Revista Virtual de Química

ISSN 1984-6835

Artigo

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Rev. Virtual Quim., **2017**, *9* (2), *no prelo*. Data de publicação na Web: 20 de fevereiro de 2017

http://rvq.sbq.org.br

Método Analítico Multiclasses Para Determinação de Agrotóxicos Em Água Utilizando DLLME e CG-MS

Abstract: This work aimed to develop and validate a method for the determination of trifluralin, metribuzin, metolachlor, endosulfan, λ-cyhalothrin, permethrin and malathion in water using dispersive liquid-liquid microextraction (DLLME). The mixture n-hexane/toluene (60:40 v/v) showed high extraction capacity and was used in the method validation. The method was applied to different surface water samples and a matrix effect was not observed. The DLLME provided recovery of 47–115 % with a RSD up to 16%. The detection limits ranged between 0.0063 and 0.046 μg L^{-1} whereas the method quantitation limit was 2.0 μg L^{-1} for all analytes. After validated, the method was applied to water samples collected in an agricultural region of Mato Grosso State, Brazil and only permethrin was detected. The proposed method can be an alternative for the simultaneous determination of multiclass pesticides in water, with the advantage of being fast and using a very small amount of solvent.

Keywords: Water; microextraction; pesticide.

Resumo

Este trabalho teve como objetivo desenvolver e validar um método para a determinação da trifluralina, metribuzin, metolacloro, endosulfan, λ-cialotrina, permetrina e malationa em água usando microextração líquido-líquido dispersiva (DLLME). A mistura de n-hexano / tolueno (60:40 v/v) mostrou elevada capacidade de extração e foi utilizada na validação do método. O método foi aplicado a diferentes amostras de água superficial e não foi observado um efeito matriz. A DLLME proporcionou uma recuperação de 47-115% com um RSD de até 16%. Os limites de detecção variaram entre 0,0063 e 0,046 mg L^{-1} enquanto que o limite de quantificação do método foi de 2,0 mg L^{-1} para todos os analitos. Depois de validado, o método foi aplicado em amostras de água coletadas em uma região agrícola de Mato Grosso, Brasil e somente a permetrina foi detectada. O método proposto pode ser uma alternativa para a determinação simultânea de pesticidas multiclasses em água, com a vantagem de ser rápido e utilizar uma pequena quantidade de solvente.

Palavras-chave: Água; microextração; agrotóxicos.

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DOI:

Volume 9, Número 2

Março-Abril 2017

Revista Virtual de Química
ISSN 1984-6835

A Multiclass Analytical Method for Pesticides Determination in Water using DLLME and GC-MS

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Recebido em 10 de março de 2016. Aceito para publicação em 20 de fevereiro de 2017

1. Introduction

Water contamination by pesticides may pose a risk to aquatic biota as well as to human health.[1,2] Furthermore, water is one of the main means of transport of contaminants to other environmental compartments, which emphasizes the importance of studies regarding this matrix.[3-5] For the determination of pesticides in water, as well as in other matrices, it is essential to use analytical methods that are exact, precise, economically viable and that generate a low amount of residue.[6,7]

Classical methods used for this purpose consist of solid phase extraction (SPE) and liquid-liquid extraction (LLE).[8] Although considered exact and precise, such methods usually utilize large amounts of organic solvents and involve several steps in the extraction process, making them more prone to experimental errors.[7,9,10] These characteristics have stimulated the development of alternative methods whose main premise is the miniaturization of the extraction process in order to reduce costs and analysis time, in addition to reducing organic solvents volume.[8,10,11]

Among those methods, the DLLME has been proposed as an alternative to classical methods for the determination of pesticides in water due to its simplicity, quickness, low cost, low consumption of organic solvents, accuracy and precision.[8, 12,13] DLLME is based on the addition of a dispersing solvent (miscible with the extracting solvent and the sample), which promotes dispersion of the extracting solution (immiscible with the aqueous phase) in the sample providing a large area of contact between the aqueous phase and the extracting solvent.[13,14] This technique has been used to determine plasticizers, pharmaceuticals, pesticides and inorganic compounds in several matrices.[15-18]

In DLLME, the extracting solvent is the main parameter to be defined for the optimization of the extracting procedure and its choice should be based on its density, extracting efficiency of the studied analytes and adequacy to the chosen analytical technique.[19] The most used extracting solvents are the organochlorines carbon tetrachloride, chloroform and dichloromethane, which are more dense than water and when separated from the aqueous phase, they deposit at the bottom of a conical tube. However, several variations of the technique were proposed using solvents less dense than water such as iso-octane, n-heptane and n-hexane with the collection of the organic phase at the top surface of the extracting tube.[19,20] The most used dispersing solvents are methanol, ethanol, acetone and acetonitrile.[20]

