Journal of Chromatography B 1156 (2020) 122296

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Validation of a methodology by LC-MS/MS for the determination of triazine, triazole and organophosphate pesticide residues in biopurification systems

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ARTICLE INFO

Keywords:
Biopurification system
Pesticides
Validation methodology
Biomixture
LC-MS/MS

ABSTRACT

Biopurification systems are useful in the management of pesticide residues and provide an option to dispose wastewaters of agricultural origin derived from pesticide application practices. The analysis of pesticide residues in the biopurification system biomixture is necessary to determine whether the removal of the target compounds occurs with reliable results. In this study, the pesticide extraction methodology was optimized and validated in a biomixture composed of coconut fiber, compost and soil, to determine a total of 43 molecules, distributed among triazines (10), triazoles (13) and organophosphates (20) using liquid chromatography coupled to a triple quadrupole mass spectrometer. For the validation, the parameters of linearity, matrix effect, limit of determination (LOD), specificity, selectivity, precision, trueness and robustness in the proposed biomixture were evaluated. The analyses of those parameters revealed satisfactory results of the method for most of the compounds, with the exception of diclorvos and ciromazine, for which the development of an alternative method is recommended. Once the extraction methodology was validated, the removal of eight molecules was assayed in a biopurification system used for the simultaneous treatment of a mixture of pesticide commercial formulations. Although most of the compounds were at least partially removed, none of them was eliminated at levels below the LOD. The removal pattern of ametryn, atrazine, chlorpyrifos, malathion and terbutryn was comparable to those obtained in other efficient biomixtures, and the highly recalcitrant triadimenol was eliminated; nonetheless, tebuconazole and diazinon were not significantly removed.

1. Introduction

The advances in agricultural science (e.g., improved soil and water management practices and the use of agrochemicals, organic fertilizers, biological control and pesticides) allowed the enhancement of food production. Although the use of pesticides was initially intended to minimize the effect of pests on crops and enhance their productivity, pesticide application may also cause undesirable effects on human health and the environment as they inevitably reach non-target organisms [1–4].

The presence of pesticides in the environment is worrisome because of their toxicity and persistence [3,4]. The effects on humans and other organisms are compound-specific and vary depending on toxicity, route and time of exposure [3,4]. In the environment, aerial fumigations, superficial runoff, and infiltrations to groundwater are some common causes of contamination by pesticides [5–14]. Implementing good management practices and adequate treatment systems can mitigate point-source pollution by pesticides, hence, reducing its environmental impact. For instance, in situ treatment can reduce pesticide residues remaining in knapsack sprayers. However, this requires treatment systems that are accessible to farmers and easy to operate [6,15–17].

Bioremediation is regarded as a feasible treatment of wastewaters containing high loads of pesticides [15–18]. Particularly, biopurification systems (BPS) stand out for their low cost and maintenance, easy construction and versatility. BPS include biofilters, Phytobac® and biobeds [6,9,11,12,19,20]. These configurations use a biological active matrix that retains the contaminants and stimulates the rapid degradation of the compounds by microbial activity [6,7,9,10,19,21]. The biological matrix is a biomixture that comprises soil, compost or peat and a lignocellulosic material at a 1:1:2 volumetric ratio [6,7,10]. Soil is the main source of degrading microorganisms; thus, it is desirable to use soils pre-exposed to the target pesticides. Compost or peat is added for enhancing the adsorption capacity, also helping to control the temperature and humidity of the system. The lignocellulosic material is

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https://doi.org/10.1016/j.jchromb.2020.122296
Received 27 May 2020; Received in revised form 25 July 2020; Accepted 29 July 2020
Available online 01 August 2020
1570-0232/

a lignin rich source for microorganisms, which favors the growth and activity of ligninolytic fungi, known for their wide capacity to degrade organic pollutants; in this respect, the use of different components such as straw, bagasse, coconut fiber, citrus peel, branches, olive leaves, wood curl, paper, rice pellets, among others [6,7,9,10,17,22], has been applied depending on geographical availability.

BPS are effective degrading molecules with different action modes like carbamates [8,23,24], organophosphates [25–28] and triazines [2,27,29–32]. Pesticides containing such molecules are not applied simultaneously, but rather in cycles in each crop. Therefore, pesticide active ingredients and their co-formulated materials can be treated in the BPS throughout the season as they are used in the crops [9,33]. Thus, the objectives of this study were (i) to develop and validate a LC-MS/MS multiresidue methodology for the determination of more than 40 pesticides in a conventional biomixture, and (ii) to evaluate the efficiency of a BPS during the treatment of wastewater containing commercial formulations of diverse pesticides, applying the validated methodology.

