

Multiresidue Determination of Pesticides in Drinking Water by Gas Chromatography-Mass Spectrometry after Solid-Phase Extraction

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Neste trabalho, um método usando extração em fase sólida e cromatografia a gás acoplada à espectrometria de massas no modo de monitoramento seletivo de íons, foi desenvolvido e validado para a determinação multiclasse de 20 pesticidas regulados pela legislação brasileira para água potável. Como estes pesticidas devem ser determinados em baixas concentrações, um elevado fator de pré-concentração associado à sensibilidade elevada da análise cromatográfica foi necessário. O método apresentou limites de quantificação entre 0,003 e 0,093 $\mu\text{g L}^{-1}$. A maioria dos compostos apresentou recuperações médias entre 51 e 116%. Embora a natureza química distinta dos pesticidas analisados dificulte a obtenção de boa recuperação para todos os compostos avaliados, a precisão dos resultados foi excelente. A seletividade do método foi avaliada através da intensidade relativa dos íons de quantificação e de qualificação, sendo considerada adequada. A análise em amostras reais cumpriu os critérios para a qualificação instrumental e a avaliação da conformidade do sistema.

In this work, a method using solid-phase extraction and gas chromatography coupled mass spectrometry in the selective ion monitoring (SIM) mode was developed and validated for the multi-class determination of 20 pesticides regulated by the Brazilian legislation for drinking water. Because these pesticides must be determined at low concentrations, a high preconcentration factor associated to the high sensitivity of chromatographic analysis was necessary. The method presented quantification limits between 0.003 and 0.093 $\mu\text{g L}^{-1}$. Most of the compounds presented mean recoveries between 51 and 116%. Although the differing chemical nature of the pesticides analyzed difficult the attainment of good recovery for all of the compounds evaluated, the precision of the results was excellent. The selectivity of the method was evaluated through the relative intensity of quantification and qualification ions and was considered adequate. Analysis in real samples met criteria for instrumental qualification and the system suitability evaluation.

Keywords: solid-phase extraction, GC-MS, pesticide, multiresidue, water

Introduction

Although pesticides increase agricultural productivity, their use can produce serious environmental problems through the contamination of water resources. Therefore, legal limits and alert levels for drinking water have been introduced.¹ In Brazil, the Ministry of Health, through Regulation MS 518 of March 25, 2004 established procedures and requirements for the control and vigilance of water quality for human consumption and drinking water standards, including limits for some pesticide residues.² The European Union (EU) Directive on the quality of drinking water has set the maximum admissible concentrations of each pesticide at 0.1 $\mu\text{g L}^{-1}$, and the total concentration of

all pesticides at 0.5 $\mu\text{g L}^{-1}$.³ The WHO threshold values for concentrations of pesticides in drinking water, based on toxicological considerations, are less strict than the maximum concentrations allowed by EU.¹

Although the importance of the determination of pesticide residues in drinking water, only few works with samples from Brazil were published and most studies were focused on a limited scope like organochlorine pesticides^{4,5} and triazine herbicides.^{6,7}

In this work, pesticides were chosen based on their necessity for regulation in water for human consumption as established by current Brazilian legislation. The pesticides selected (Figure 1) present quite distinct physicochemical and chromatographic characteristics.

The physicochemical properties of pesticides can be used as a criterion in the evaluation of risk to the aquatic