Since DLLME is a relatively new technique, its application still requires improvement. Most applications of this technique still use manual chromatographic injection due to the small volume of the final extract, which has been pointed out as a considerable disadvantage of the method.[12] Also, few studies have reported the application of DLLME to simultaneous extraction of several pesticides belonging to different chemical classes. Moreover, the use of organochlorine solvents predominates, which is a considerable drawback since they can be as toxic as the analytes.[12]

Thus, the present study aimed to contribute to the improvement of DLLME through the development and validation of an analytical method for the determination of different classes of pesticides in water, using this technique followed by gas chromatography-mass spectrometry. Besides the commonly studied extraction parameters such as agitation mode, addition of dispersing solvent and ionic strength other factors such as utilization of non-chlorinated solvents, influence of matrix effects on extraction efficiency and use of a volume reducer (insert) in the vial to allow automation of chromatographic injections were also evaluated.

2. Experimental

2.1. Chemicals and water samples

Toluene (Panreac), n-hexane and acetone (M Tedia) HPLC grade were used for the preparation of standard solutions and for extraction of pesticides. Extraction essays also used chloroform PA (Synth 99.80%). Primary standards of the analytes trifluralin – Trif. - (99.1%), malathion (97.2%), metolachlor – Metol.- (98.0%), beta-endosulfan - β-End. - (99.8%) and permethrin – Permeth. - (98.0 %) were acquired from Supelco, while the standards of metribuzin - Metrib. -, alpha-endosulfan - α -End.-, endosulfan sulfate – End. Sulf. - (99.5%) and lambda-cyalothrin - λ-Cial.- (98%) were purchased from Dr. Ehrenstorfer GmbH. Deuterated phenanthrene D_{10} – Phen-D_{10} - (98.0%) was acquired from Sigma Aldrich. For ionic strength adjustment, sodium chloride (Quemis PA) was used.

Deionised water (18.2 MΩ.cm) and water samples collected at Cuiabá and Coxipó Rivers and at a lagoon localized at Federal University of Mato Grosso—all in Cuiabá, Mato Grosso, Brazil—were used for method development and evaluation of the matrix effect. After validation, the method was applied to a water sample from Cuiabá River in the municipality of Nobres, an important agricultural region in Mato Grosso state.

2.2. Instrumentation

Adjustable volume micropipettes in the range of 100–1000 µL and of 20–200 µL (Gilson) with precision of ± 0.60 and ± 0.20 µL, respectively, were used for standard solutions preparation. A vortex mixer from Labnet International and a refrigerated centrifuge, Heittech Zentrifugan model Universal 320R, were used in the extraction procedure. pH and electrical conductivity of the water samples were determined in the field using a multiparameter meter HQ 40D Multi HACH. For total organic carbon determination in the water samples, a carbon analyzer, Analytical Aurora model 1030, equipped with automatic sampler from A. I. Analytical was used.

A gas chromatograph Agilent model 6890 equipped with an automatic injector 7683B series and a selective mass spectrometer detector HP 5973 was used for pesticides determination. Pesticides separation was carried out in a capillary column HP-5MS (30 m long, 0.25 mm of internal diameter and 0.25 µm of phase thickness), using helium 6.0 (99.9999 % purity) as carrier gas. Injection volume was 1.0 µL with a carrier gas flow rate of 1.0 mL min^{-1}. Initial oven temperature was 92 °C hold for 2.5 min, followed by heating at 15 °C min^{-1} to 175 °C, which was kept constant for 13 min, followed by another ramp of 20 °C min^{-1} up to 280 °C, which was kept constant for 3 min. Injector and transfer line temperatures were 280 °C and 290 °C, respectively. In the mass detector, electron impact ion source operated at 70 eV at 250 °C and quadrupole temperature was adjusted to 150 °C. Pesticides quantification was done using an internal standard, and detector was operated in the selected ion monitoring mode (SIM), using the target ion for quantification, and two secondary ions for identification. Table 1 presents the retention time, monitored ions by GC-MS, water solubility (WS) and octanol-water partition coefficient (Kow) for all evaluated analytes.

Table 1. Retention time, monitored ions by GC-MS, water solubility and octanol-water partition coefficient of analytes and Phenantrene-d_{10}, used as internal standard

Pesticide	t_r[a]/ (min.)	m/z			WS[b]/ (mg L^{-1})	LogK$_{OW}$[c]
		Target ion	Secondary ion I	Secondary ion II		
Trifluralin	11.02	264.0	290.0	306.0	0.221	5.27
α-Endosulfan	23.72	240.9	238.9	242.9	0.32	4.74
β-Endosulfan	25.05	236.8	238.8	195.0	0.32	4.74
Endosulfan sulfate	27.21	271.6	273.8	228.8	0.48	3.66
Malathion	19.80	125.0	127.0	173.1	148	2.75
Metolachlor	19.97	162.1	238.1	240.1	530	3.40
Metribuzin	15.95	198.0	199.0	182.0	1165	1.65
Permethrin	28.37	163.0	183.0	165.0	0.2	6.1
λ-cyalothrin	25.82	181.0	197.0	208.0	0.005	5.5
Phenantrene-d_{10}	13.20	184.1	188.1	189.1	-[d]	4.57

[a]Analytes retention time; [b]WS – water solubility;[21] [c]Kow – octanol-water partition coefficient;[21,22] [d]data not available.