2. Materials and methods

2.1. Chemicals and reagents

The analytical standards anilophos (98.9%), azinphos-methyl (98.8%), cadusafos (97.2%), chlorpyrifos (99.5%), dichlorvos (98.4%), dimethoate (99.5%), edifenphos (98.5%), ethoprophos (98.8%), fenamiphos (99.0%), phoxim (99.4%), heptenophos (98.6%), isazofos (99.2%), isofenphos (99.5%), malathion (99.5%), methamidophos (99.5%), monocrotophos (99.5%), pirimiphos-methyl (99.5%), cyromazine (99.5%), prometon (99.5%), prometryn (99.5%), simetryn (99.5%), terbutryn (98.1%), bitertanol (99.5%), cyproconazole (99.5%), epoxiconazole (99.5%), fenbuconazole (99.5%), flusilazole (98.6%), hexaconazole (99.3%), myclobutanil (98.0%), tebuconazole (98.0%), triadimefon (99.5%), triadimenol (98.7%) were purchased from ChemService (Penssylvania, U.S.). Standards acephate (99.0%), coumaphos (99.0%), fenthion (99.0%), triazophos (80.0%), amethryn (98.0%), atrazine (99.0%), cyanazine (98.5%), simazine (98.0%), terbuthylazine (98.5%), difenoconazole (98.7%), paclobutrazole (98.5%), propiconazole (99.0%), carbofuran-d_3 (98.0%) and linuron-d_6 (98.5%) were acquired from Dr. Ehrenstorfer (Augsburg, Germany).

Commercial formulations of atrazine (Atranex®, 90% w/w), ametryn (Agromart®, 50% w/v), chlorpyrifos (Solver™ 48% w/v), diazinon (Zinoncoop 60 EC, 60% w/v), malathion (Bioquim malathion, 5% w/w), tebuconazole/triadimenol (Silvacur® Combi 30 EC, 22.5% and 7.5% w/v, respectively) and terbutryn (Terbutrex®, 50% w/v) were acquired at local markets.

Distilled and deionized (DDI) water (< 18 mΩ) was produced in the laboratory, formic acid (ACS, ISO, Reag. Ph Eur, 98–100%), glacial acetic acid (ACS, ISO, Reag. Ph Eur 100%), acetonitrile (LichroSolve®, > 99.8%) and methanol (LichroSolve®, 99.8%) were purchased from Merck (Darmstadt, Germany). Anhydrous magnesium sulfate ($MgSO_4$, > 99.5%) and sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$, > 99.5%) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.), bondesil-PSA (40 μm particle size) from Agilent (Santa Barbara, CA, U.S.), Sepra-C18 from Phenomenex (Torrance, CA, U.S.) and sodium chloride from JT Baker (PA, U.S.).

2.2. Analytical solutions

Stock solutions of individual analytes ranging from 700 to 3800 mg/L were prepared, depending on their solubility, in methanol or acetonitrile. Primary dilution standards (PDS) at 10 mg/L were prepared using acidified acetonitrile (0.1% formic acid). Calibration standards from 1 to 500 μg/L were prepared in a mixture (1:1 v/v) of acetonitrile:water acidified with 0.1% formic acid, and in the matrix extract (matrix-matched standards). The stock solutions were stored at −15 °C in a freezer while the PDS and the calibration standards at < 6 °C.

2.3. Samples and sample preparation

The biomixture (pH 6.4; C 4.83%; N 0.32%; C/N 15.2; P 0.22%; Ca 0.48%; Mg 0.71%; K 0.19%; S 0.07%; Fe 31 192 mg/kg; Zn 91 mg/kg; Mn 521 mg/kg; B 66 mg/kg; EC 0.6 mS/cm) employed consisted of coconut fiber, compost and soil (45:12:43, volumetric ratio). This biomixture was previously optimized for the removal of carbofuran [8]. The biomixture samples were fortified with the target pesticides during the optimization and validation of the method. Pesticides were extracted following a QuEChERS modified procedure described elsewhere [8]. Carbofuran-d_3 and linuron-d_6 were used as surrogate and internal standard, respectively. Quality controls included blank samples (pesticide-free biomixture), blanks for calibration curve (pesticide-free biomixture without surrogate or internal standard; extract used for the calibration curve in the matrix) and solvent reference (procedure reagents without sample).