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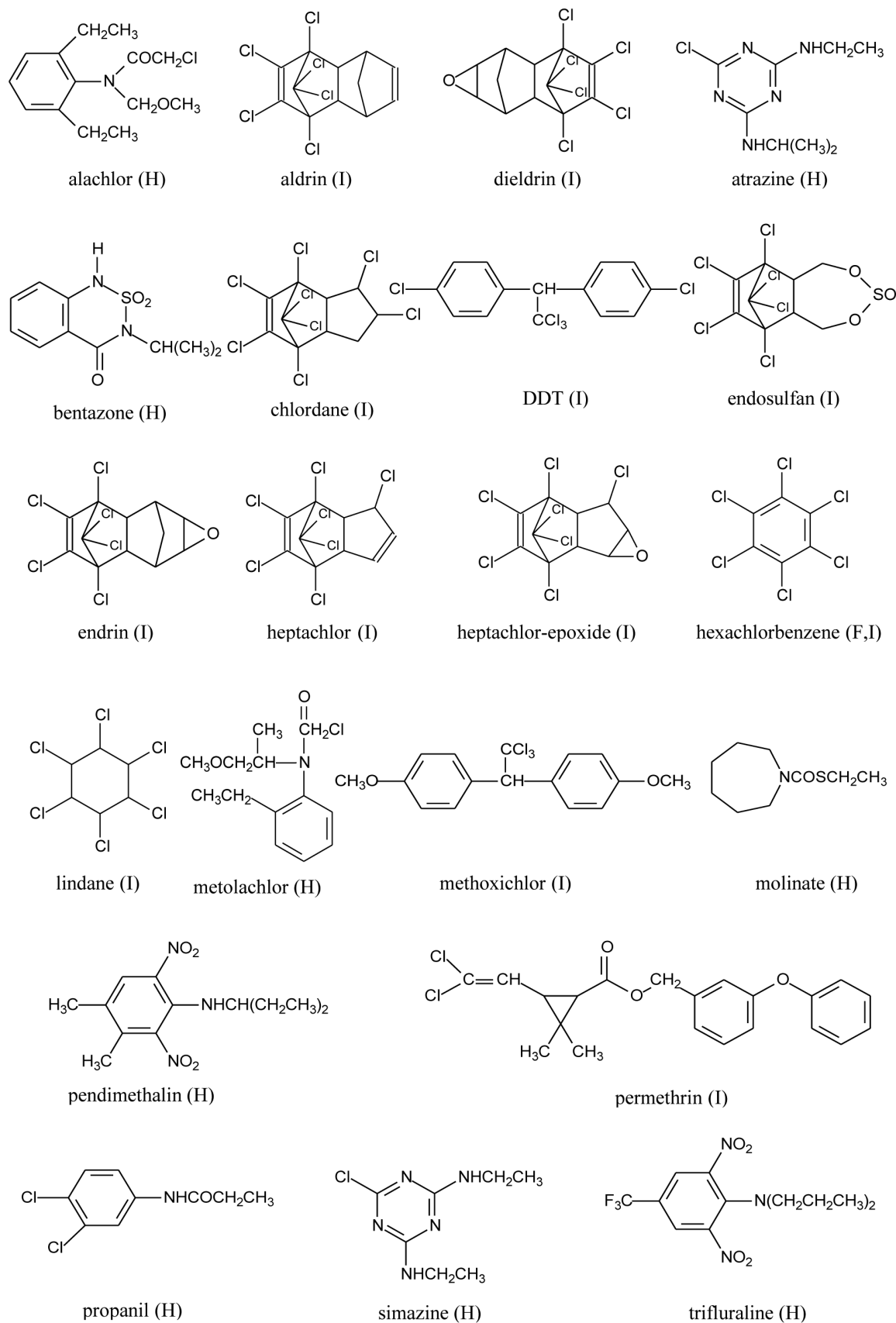


Figure 1. Structure of selected pesticides (H = Herbicides; I = Insecticides; F = Fungicides).

environmental,⁸ but they also provide important information as to behavior during the stages of preconcentration, solvent evaporation, stability in water and adsorption on organic material present in the samples. For chromatographic determination it is necessary to investigate the analytes' physicochemical properties to achieve high selectivity and sensitivity. Sample preparation is the first step to develop a good analytical method. The literature has shown excellent advantages of using solid-phase extraction for multiresidue pesticide analysis in water, including shorter analysis times, low solvent consumption, high preconcentration factor, good recoveries and precisions, applicability in a wide range of polarities and selective extractions.⁹⁻¹¹ Huck and Bonn¹² reported solid-phase extraction with polymeric-based sorbents with better results than those obtained with C-18. Pichon *et al.*¹³ described the difficulty of working with analytes with a wide range of polarities, underlining the problems of elution and strong retentions.

For the determination of relatively volatile and thermally stable apolar or medium polarity compounds, GC-MS has given excellent results, especially simultaneous determinations of compounds with differing classes and properties.^{11,14} The advantages of GC-MS in SIM mode, including high sensitivity, selectivity and robustness, have been pointed out by several authors.^{15,16}

Therefore, this study aims to develop and validate a reliable, highly sensitive, low-cost method with a short analysis time. The method, based on solid-phase extraction and quantification by gas chromatography coupled to mass spectrometry (GC-MS) in the selective ion monitoring (SIM) mode was validated for a number of parameters including system suitability for routine analysis.