2.3. DLLME procedure

For DLLME, 750 µL of acetone (disperser solvent) and 150 µL of chloroform (extracting solvent) were added simultaneously to 7.50 mL of sample in a glass test tube (15 x 100 mm), which was closed with a polypropylene stopper, and agitated by vortexing for 10 seconds and then centrifuged at 4000 rpm and -4 °C for 15 min. Afterwards, 100 µL of the supernatant was collected and transferred to a 2 mL auto-sampler vial containing 300 µL volume reducer (insert) for automatic injection. For extracting solvent selection, other experiments, were carried out replacing chloroform by equal volumes of n-hexane or a toluene:n-hexane mixture (40:60 v:v), maintaining the same extraction conditions previously described. The extracting solvent selection was made using the Hildebrand/Hansen solvency theory.[23] The extraction efficiency of solvents was evaluated by comparing the slope (*b*) values of analytical curves constructed by the matrix superposition method, obtained with the different evaluated extractors.

To assess the effects of the sample ionic strength, agitation mode and addition of dispersing solvent, a 2^n full factorial design where n is the number of involved variables, was used.[24] These variables were evaluated in two levels: agitation mode (manual and mechanical in vortex); with and without addition of dispersing solvent; with and without addition of salt at 10% (m/v) for ionic strength modification prior to extraction. Afterwards, an experimental matrix was designed in order to allow evaluation of the effect of each variable as well as the combined effects. For the effects calculation, the linear correlation coefficient (*r*) and slope (*b*) of analytical curves constructed from water samples spiked at different concentration levels for each analyte (2.0, 6.0, 12, 18, 24, 30 and 40 µg L^{-1}) were considered as response. In all experiments, quantification was carried out using matrix superposition [25] with internal standardization using phenantrene-d_{10} at 20 µg L^{-1}. Internal standard was added directly to the matrix before the extraction procedure.

2.4. Method validation

The linearity of instrumental response was evaluated by means of linear correlation coefficient (r) and visual inspection of the analytical curve.[26] Instrumental detection and quantification limits (LDI and LQI, respectively) were determined by the signal to noise ratio.[27] Instrumental precision was determined from ten consecutive injections of standard solutions at three concentrations: 100, 900 and 2000 µg L^{-1}, within the analytical curve interval.

Selectiveness was evaluated by comparing the chromatogram of the extract obtained from a non-contaminated sample with that of a 40 µg L^{-1} standard solution of the pesticides prepared in toluene. Method linearity was assessed by means of the linear correlation coefficient (r) and visual inspection of analytical curves plotted using matrix superposition at concentration interval from 2.0 to 40 µg L^{-1}.[26]

For the matrix effect assessment, standard solutions prepared by matrix superposition using deionized water and non-contaminated natural water samples were used. These water samples were submitted to DLLME and the obtained extracts were used for plotting the analytical curves. The matrix effect was then evaluated by statistical comparison (t-test at 95 %) of the angular coefficients (*b*) of those curves.

Method accuracy and precision were verified by means of standard addition at three fortification levels (2.0, 18 and 40 µg L^{-1}) to different water samples. The fortified samples were left to rest for 24 h before the extraction by DLLME. These essays were carried out in triplicate, over three consecutive days, giving a total of nine replicates at each fortification level. The method detection and quantification limits

(MDL and MQL, respectively) were determined according to Thier and Zeumer (1987).[28]

After validation, the method was applied to a river water sample previously filtered through a glass fiber membrane (0.45 µm) to eliminate solid suspended material. These determinations were carried out in triplicate together with an analytical blank.

3. Results and discussion

3.1. Instrumental parameters

The chromatographic operating conditions allowed the elution of the analytes in 29 min (Figure 1). With the exception of the malathion/metolachlor pair, the resolutions were above 4.60 indicating that separation of the analytes was adequate.[26] Although the resolution for malathion/metolachlor peaks was lower than 1.0, the reliability of the quantification was not impaired since the detection was done by mass spectrometry operated in selected ion monitoring (SIM) mode. For malathion monitoring, the selected ions were m/z 125.0, 127.0 and 173.1 and for metolachlor were m/z 162.1, 238.1 and 240.1, which were not present with a significant intensity in both spectrum simultaneously. Quantification was carried out using the area of the target ion in the selected ion chromatogram, allowing quantification without the interference of one pesticide in the other. The peaks 10 and 11 (Figure 1) refer to the permethrin isomers (cis/trans). Quantification was made in terms of total permethrin.