2.4. Chromatographic conditions

LC-MS/MS analyses were carried out using an Agilent 1290 Infinity II LC System (Santa Clara, CA, U.S.) Ultra-high performance liquid chromatography (UHPLC) coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was done at 40 °C injecting 6 μL of the sample (2 μL loop) in a Poroshell 120 EC-C18 column (100 mm × 2.1 mm i.d., particle size 2.7 μm) and using a binary mobile phase consisting of acidified water (formic acid 0.1% v/v, solvent A) and acidified methanol (formic acid 0.1% v/v, solvent B) at a flow rate of 0.3 mL/min. The conditions were as follows: 30% of solvent B for 3 min, 15 min linear gradient to 100% solvent B, 4 min at 100% solvent B, 0.1 min gradient back to 30% of solvent B, and 5 min at initial conditions. The mass spectrometer used a jet stream (electrospray) ionization source operating at a gas temperature of 300 °C; gas flow 7 L/min, nebulizer 45 psi; sheath gas temperature 250 °C; sheath gas flow 11 L/min. The other conditions were capillary voltage 3500 V; nozzle voltage 500 V; heater MS1 and MS2 100 °C. Data acquisition was performed using the MassHunter software (Santa Clara, CA, U.S.).

2.5. Optimization of the transitions

Each molecule was injected individually into the LC-MS/MS system to optimize the fragmentor voltage and the collision cell energy for all the transitions. The optimization was done in five acquisition modes including (i) MS2 Scan to find the precursor ions (in positive and negative electrospray ionization), (ii) Product ion to find the optimized fragmentor voltages (ranging from 50 to 210 V) and the main fragments, (iii) multiple reaction monitoring (MRM) for optimizing the collision cell energies (range of 1 to 45 V) of each fragment; all those methods were done without column; (iv) MRM with column to find the retention time, and (v) dynamic MRM (dMRM) to define a specific acquisition time range; this acquisition method was applied with the chromatographic gradient conditions.

2.6. Optimization of the analytical method

A 2^3 full-factorial design was used to study the effects of stirring (manual vs. automated), amount of water added to the sample (5 mL vs. 10 mL) and the amount of magnesium sulfate added for cleaning up (450 mg vs. 900 mg) on the extraction process. Each experiment was performed in duplicates. The recovery for each evaluated condition was the measured response.

2.7. Method validation

The validation of the method was conducted following the

guidelines of the European Commission-Directorate General for Health and Food Safety [34]. Analytical parameters evaluated included linearity, limit of determination (LOD), matrix effects, trueness, precision, robustness, application range, specificity and selectivity. Every sample was analyzed applying the methodology described in 2.3. Specificity and selectivity were calculated using the transitions, retention time and ion ratio for each compound in each analyzed sample. The linearity of the calibration curves was evaluated with analytical standard solutions at ten concentration levels (5, 10, 20, 50, 90, 135, 170, 200, 250 and 500 μg/L). The standards were prepared in blank biomixture extract and in acidified (0.1% formic acid) water-acetonitrile (1:1). Each calibration curve was prepared in triplicate. LOD was estimated on a signal to noise ratio (S/N) ratio > 10. LOD was the lowest spiked level with good criteria of trueness and precision. For LOD, seven blank samples were spiked at 10 μg/kg.

Matrix effects (ME) were calculated as the correlation percentage between the slopes of the solvent calibration curve and the calibration curves prepared with blank biomixture extracts, using the expression $ME\ (\%) = [(Slope_{matrix}/Slope_{solvent})\text{-}1] \times 100$.

Trueness and precision were evaluated spiking the blank biomixture at four different concentrations (10, 50, 150 and 350 μg/kg), with seven replicates for each spiked level (n = 7) and three analysts, for a total of 21 samples. Trueness and precision were determined as recovery and relative standard deviation percentages (RSD), respectively.

The robustness was measured applying the Youden-Steiner test [35]. It was applied at two effect levels of seven factors or conditions. To apply the test, seven changing factors in two conditions were employed (Table 1). This study was developed according to the experiment guide in Table 2. Capital letters indicate the experiment was applied with the values of the condition "HIGH", and the lower-case letters with the value of the condition "low".