Experimental

Standards, reagents and solvents

Analytical standards of the pesticides evaluated (purity between 94.0 and 99.7%) were acquired from Dr. Ehrenstorfer (Augsburg, Germany). The internal standard, 1,3-diphenoxybenzene, was provided by Sigma-Aldrich (Poole, United Kingdom). Surrogate standards, 1,4-dichlorobenzene-d4, naftalene-d8, acenaftene-d10, anthracene-d10, crisene-d12 and perilene-d12, were obtained from Chem Service (West Chester, USA). Working standard solutions were prepared in ethyl acetate.

Pesticide grade/analytical reagent grade ethyl acetate, dichloromethane and methanol were purchased from JT Baker (Phillipsburg, USA). Milli-Q purified water was obtained from Millipore (St. Quentin-in-Yvelines, France) and helium 99.999% was obtained from AGA (Barueri-

SP, Brazil). The following SPE cartridges were used for compound preconcentration: Strata-X[®] 30 mg *per* 3 mL (Phenomenex, Torrance, USA), Nexus[®] 60 mg *per* 3 mL (Varian, Palo Alto, USA), Oasis[®] 30 mg *per* 3 mL (Waters, Milford, USA) and C18 500 mg *per* 6 mL (Phenomenex, Torrance, USA).

Apparatus

Determination was performed using an Agilent GC system (model HP 6890N) equipped with a split/splitless injector, liner of silanized quartz with 4 mm i.d. (effective volume 0.49 mL), and a 7683 autosampler for 100 vials (Wilmington, EUA). Chromatographic conditions were: HP5-MS low bleed capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness); helium was used as a gas carrier; flow constant: 1.0 mL min⁻¹; injection volume: 2 μL (pulsed splitless: 15 psi for 1.5 min); injector temperature 250 °C; GC temperature program: 50 °C (2 min), 30 °C min⁻¹ up to 160 °C (5 min), 5 °C min⁻¹ up to 180 °C and 10 °C min⁻¹ up to 270 °C (6 min). A HP 5973N Agilent quadrupole mass spectrometer (Palo Alto, EUA) was operated in selective ion monitoring (SIM) mode, with ionization source by electron impact at 70 eV, transfer line at 280 °C, ion source at 230 °C and quadrupole at 150 °C.

In the SPE sample preparation stage, a manifold system with capacity for 12 cartridges (Phenomenex; Torrance, USA) and a vacuum pump (model TE-058, Tecnal; Piracicaba, Brazil) were used.

Chromatographic separation and ion selection

To determine the best temperature program, a retention time behavioral study was performed for all compounds, based on which, a multi-linear temperature program was adopted.

Selective ion monitoring (SIM) mode was utilized in order to achieve high selectivity and sensitivity. One ion was chosen for quantification and another for qualification, based on the best signal-noise ratios and the highest masses.

SPE preconcentration

Initially, the retention capacity results obtained for the sorbents Strata-X[®], Nexus[®], Oasis[®] and C18 were evaluated. Ethyl acetate and dichloromethane were tested as elution solvents. The following SPE steps were performed: (i) Conditioning: each cartridge was conditioned with two cartridge volumes of methanol and two volumes of purified water, avoiding drying of the sorbent; (ii) percolation of the sample: 200 mL of each sample, after adding the surrogate standards, were percolated at approximately 2 mL min⁻¹.

Then, the cartridges were washed with a cartridge volume of purified water and dried for 30 min in the manifold system under vacuum; (iii) elution and redissolution: the analytes were eluted with 2 mL solvent in two steps (1 + 1 mL), with an interaction time of 1 min for each step. The solvent was removed by drying in water bath at 55 °C for approximately 20 minutes. The analytes were redissolved by adding 0.5 mL of the redissolution solution containing the internal standard in ethyl acetate.

Method validation and quality assurance

Analytical curves were established by injecting 7 times each analytical solution containing the pesticides at concentrations between 1 and 1000 µg L⁻¹. Linear regression and polynomial equations were generated as a model of prediction. Internal standardization was adopted and application range was defined based on method linearity, quantification limits and maximum limits allowed in drinking water by Brazilian legislation.