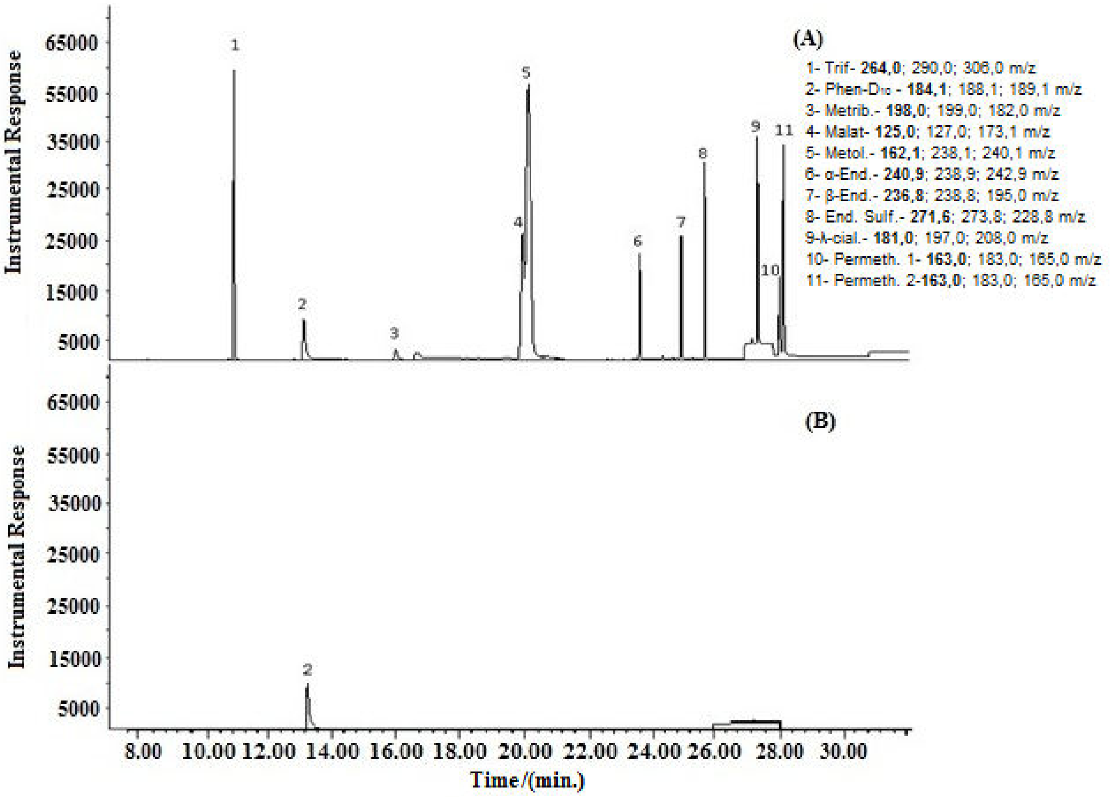

Figure 1. Chromatograms (A) of a standard solution of the analytes at 2000 µg L^{-1} with internal standard at 100 µg L^{-1} and (B) of an analytical blank with internal standard. Above right, description and identification of the peaks and monitored ions, with the main ions in bold

The comparison between the chromatograms of the analytical blank and of a standard solution at 2000 µg L^{-1} showed an absence of interferents in the retention time of the studied analytes. Visual inspection of the analytical curves (Figure 2) and r values higher than 0.99 indicate adequate instrumental linearity for all analytes.[26]

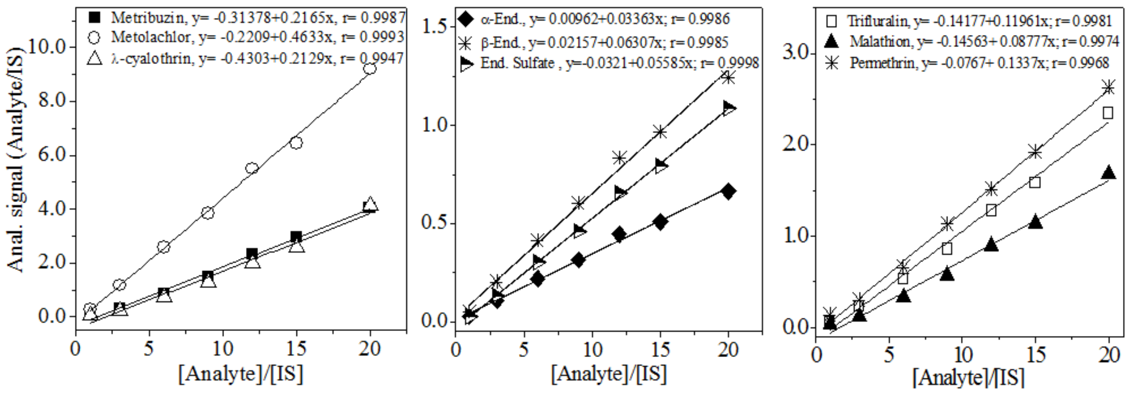

Figure 2. Analytical curves constructed for evaluation of instrumental linearity using standard solutions prepared directly in solvent. The [analyte]/[IS] is the ratio of analyte and internal standard concentration