The robustness was calculated by comparing the difference of the values of each factor, according to the experiment, in relation to the value calculated as a critical value, which depends on the total standard deviation of the experiment. To evaluate selectivity and specificity, five blank samples and five spiked samples at 25 μg/kg were prepared, then, the signals derived from both kinds of samples were compared to differentiate between signals provided by the matrix and the analyte, respectively.

The application range was calculated with an initial concentration of 2000 μg/kg in samples that were subsequently diluted to intermediate concentrations of the calibration curve, to evaluate the efficiency of the method to achieve good results at high concentrations.

2.8. Determination of pesticide removal in a functional BPS

A pilot-scale functional BPS conformed by a 204 L plastic barrel containing 104 L (~56.2 kg) of the biomixture was employed to assay the performance of the method at field relevant concentrations. A pesticide solution containing commercial formulations of ametryn, atrazine, chlorpyrifos, diazinon, malathion, tebuconazole, terbutryn and triadimenol was disposed into the biomixture. Disposal was performed using a watering can; uniform application allowed a free drip flow, with a more homogeneous distribution on the contact surface; the biomixture was thoroughly mixed immediately after pesticide application. Composite samples were collected at 0, 9, 14, 21, 28, 38, 47 and 53 d after pesticide disposal, by withdrawing small portions of biomixture with basin and shovel from the upper, middle and lower parts of the system in four different points distributed randomly at each sampling time; subsamples were pooled to collect around 200 g. At least 100 g of the biomixture was kept in custody and stored at −20 °C. The remaining biomixture was reincorporated into the BPS. When possible, removal data for each compound was modeled according to a first order model (SigmaPlot 14.0) to estimate removal half-life (DT_{50}) values.

Table 1
Experimental design for the determination of the robustness test using the Youden-Steiner test.

ID Factor	Factor	Condition 1	Condition 2
F1	Water rest time (min)	20 (A)	30 (a)
F2	Acetonitrile stir time (min)	2 (B)	5 (b)
F3	Type of magnesium sulfate (brand)	Sigma (C)	Fluka (c)
F4	Shaker agitation time (min)	30 (D)	15 (d)
F5	Centrifuge time (min)	7 (E)	3 (e)
F6	Centrifuge speed (rpm)	4000 (F)	2500 (f)
F7	Water bath temperature (°C)	30 (G)	40 (g)

Table 2
Distribution of the experimental conditions of the study factors for the Youden-Steiner test.

ID Factor	E1	E2	E3	E4	E5	E6	E7	E8
F1	A	A	A	A	a	a	a	a
F2	B	B	b	B	B	B	b	b
F3	C	c	C	C	C	c	C	c
F4	D	D	d	D	d	d	D	D
F5	E	e	E	E	e	E	e	E
F6	F	f	f	F	F	f	f	F
F7	G	g	g	G	g	G	G	g
RESULT	**s**	**t**	**u**	**V**	**w**	**x**	**y**	**z**

3. Results and discussion

3.1. Optimization of pesticide molecules

Physicochemical properties of each compound were used to decide on the optimization experiments to be developed (Table S1, Supplementary Material) and to define the ionization mode, precursor ion and the solubility of the pesticide in the organic solvent of the methodology. Then, the fragmentor voltages and the collision cell energies were optimized for the precursor and the product ions for each molecule. The optimization results are shown in Table 3.

Each compound was first injected individually without the analytical column for identifying the best working conditions. MS2 Scan acquisition mode was conducted to identify the precursor ion of each molecule at positive or negative electrospray ionization mode (ESI^+ or ESI^-). All tested molecules showed better results in ESI^+ and worked with the protonated molecules $[MH]^+$. The use of adducts of sodium, potassium or ammonium was avoided, as they produce a lower sensibility in the next optimization steps [36] (Fig. 1).

Then, the main fragments of the precursor ion were determined in Product Ion acquisition mode. Likewise, the fragmentor voltage that gives a signal with a greater intensity was selected. At least two product ions were selected for each molecule (Fig. 1).

A third acquisition mode, MRM without column, was used to ensure the product ions or main fragments were selected at the maximum voltage signal (Fig. 1). Finally, the MRM mode with column was applied with the chromatographic column conditions and a proposed mobile phase gradient, at optimized values for the individual injection of each molecule. Thus, the retention time of each molecule and the evaluation of the transitions, as well as possible interference signals for other molecules were obtained. Table 3 summarizes the optimized values for all molecules.