Method selectivity was ensured by using SIM and by the absence of quantification and qualification ions in analyte retention time of a blank sample. In real samples, selectivity was ensured by the ratio between quantification and qualification ions with a tolerance of 20%. This tolerance can be increased to 30% close to the quantification limit.¹¹

The limits of detection (LOD) and of quantification (LOQ) were established from the signal-noise ratio of quantification ions and qualification ions selected for each compound studied. Seven injections of the standard solution 10 µg L⁻¹ were performed, with the exception of chlordane, DDT and permethrin which are isomeric mixtures considered individually as fractions proportional to the peak heights of their respective isomers. Concentration levels near to the estimated quantification limits were chosen. The mean results for peak height were used for the coefficient of sensitivity calculation, which convert the signal into the concentration. The blank sample was injected 7 times. Mean standard deviation from the noise was determined. The lowest noise was established as its own unit due to the high signal stability in SIM mode, which presented lower deviation for all compounds than the unit of abundance. The LOD was established as the concentration where the ion of lowest abundance presented a peak height corresponding to 3 times the signal-noise ratio. For the LOQ, 10 times the signal-noise ratio was considered for quantification ions, as long as the qualification ion was at least 6 times this ratio. Therefore, both limits are guaranteed in a confirmatory way.¹⁷

Instrumental precision was evaluated from the results of 7 replicates for each concentration of the linearity

study. Relative standard deviation (RSD) was compared with the Horwitz curve.¹⁸ To evaluate the accuracy and precision of the method in terms of repeatability and intermediate precision, blank samples were fortified at three different concentration levels 0.030, 0.100 and 0.300 µg L⁻¹, identified as F1, F2 and F3, respectively. These concentrations represent 1, 3.3 and 10 times the lowest limits established by Brazilian legislation for drinking water and allowed the evaluation of accuracy, in terms of recovery, and precision of the proposed method. Intermediate precision was evaluated in order to simulate a more real routine analysis situation, and was determined by six repetitions of the complete procedure using the same three concentration levels on three different days.

For routine assays, certain parameters should be periodically verified to guarantee system suitability and validity of results. The parameters chosen for this purpose were: peak area and retention time of the internal standard, efficiency, sensitivity, coefficient of determination, recovery of surrogate standards and selectivity. However, the detection of very low levels may not always be essential and it may be acceptable in some circumstances to define a practical reporting limit based on the proposed use of the analytical data.^{19,20} This would have the advantage of reducing the technical difficulty of obtaining data and of reducing costs.

Application in real samples

The method was tested with 5 real samples with different characteristics: two samples of untreated water (water from superficial springs and wells) and three samples of water treated at water treatment facilities (water from creeks, rivers and lakes). The purpose was to include samples with different characteristics and differing levels of mineral salts, pH, turbidity, fulvic and humic acids, among other features. Samples were collected in glass recipients and stored in a dark place at temperatures between 1 and 4 °C.

Results and Discussion

Chromatographic system

The chromatogram in Figure 2 shows the good separation obtained after the retention time and oven temperature studies.

The optimization of the chromatographic analysis by ideal gas law application allowed a better chromatographic response and, therefore, a 4-fold reduction of the preconcentration factor in the sample preparation, which reduces extraction time.