The RSD % values, resulting from ten sequential injections of standard solutions at concentrations of 100, 900 and 2000 µg L^{-1} were smaller than 12 % indicating acceptable instrumental precision.[26] The calculated IDL varied from 2.5 to 100 µg L^{-1} and the IQL from 8.3 to 330 µg L^{-1}, with the smallest value obtained for α-endosulfan and the highest for metribuzin (Table 2). These limits were obtained from the analytical curve parameters acquired from standard solutions prepared in toluene:hexane (40:60 v/v).

Table 2. Instrumental detection and quantification limits and precision of chromatographic responses.

Analytes	Instrumental precision/ (RSD %)			Instrumental limits/ (µg L^{-1})	
	Concentration levels/(µg L^{-1})			IDL[a]	IQL[b]
	100	*900*	*2000*		
Trifluralin	2.3	3.2	4.7	5.0	17
Metribuzin	9.5	4.8	4.7	100	330
Malathion	12	3.7	4.1	50	170
Metolachlor	2.8	5.2	7.2	5.0	17
α-endosulfan	1.9	3.4	7.6	2.5	8.3
β-endosulfan	1.6	3.9	8.8	2.5	8.3
Endosulfan sulfate	3.9	5.1	9.5	2.5	8.3
λ-cyalothrin	3.7	2.7	1.9	50	160
Permethrin	2.0	2.8	2.8	25	82

[a] Instrumental detection limit; [b] Instrumental quantification limit.

In the DLLME experiments, the ratio of the sample volume (7.5 mL) to the extracting solution (150 μL) gives a concentration factor of 50, and thus, they allow detection/quantitation of the analytes in water at concentrations around 50 times lower than the values presented in Table 2.

The use of volume reducer (insert) in the sampling vial did not affect the linearity and instrumental precision, and can be used in automated injection of extracts.

3.2. Selection of extracting solvent

No significant differences were observed (t 95%) among the *b* values of the analytical curves of the pesticides trifluralin, λ-cyalothrin, α-, β-, and endosulfan sulfate obtained using extractions with the different solvents evaluated (Figure 3). This behavior may be due to the low water solubility and high Kow of these pesticides (Table 1), indicating a higher affinity to the organic phase. Such results suggest that any of the evaluated solvents may be used for the extraction of these compounds.

In the case of malathion and metolachlor, an increasing *b* value was observed from n-hexane to toluene:n-hexane (40:60 v/v) and to chloroform (Figures 14-F and 14-G). The Kow values of malathion and metolachlor (Table 1) indicate that these compounds are more polar than trifluralin, λ-cyalothrin, α, β, and endosulfan sulfate, which justifies the higher extraction efficiency with increasing polarity of the extracting solvent. Differently from the less polar pesticides, the extracting solvent polarity greatly influences the extraction of more polar ones.

The *b* value of the metribuzin analytical curve (Figure3-H) obtained using chloroform as an extractor was about 10 times higher than the one obtained with toluene:n-hexane mixture (40:60 v/v). Metribuzin is the most polar among the studied pesticides as indicated by its low Kow (Table 1), justifying the great difference observed between these *b* values, as well as the fact that this analyte was not extracted in n-hexane.

On the other hand, for permethrin, greater *b* values were obtained using n-hexane (Figure 3-I). No significant difference between the *b* values was observed when using chloroform and the mixture toluene:n-hexane (40:60 v/v).This pesticide is less polar, and as expected, the *b* value increased as the solvent polarity decreased.

In general, it was observed that for pesticides with very high Kow, the extractor nature did not influence extraction efficiency. However, as Kow decreases, solvent polarity tends to be more important for obtaining an adequate extraction. The three studied extracting solvents provided good linearity for all the analytes. The toluene:n-hexane mixture (40:60 v/v) provided extraction efficiency comparable to that of chloroform, except for metribuzin, and so it was chosen for the next steps of method evaluation. Although toluene is as toxic as chloroform, only a fraction of it is used in the extracting solution.