3.2. Method optimization

The matrix of study was a biomixture, a sample with high organic and low water content (< 30%). Pesticide extraction from this type of matrix with low water content was previously evaluated with QuEChERS methodologies [2,7,8,23,27,37].

A 2^3 full-factorial design was used to determine the method with

Table 3
Optimization of transitions for the quantification (Q) and confirmation (q) of the studied analytes by LC-MS/MS.

Compound	Transition		Fragmentor (eV)	Collision energy (eV)	Retention time (min)	Type of transition
	Precursor ion	Product ion				
Acephate	184	143	60	5	0.91	Q
		95		25		q
Amethryn	228	186	106	17	8.05	Q
		96		25		q
Anilophos	368	199	70	10	13.58	Q
		171		15		q
Atrazine	216	174	106	17	9.42	Q
		96		25		q
Azinphos-methyl	318	132	60	13	10.65	Q
		125		17		q
Bitertanol	338	99	82	13	14.05	Q
		269		5		q
Cadusafos	271	159	70	5	14.32	Q
		131		20		q
Chlorpyrifos	350	97	90	30	15.74	Q
		198		15		q
Coumaphos	363	277	116	25	13.67	Q
		307		13		q
Cyanazine	241	214	100	15	7.05	Q
		104		30		q
Cyproconazole	292	70	110	15	12.42	Q
		125		30		q
Cyromazine	167	85	104	17	0.87	Q
		60		21		q
Dichlorvos	221	109	104	13	7.40	Q
		79		29		q
Difenoconazole	406	251	126	25	14.42	Q
		337		13		q
Dimethoate	230	199	70	3	3.45	Q
		125		20		q
Edifenphos	311	111	90	20	13.46	Q
		283		10		q
Epoxiconazole	350	121	106	25	12.75	Q
		101		40		q
Ethoprophos	243	97	84	33	12.52	Q
		131		17		q
Fenamiphos	304	217	138	21	13.13	Q
		234		13		q
Fenbuconazole	337	70	116	17	12.95	Q
		125		40		q
Fenthion	279	247	104	9	13.83	Q
		105		25		q
Flusilazole	316	165	110	25	13.14	Q
		247		15		q
Hexaconazole	314	70	116	21	13.85	Q
		159		33		q
Heptenophos	251	127	110	10	10.23	Q
		109		30		q
Isazofos	31	120	94	29	12.25	Q
		162		13		q
Malathion	331	99	82	25	11.83	Q
		127		9		q
Methamidophos	142	94	90	10	0.98	Q
		125		10		q
Monocrotophos	224	127	62	13	1.85	Q
		193		5		q
Myclobutanil	289	70	106	17	11.86	Q
		125		37		q
Paclobutrazol	294	70	110	15	11.81	Q
		125		35		q
Phoxim	321	192	94	9	13.88	Q
		115		21		q
Pirimiphos-methyl	306	164	90	20	13.38	Q
		108		30		q
Prometon	226	142	116	21	7.15	Q
		184		17		q
Prometryn	242	200	126	17	9.71	Q
		158		21		q
Propiconazole	342	159	126	29	13.61	Q
		69		21		q
Simazine	202	124	106	17	7.42	Q
		104		25		q

(*continued on next page*)

Table 3 (*continued*)

Compound	Transition		Fragmentor (eV)	Collision energy (eV)	Retention time (min)	Type of transition
	Precursor ion	Product ion				
Simetryn	214	124	106	17	6.08	Q
		96		25		q
Tebuconazole	308	70	106	21	13.55	Q
		125		40		q
Terbuthylazine	230	174	104	13	11.33	Q
		96		29		q
Terbutryn	242	186	96	17	9.83	Q
		91		29		q
Triadimefon	294	197	94	13	11.74	Q
		69		21		q
Triadimenol	296	70	72	9	12.35	Q
		99		13		q
Triazophos	314	162	100	15	12.25	Q
		119		35		q
Linuron-d_6 (IS)	255	160	92	17	11.12	Q
		185		13		q
Carbofuran-d_3 (SS.)	225	165	86	9	7.67	Q
		123		21		q

IS: internal standard; SS: surrogate standard.

best recovery for more molecules in the same experiment. Three critical factors of the methodology were evaluated: the amount of water added to 5 g of the biomixture (5 mL vs. 10 mL), the type of agitation employed during extraction (automated vs. manual), and the amount of magnesium sulfate added for sample drying in the cleaning stage (450 mg vs. 900 mg). The experimental conditions are shown in Table S2 (Supplementary Material). The examination of the effects of the main factors and the interactions between factors revealed that individual factors produce the greatest inference on the analysis methodology.

