ground for the next crop.

The developed SPE and GC-MS/MS method was validated to cover the Brazilian legislation. The proposed method was rapid and efficient, allowing the determination of 70 pesticides with different physicochemical properties in drinking water. Caldas *et al.*,[35] in 2013, also investigated the presence of 20 pesticide residues in surface water in South of Brazil and detected carbendazim, atrazine, epoxiconazole and tebuconazole. These authors also detected epoxiconazole and tebuconazole in drinking water. Sabin *et al.*,[36] in the same year, used SPE and GC-MS operating at selective ion monitoring for the multi-class determination of 20 pesticides regulated by the Brazilian legislation for drinking water, no positive samples were found over the LOQ range (0.003 and 0.093 μg L^{-1}).[36] An efficient method based on SPE and LC-MS/MS was developed by Montagner *et al.*,[27] in 2014, for simultaneous determination of 12 pesticides at trace levels in surface and drinking water from the State of São Paulo (Brazil). The rivers investigated presented nine of the twelve compounds analyzed. Chlorpyrifos, profenofos and fipronil were under their limits of quantification. For drinking water samples, three of the twelve pesticides (tebuconazole, atrazine and carbendazim) were determined in concentrations from 4 to 87 ng L^{-1}. In our work, the determination of residues of the selected pesticides by LC-MS/MS and GC-MS/MS were satisfactory, allowing the confirmation and the quantification through the SRM acquisition mode for most of the compounds. The greatest advantage of this method was the possibility of simultaneous determination of different pesticide classes (acaricides, insecticides, fungicides and herbicides) at low levels.

Conclusions

The SPE procedure with the polymeric sorbent Oasis HLB demonstrated to be an excellent technique for the preparation of water samples and essential for achieving the required limits for all analytes, by providing a high concentration factor. It is a fast method because it allows the simultaneous preparation of various samples at

the same time. Also, has low solvent consumption and provides adequate recoveries. The same extract allowed the determination by GC-MS/MS and LC-MS/MS resulting in a rapid and cheap procedure.

The GC-MS/MS and LC-MS/MS techniques with triple quadrupole mass analyzer have shown to be the most suitable tool for the determination of pesticide residues, since the coupling of the chromatography with the mass spectrometry enables high detectability and selectivity. Moreover, it allows obtaining a quantitative and qualitative analysis of the selected fragments of each analyte in the acquisition mode SRM. The chromatographic conditions optimized in both LC and GC systems allowed the identification and quantification of the compounds under study.

Therefore, it can be concluded that the proposed method for the determination of pesticide residues in drinking water using GC-MS/MS and LC-MS/MS proved to be effective to meet the validation parameters of chromatographic methods. Considering the obtained results, it can be concluded that the method is effective and rapid, and can be applied in routine analyses as an excellent tool for monitoring pesticide residues in water samples.

Supplementary Information

Supplementary data, including optimized parameters for the analyzed pesticides by LC-MS/MS and GC-MS/MS, as well a SRM chromatogram of positive water sample, are available free of charge at http://jbcs.org.br as a PDF file.

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