3.3. Effect of agitation mode (AG), dispersing solvent addition (SD) and ionic strength (SF) on the extraction efficiency

From the experimental matrix, eight experiments were carried out giving analytical curves, constructed by the matrix superposition method, for each analyte. The *b* values of these curves were used as response to calculate the main and combined effects.

The agitation mode did not have a significant effect (t 95%) in the extraction of permethrin, α- and β-endosulfan. For the other analytes, a 24% increase, on average, was observed in the *b* value when vortex agitation was used, pointing to an increase in extraction efficiency. Yiantzi and colleagues evaluated the determination of bisphenol A, octylphenol and nonylphenol in water by vortex-assisted liquid–liquid microextraction

(VALLME), and highlighted the gain in extraction efficiency provided by vortex agitation.[29] According to these authors, the effect of a vortex agitation is to swirl the fluids and create a vortex that in elevated speeds generally results in the breaking up of the extractor solvent into fine droplets, thus improving the extraction efficiency. Ojeda and colleagues also highlight the contribution of the vortex agitator in DLLME. [30]

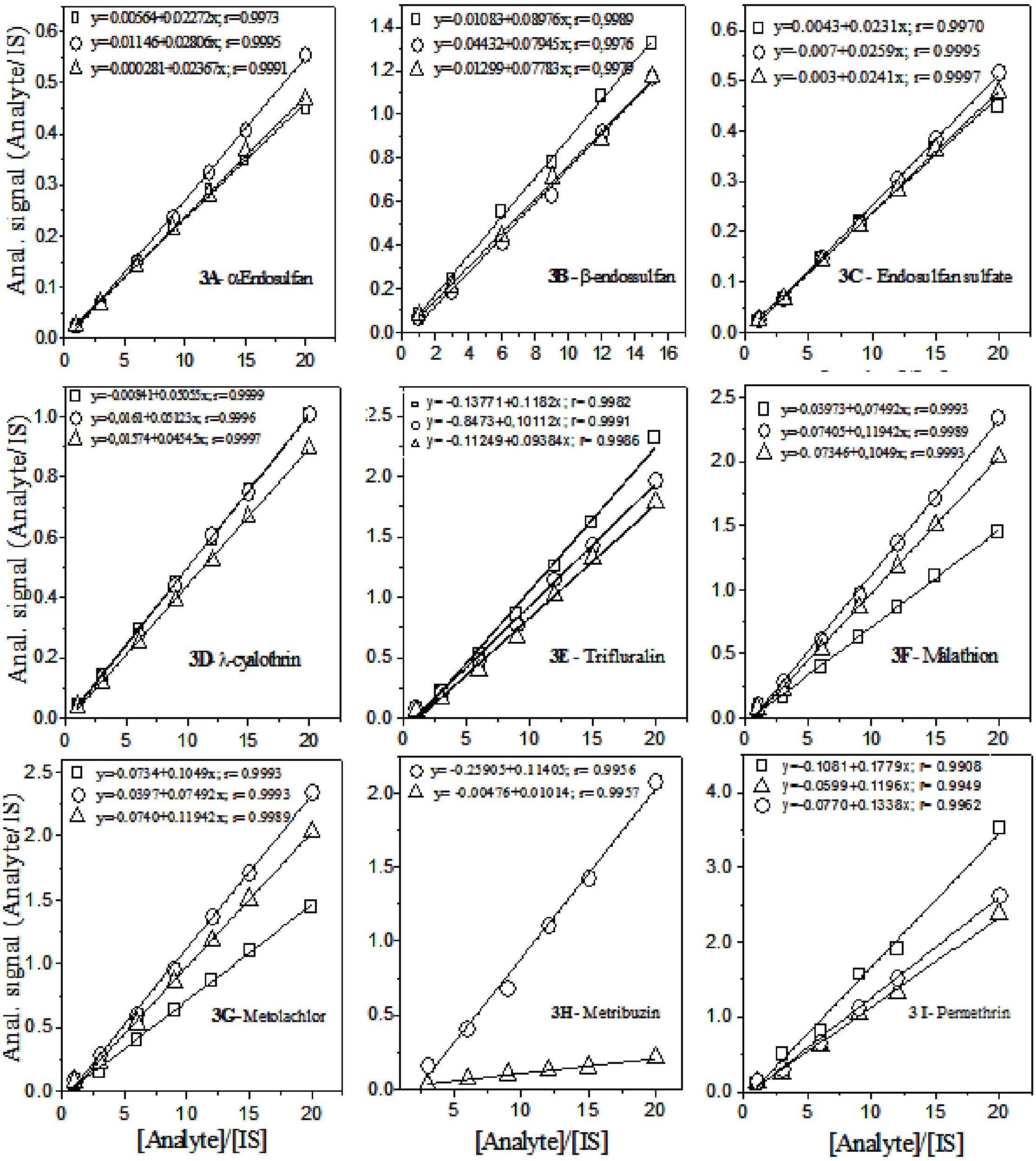

Figure 3. Analytical curves of the analytes obtained by matrix superposition with extraction using different solvents: ○ - chloroform; □ - n-hexane, Δ - toluene:n-hexane (40:60 v/v). The [analyte]/[IS] is the ratio of analyte and internal standard concentration