The first factor evaluated was the amount of water added to the matrix (Fig. 2); the recoveries exhibited differences of up to 5% for triazines and triazoles and up to 19% for organophosphates. The addition of 10 mL of water enhanced the extraction recovery rates of 3–5% out of 37 molecules, particularly organophosphates. This effect was attributed to the time of hydration of the matrix during the extraction, since it allows the opening of the pores of the matrix which leads to a better extraction of the molecules by the solubility of the acetonitrile in water medium [38,39].

Recoveries for cyromazine, cyproconazole, acephate and methamidophos decreased as the volume of water was increased. This is attributed to the high water solubility of these molecules, which causes a greater affinity to the aqueous phase and subsequent losses by the addition of sodium sulfate to dry the sample. However, other molecules

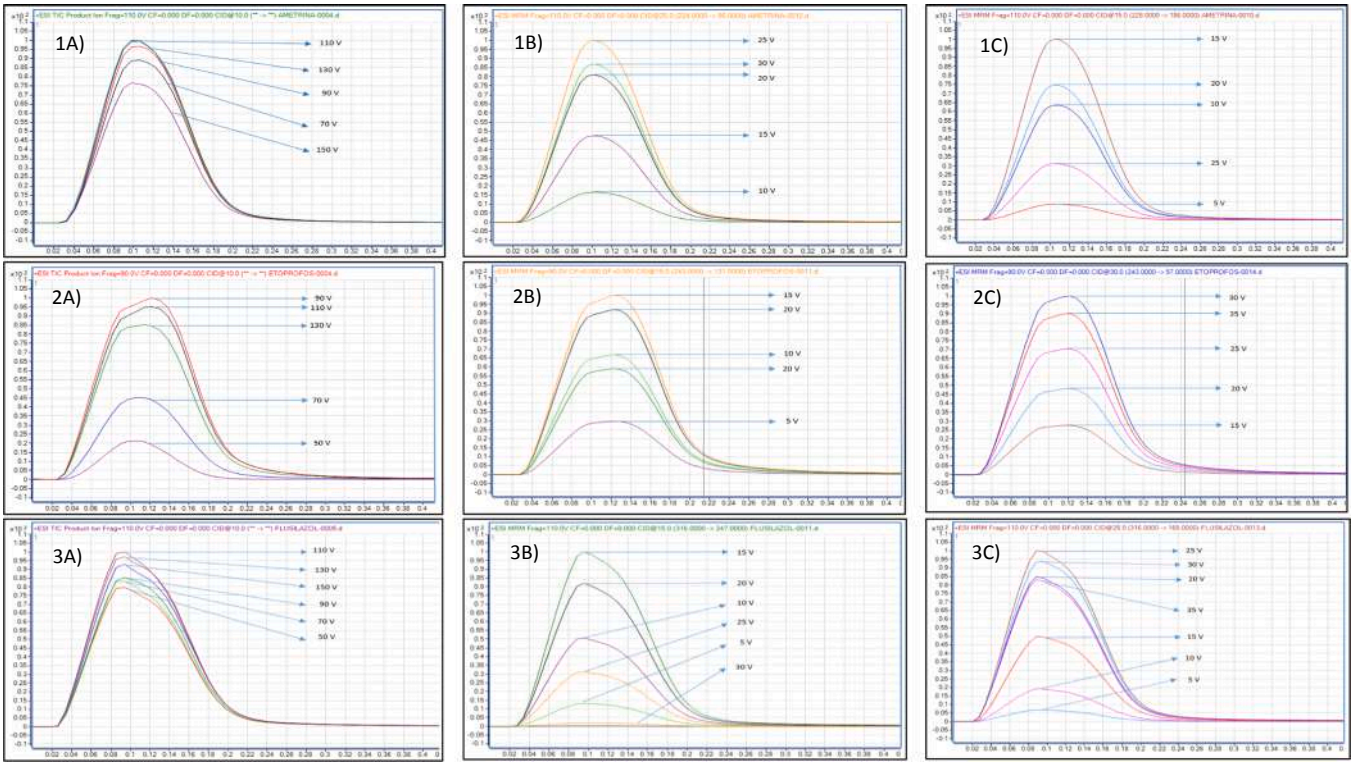

Fig. 1. Optimization of voltage fragmentor and precursor ion (A); the quantification product ion (B); and confirmation product ion (C) for ametryn (1), ethoprophos (2), and flusilazol (3).