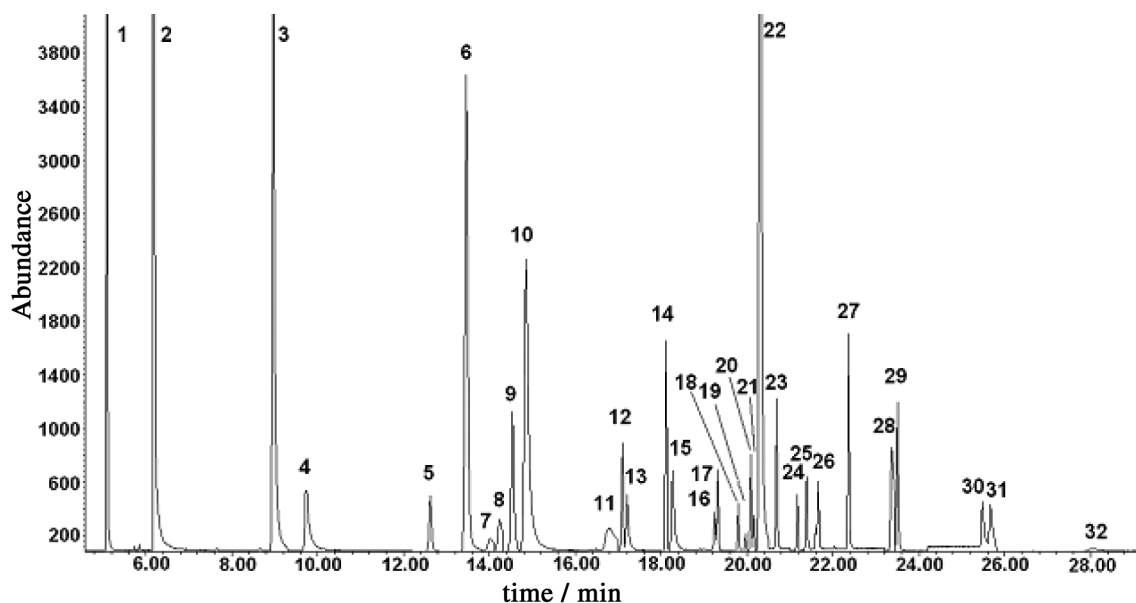


Figure 2. Typical chromatogram of a blank water sample fortified at $0.300 \mu\text{g L}^{-1}$. Compound identification is shown in Table 1.

SPE preconcentration

The polymeric sorbents Strata-X[®] (30 mg per 3 mL) presented consistent good recoveries for the most of the studied pesticides and it was chosen for this method. Dichloromethane presented best results than ethyl acetate as eluent. The analytes were eluted with 2 mL dichloromethane in two aliquots of 1 mL and an interaction time of 1 min for each step. Dichloromethane is more volatile than ethyl acetate and permitted an easier evaporation step before GC-MS analysis.

Method validation

Method selectivity was shown to be adequate for the determination of the pesticide studies. Table 1 shows percentual difference between the recovery obtained for the quantification ion and the qualification ion of each compound. Maximum tolerance was 20% for levels F2 and F3 and 30% for level F1 and should be evaluated critically for each case.¹¹

Although lindane had a systematic matrix effect on the qualification ion, it had a maximum residue limit (MRL) of $2 \mu\text{g L}^{-1}$ making the matrix effect negligible. Molinate and permethrin also had a matrix effect on the qualification ion, but only for F1, which does not denote a problem for determinations with MRLs near 6 and $20 \mu\text{g L}^{-1}$, respectively.

Most of the pesticides presented r^2 values greater than 0.99. Alachlor, atrazine, metolachlor, permethrin, propanil and simazine presented r^2 values of 0.98, 0.97, 0.98, 0.98,

0.97 and 0.97, respectively. When polynomial equations were used a minimum r^2 of 0.9997 was verified. In the range studied, linearity was adequate for all compounds. However, for routine analyses a curve of three levels (12, 40 and $120 \mu\text{g L}^{-1}$), which correspond to the concentrations in water of 0.030, 0.100 and $0.300 \mu\text{g L}^{-1}$, was used in order to prioritize the best quantification of lower concentration levels. When concentrations above the working range are verified, a dilution of the extract for quantification should be performed.

Results for instrumental precision are shown in Table 2. RSD values that meet Horwitz curve criteria are shown in the shaded area of Table 2. It is important to note that the lowest concentration level with acceptable precision is lower than 50% of the MRL, rendering instrumental precision adequate. Repeatability, intermediate precision and method recovery can be seen in Table 3. According to the Horwitz curve, for the concentration level of $0.030 \mu\text{g L}^{-1}$, the adequate RSD for repeatability (RSD_r) is 38% and adequate RSD for intermediate precision (RSD_{pi}) is 76%. For the concentration level of $0.300 \mu\text{g L}^{-1}$, adequate RSD_r is 27% and RSD_{pi} is 54%.

The results in bold type refer to compounds with MRL values at the F1 concentration level. Precision results were considered excellent. The other compounds were evaluated by the F3 concentration level as they presented MRLs over $0.300 \mu\text{g L}^{-1}$. Method precision was considered excellent. Recoveries for each compound at the three fortification levels were very consistent. Hexachlorobenzene, aldrin, DDT and permethrin presented low recoveries.