The addition of dispersing solvent, except for permethrin, did not have a significant effect (t 95%) on the analytes extraction. The vortex agitation is effective for dispersion of low density solvents in micro-droplets, and the use of dispersive solvent in the liquid-liquid microextraction procedures can be dispensed for several analytes.[29,30] In fact, the high recovery of most analytes in tests made with vortex, without the addition of dispersing (acetone), shows that the stirring mode is effective in dispersing the extractor. For permethrin, acetone increased the extraction efficiency by about 50%. In this case, acetone may have contributed to the processes of mass transference, and its role as disperser may have been secondary.

In DLLME, the increase in ionic strength may improve phase separation contributing to organic phase recovery, since the presence of salt decreases solvent solubility in the aqueous phase. In addition, the salting out effect may cause the reduction of analytes solubility in the aqueous phase, increasing its partition to the organic phase.[18] However, in this work the increase in samples' ionic strength, except for permethrin and trifluralin, had no significant effect (t 95%) on the extraction efficiency of the analytes. For permethrin and trifluralin, the increase in sample ionic strength caused a decrease in the extraction efficiency of about 30 % and 50%, respectively.

Several studies have reported similar results in the extraction of different chemical groups. Wu *et al.* observed a significant decrease in the extraction percentage of carbofuran, carbaryl, pirimicarb and diethofencarb as salt was added to the water sample.[31] A similar behavior was also reported by Alves et al., who showed that the addition of 1, 5 and 10% NaCl to the aqueous samples caused a decrease in organophosphate extraction.[32]

These results indicate that factors such as solubility and polarity, usually used to explain the partition of the analyte between the matrix and the extraction solution, may not be sufficient to completely justify observed differences in analytes recovery. In the present work, the eight analytes studied belong to six different chemical classes with different heteroatoms and functional groups. These differences may provide a large number of complex interactions that do not allow a direct evaluation of the extraction process.

Considering the above presented results, the subsequent experiments were carried out without salt addition, using vortex agitation and dispersing solvent addition, due to its effect on the extraction of permethrin.

3.4. Method validation

The r values obtained from curves constructed by matrix superposition method and internal standardization were higher than 0.99 (Table 3), indicating a high linear correlation between analytes concentration and analytical signal.

Table 3. Parameters of the analytical curves obtained by matrix superposition and method limits of detection and quantification

Analyte	Analytical curves parameters	r	MDL[a]/ (µg L^{-1})	MQL[b]/ (µg L^{-1})
Trifluralin	y = 0.1022x -0.1265	0.9940	0.021	2.0
Malathion	y = 0.0912x – 0.1091	0.9929	0.045	2.0
Metribuzin	y = 0.0732x – 0.0025	0.9978	0.028	2.0
Metolachlor	y = 0.4075x – 0.089	0.9992	0.17	2.0
α-endosulfan	y = 0.0238x – 0.0088	0.9991	0.0070	2.0
β-endosulfan	y = 0.0194x – 0.0073	0.9995	0.0063	2.0
Endosulfan sulfate	y = 0.0382x – 0.0244	0.9987	0.018	2.0
λ-cyalothrin	y= 0.0128x- 0.0522	0.9909	0.025	2.0
Permethrin	y= 0.0372x- 0.0286	0.9900	0.046	2.0

[a]MDL: Method detection limit, [b]MQL: Method quantification limit.

According to Thier and Zeumer, MQL coincides with the concentration of the lowest fortification level that results in a recovery percentage higher than 70% with RSD less than 20%. Thus, for all the studied analytes, the MQL value was 2.0 μgL^{-1}. [28]

It is important to emphasize that with the comparison of MQL values with regulatory limits for pesticide residue in drinking water, we should consider that these limits must be appropriate for national, regional and local circumstances, which requires adaptation to environmental, social, economic and cultural circumstances and priority settings.[33] Thus, it is common that regulatory agencies contemplate different pesticides or establish different limits for the same pesticide. For instance, the WHO guidelines value for drinking water, using health based criteria for metolachlor, trifluralin and permethrin are 10, 20 and 300 μg L^{-1}, respectively. [33] The guideline values for Canadian drinking water quality, using the same criteria, listed 50, 45, 190 and 80 μgL^{-}1 as maximum acceptable concentrations (MAC) for metolachlor, trifluralin, malathion and metribuzin, respectively.[34] The Australian drinking water guidelines, based on 10% of acceptable daily intake (ADI) for metolachlor, trifluralin, permethrin metribuzin and endosulfan are 300, 50, 100, 50 and 30 μg L^{-1}, respectively.[35] A drinking water health advisory (DWHA), issued by USEPA, is an estimate of a concentration that would result in no known or anticipated health effects, or for carcinogens, a specified cancer risk, and is calculated from the lowest-observed-adverse-effect-level (LOAEL) in toxicity tests. The DWHA for metolachlor, trifluralin, malathion and metribuzin are 100, 5, 100 and 200 μg L^{-1}, respectively.[35]

The MQL values obtained in this study are below the regulatory limits described by all mentioned environmental agencies.