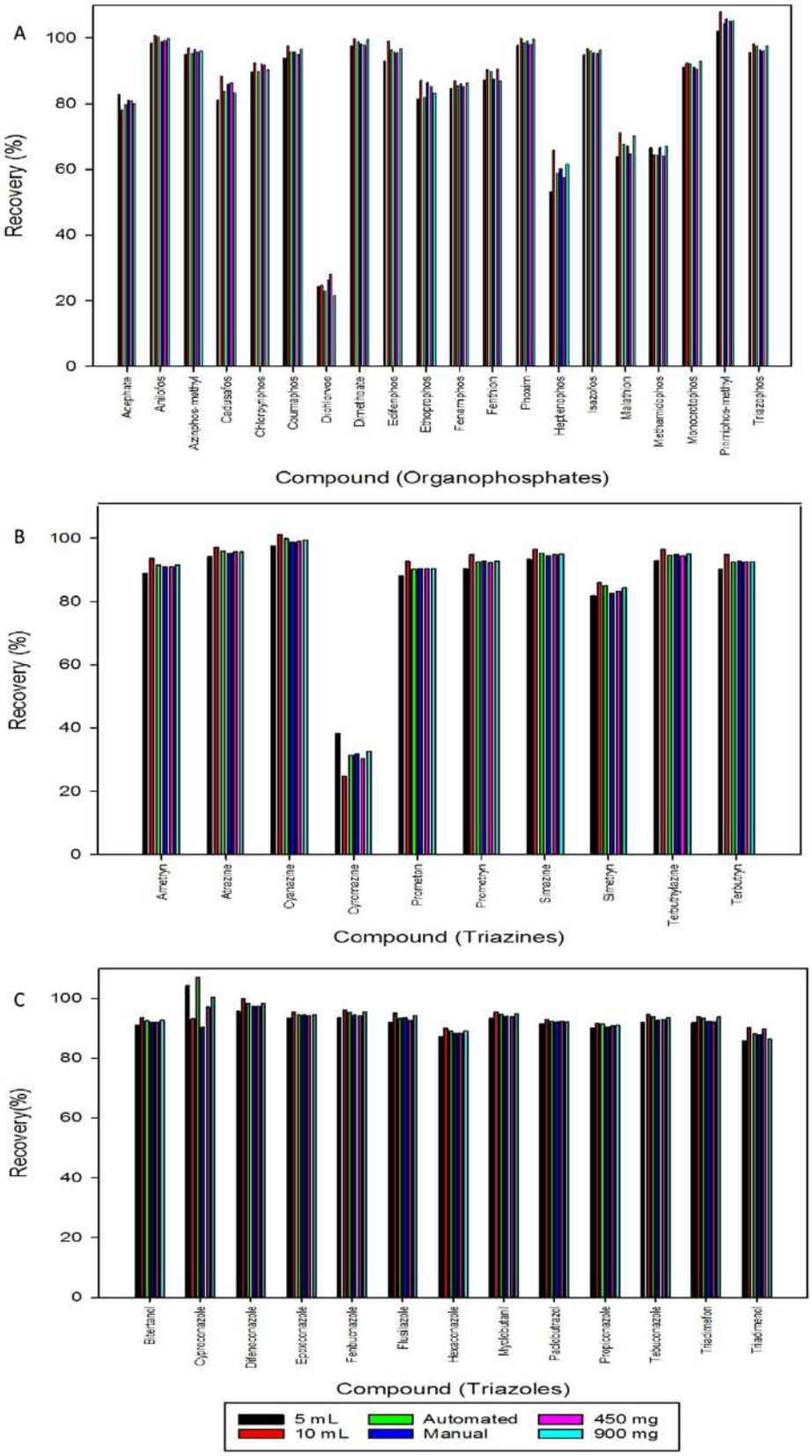

Fig. 2. Recovery obtained from the design of comparative experiments 2^3 during the optimization of methodological factors: i. addition of water (5 mL vs. 10 mL) during the extraction; ii. automated or manual agitation during extraction; and iii. the amount of sulphate magnesium (450 mg vs. 900 mg) added in the cleaning step for: (A) Organophosphates; (B) Triazines, (C) Triazoles.

with higher water solubility like monocrotophos did not exhibit this effect.