All the pesticides presented signals greater than their

Table 1. Percentual difference between the recoveries obtained for the quantification ion and the qualification ion

Elution order	Standards	Quantification	Qualification	F1	F2	F3
		Ion	Ion	0.030 µg L ⁻¹	0.100 µg L ⁻¹	0.300 µg L ⁻¹
1	1,4-Dichlorobenzene-d4 (SS1)	150	152	0	0	0
2	Naftalene-d8 (SS2)	136	137	3	0	-11
3	Acenaftene-d10 (SS3)	162	164	-4	0	-1
4	Molinate	126	187	44	3	2
5	Trifluraline	306	264	5	0	-2
6	Hexachlorobenzene	284	286	4	-2	0
7	Simazine	201	186	-8	-2	1
8	Atrazine	200	215	-6	-1	1
9	Lindane	181	219	-324	-79	-24
10	Antracene-d10 (SS4)	188	189	0	0	-1
11	Propanil	161	163	-4	3	3
12	Heptachlor	272	274	-3	0	0
13	Alachlor	160	188	-5	0	0
14	Aldrin	263	261	-1	-1	2
15	Metolachlor	162	238	4	2	-1
16	Pendimethalin	252	253	-13	-5	-4
17	Heptachlor epoxide	183	353	12	-2	1
18	Chlordane I	373	375	7	-4	1
19	Chlordane II	373	375	26	-1	-1
20	Chlordane III	373	375	15	-5	-5
21	Endosulfan I	195	241	4	0	4
22	1,3-Diphenoxybenzene (IS)	141	262	-	-	-
23	Endosulfan II	195	241	-3	2	1
24	Dieldrin	79	277	18	4	5
25	Endrin	263	261	2	-2	-1
26	DDT I	235	165	-16	4	1
27	DDT II	235	165	-7	-1	1
28	Crisene-d12 (SS5)	240	236	2	-1	-1
29	Methoxychlor	227	228	0	-2	-1
30	Permethrin I	183	163	-76	-4	1
31	Permethrin II	183	163	-29	-7	-3
32	Perilene-d12 (SS6)	260	264	3	1	1

SS = surrogate standard; IS = internal standard.

Table 2. Instrumental precision evaluated for 10 concentration levels (n= 7)

Standards	Concentration / (µg L ⁻¹)	Horwitz curve	Instrumental Precision-Relative Standard Deviation / (%)																								
			Alachlor	Aldrin	Dieldrin	Atrazine	Chlordane I	Chlordane II	Chlordane III	DDT I	DDT II	Endosulfan I	Endosulfan II	Endrin	Heptachlor	Heptachlor epoxide	Hexachlorobenzene	Lindane	Metolachlor	Methoxychlor	Molinate	Pendimethalin	Permethrin I	Permethrin II	Propanil	Simazine	Trifluralin
P1	1	22	#	12	#	#				#					#	7	46	10	##	8	##	##	##	#	#	#	16
P2	2.5	20	46	4	10	32	13	35	14	6	4	21	11	44	4	6	16	4	9	5	24	##	##	#	#	#	9
P3	5	18	12	1	10	13	5	16	7	17	4	5	6	5	14	8	2	3	2	2	4	14	4	4	7	5	16
P4	10	16	10	4	9	4	4	8	6	18	6	9	9	2	5	8	5	5	7	8	6	9	12	16	9	9	7
P5	25	14	7	3	3	3	3	5	4	6	2	4	5	2	5	4	4	5	4	5	5	5	6	6	6	5	4
P6	50	12	1	3	4	1	3	3	6	5	4	1	2	1	4	3	4	3	2	7	5	2	9	11	4	4	4
P7	100	11	3	2	2	4	2	6	3	3	1	4	2	2	3	1	3	2	3	3	4	2	6	6	5	4	3
P8	250	10	3	7	3	3	2	3	3	8	2	1	1	2	3	2	3	2	2	4	4	1	7	7	5	3	4
P9	500	9	4	4	1	3	2	3	4	2	3	3	2	1	5	3	6	4	3	5	6	3	9	10	6	5	5
P10	1000	8	6	3	2	3	2	1	2	5	2	3	1	1	4	2	3	3	5	5	4	4	11	11	4	5	6

Table 3. Results for precision and accuracy for F1 (0.030 µg L⁻¹) and F3 (0.300 µg L⁻¹) fortification levels