The recoveries obtained in the addition and recovery test ranged from 47 to 115% with RSD less than 16% (Table 4) for all studied analytes, indicating acceptable accuracy and precision.[26]

Table 4. Recovery percentages and RSD values obtained for the pesticides studied

Analytes	Recovery ± RSD[a] (%)		
	Level[b] 1 (2 μg L^{-1})	Level 2 (18 μg L^{-1})	Level 3 (40 μg L^{-1})
Trifluralin	60±12	87± 11	97±5
Metribuzin	78±16	106±5	115±4
Malathion	82±5	110±6	107±8
Metolachlor	91±7	109±8	100±2
α-endosulfan	86±9	90 ± 4	81±5
β-endossulfan	70±5	93 ± 7	85±9
Endosulfan sulfate	74±6	108±7	100±5
λ-cyalothrin	47±12	92± 9	101±6
Permethrin	60±15	89± 10	98±8

[a] n= 9; [b] fortification levels used in the recovery essays.

The acceptable recovery is dependent, among other factors, on the concentration level of the analyte in a given matrix. For a concentration level in the order of μg L^{-1}, AOAC and other works that address validation methods consider recoveries between 40 and 120% with RSD % of up to 45% as acceptable.[36,37] Several studies that use DLLME with non-chlorinated solvents to determine pesticides in water report recoveries between 50 and 114% for concentrations levels between 1 and 10 µg L^{-1}.[38-40]

Comparing the chromatograms of the analytical blank and of a standard solution did not reveal the presence of interferents in the retention times of the analytes studied confirming the method selectivity.

3.5. Evaluation of matrix effect

The matrix effect was evaluated by means of analytical curves constructed by matrix superposition using water samples collected in the field and deionized water (Item 2.4). These samples were characterized in terms of pH, conductivity and total organic carbon, which can influence the analytes behavior in the matrix and directly affect the extraction process.[13,41]

Samples pH ranged from slightly acidic to slightly basic, which is common in natural waters where the pH typically ranges from 6.0 to 8.5 (Table 5). Electrical conductivity remained in the range from 10 to 1000 µS cm^{-1}, commonly found in freshwater.[41]

Table 5. Properties of the samples used in matrix effect evaluation.

Sample	pH	Conductivity/ (µS cm^{-1}) at 25 ^{0}C	TOC[a]/ (mg L^{-1})
A1 - Deionized water	6.50	18.20	ND[b]
A2- Cuiabá River	6.88	71.10	3.00
A3- Coxipó River	6.85	65.30	3.69
A4- UFMT lagoon	7.39	328.00	21.85

[a]Total organic carbon [b]Note detected

The TOC concentration ranged from not detected in deionized water to 21.85 mg L^{-1} in sample A4, collected from a lagoon in eutrophication process. This sample was used to evaluate the methods' robustness under conditions of high TOC concentrations, characteristic common in impacted water bodies. In surface waters, TOC concentrations generally are less than 10 mg L^{-1}.[41]

Despite the variable properties of these samples, no significant difference ($t_{95\%}$) was observed between the *b* values of the analytical curves obtained by matrix superposition. These results indicate that the matrix had no effect on the analytes extraction.

After being validated, the method was applied to water samples collected in an agricultural region of Mato Grosso State. In these samples, only permethrin was detected. The samples were collected where these compounds are commonly used, during the maize cultivation period.

4. Conclusions

DLLME showed to be a good alternative to classical extraction methods for the multiclass pesticides studied, since it provides acceptable precision, accuracy and robustness. The toluene:n-hexane mixture (40:60 v/v) provided an extraction efficiency similar to that of chloroform, and may replace this chlorinated solvent in the extraction of analytes studied. The best extracting conditions were: use of vortex agitation, addition of acetone as dispersing solvent, without salt addition. When applied to water samples with different physicochemical properties, no matrix effect was observed, demonstrating the method robustness. The use of a volume reducer (insert) in the vial allowed the automation of chromatographic injections without compromising method accuracy overcoming one of the disadvantages of this method. The main advantages of this method are its simplicity due to the few steps and use of small amounts of sample and organic solvents, which reduces the cost of analysis and exposition of the analyst to toxic solvents.

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