	F1				F3			
	Repeatability		Intermediate precision		Repeatability		Intermediate precision	
	*R%	RSDr	*R%	RSDpi	*R%	RSDr	*R%	RSDpi
Molinate	87	76	74	79	32	46	49	48
Trifluraline	61	11	57	25	54	13	56	17
Hexachlorobenzene	36	17	36	27	34	18	38	25
Simazine	116	4	107	14	107	15	112	21
Atrazine	114	5	107	11	110	14	112	17
Lindane	72	4	72	8	72	11	71	18
Propanil	114	9	117	20	121	14	137	24
Heptachlor	57	4	52	22	53	12	49	18
Alaclor	106	6	102	11	99	10	99	11
Aldrin	17	14	25	32	50	22	43	33
Metolachlor	114	3	114	9	112	9	111	11
Pendimethalin	68	12	72	23	67	9	70	15
Heptachlor epoxide	70	9	69	19	68	7	68	10
Chlordane	61	22	58	40	58	13	60	28
Endosulfan	79	9	82	22	80	6	80	8
Dieldrin	81	12	84	25	73	7	74	9
Endrin	80	8	80	18	79	5	82	6
DDT	43	27	45	44	42	20	41	22
Methoxychlor	85	19	82	36	71	13	74	14
Permethrin	35	27	32	43	37	17	33	21

*R%: Recovery (%).

limits of quantification at fortification level of 0.030 µg L⁻¹. Table 4 shows the limits of quantification and the MRL values from Regulation MS 518/2004. The limits of quantification achieved with the proposed method are also adequate for the control of pesticide residues in drinking water in accordance to the European standard.²¹

The method developed was only incapable of adequately determining four of the pesticides from Regulation MS 518/2004: glyphosate and 2,4-D, which are not suitable for direct GC determination, and bentazone and pentachlorophenol, which presented sensitivity, but were not adequately recovered in the multiresidue extraction by SPE.

Method applicability in real samples and system suitability

Application of the method with real samples showed excellent performance in terms of system suitability. No pesticide was detected in the real samples evaluated.

At the concentration range between 0.030 and 0.300 µg L⁻¹, the lowest result obtained for coefficient of determination using the linear regression equation was 0.995. These results proved to be adequate for utilization

Table 4. Quantification limits of the proposed method and maximum residue limits established by Regulation MS 518/2004

Pesticides	Quantification limit / (µg L ⁻¹)	MRL Regulation MS 518/2004 / (µg L ⁻¹)
Alachlor	0.023	20.0
Aldrin	0.010	0.03
Dieldrin	0.023	0.03
Atrazine	0.035	2
Chlordane	0.015	0.2
DDT	0.010	2
Endosulfan	0.033	20
Endrin	0.033	0.6
Heptachlor	0.023	0.03
Heptachlor epoxide	0.033	0.03
Hexachlorobenzene	0.003	1
Lindane	0.010	2
Metolachlor	0.018	10
Methoxychlor	0.023	20
Molinate	0.018	6
Pendimethalin	0.093	20
Permethrin	0.018	20
Propanil	0.035	20
Simazine	0.053	2
Trifluraline	0.023	20

in routine analysis for any type of water that meets physicochemical parameters for potability. Soboleva and Ambrus²² make a good discussion on system suitability studies that combine parameters of injection and detection. The results obtained in our study are in accordance with those presented by these authors.

Conclusions

The present method was shown to be adequate for the determination of most pesticides in drinking water, thus meeting requirements in the Brazilian legislation. The GC-MS in the SIM mode proved to be essential for quantification with adequate sensitivity and selectivity for pesticide residues in samples from a number of origins and that present high turbidity, without significant interference. The chromatographic parameters showed excellent results in terms of efficiency, resolution and total analysis time. The method was utilized for over a year without presenting significant modifications in retention times. The preconcentration factor of 400 times of the SPE step was essential for reaching Regulation MS 518/2004 limits. In the method development, a large number of factors were considered which significantly increased sensitivity and robustness. In the evaluation of precision, the results were excellent for all concentration levels studied when compared with values given by the Horwitz curve. Intermediate precision was also considered adequate, showing no differences on different days. Recoveries were consistent, regardless of the day and concentration level. Although some analytes presented low recoveries, the consistency of the results obtained and the use of surrogate standards make it possible to reliably establish a correction factor for the analysis of these analytes in real samples.

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