When comparing the agitation types (Fig. 2) (either with a programmable automated agitation equipment vs. manual agitation), variability between both methodologies was below 2% for most of the pesticides. This finding implies that manual agitation can be used for this type of samples, as usually employed for the traditional QuEChERS methodology applied to vegetable matrices [40–42]. The application of manual agitation in each extraction stage produced similar results to the use of agitation for 30 min at 2500 rpm. Cyproconazole was the only molecule that showed an improvement of 15% when working with the programmable agitator. Considering the availability of the programmable shaker, that allows the simultaneous processing of several samples, the use of programmable agitation was selected for the purpose of the proposed method.

The third factor of study was the amount of magnesium sulfate used in the cleaning stage (Fig. 2). Magnesium sulfate is added to remove the excess of water in the samples, since the proposed methodology requires a subsequent step of concentration to dryness. Also, it reduces the number of co-extracts due to the decrease in polarity in the extraction acetonitrile phase [40]. The addition of magnesium sulfate did not show significant differences in the groups of triazines and organophosphates; however, an increase in the recoveries between 1 and 5 % was observed when using 900 mg of magnesium sulfate and the chromatogram showed less interferences. Yet, most of the triazoles were favored when using 900 mg of magnesium sulfate. This is expected as this is the group with the lowest water solubility reported. Based on this finding, 900 mg of magnesium sulfate were used for improving the recovery of most of the studied molecules.

In summary, the key factor that permitted to achieve better recovery was the addition of 10 mL of water to the biomixture, with favorable results on most of the compounds. Although the amount of magnesium sulfate added for cleaning did not result in significantly different values for the study molecules, the cleaning with 900 mg of magnesium sulfate increased the recovery of triazoles by up to 5% and eliminated interferences, which favors parameters for the methodology validation such as selectivity.

3.3. Parameters for methodology validation

After optimizing the aforementioned factors, the methodology proposed employed 5 g of sample and the addition of 10 mL of DDI water, 15 mL of acetonitrile acidified (1% v/v acetic acid), 6 g of magnesium sulfate, 1 g of sodium chloride and 2.6 g of sodium acetate trihydrate, followed by shaking for 30 min, 2500 rpm, and centrifugation (4000 rpm, 10 °C, 7 min). After centrifugation, an aliquot of 3 mL of the extract was placed in a tube with 900 mg of magnesium sulfate, 150 mg of PSA and cleaned with 75 mg of C18. The sample was then stirred and centrifuged again at the same conditions. A sample of 1.5 mL of the supernatant was gently dried with a nitrogen stream and finally reconstituted to 1.5 mL with acidified (0.1% v/v formic acid) water-acetonitrile mixture (1:1), and filtered (0.45 µm PTFE filter) before being placed into a vial.

The subsequent validation of the method included selectivity, specificity, precision, intermediate precision, LOD, trueness, linearity, application range, matrix effect and robustness.

3.3.1. Selectivity and specificity

Selectivity is the ability of the method to discriminate between the analyte of interest and other molecules present in the matrix, while specificity is the ability to obtain a negative result, when the samples do not have the analyte [34]. By carefully coupling the choosing of the solvents and reagents of the analytical extraction, to the properties of the LC-MS/MS technique to identify compounds according the optimization of their mass, the results will be most of the times selective. The retention times, transitions and fragmentation and collision cell voltages of individual molecules were optimized (Table 3). Then, a working solution containing the 43 analytes, carbofuran-d_3 and linuron-d_6 was injected. Also, the following 52 additional molecules oxamyl, carbendazim, carbendazim-d_4, amitraz, methomyl, thiamethoxam, thiabendazole, imidacloprid, picloram, imazapyr, pirimicarb, 3-hydroxycarbofuran, acetamiprid, cymoxanil, imazapic, aldicarb, 3-ketocarbofuran, monuron, metribuzin, bromacil, propoxur, hexazinone, carbofuran, thiophanate-methyl, pyrimethanil, bentazone, metsulfuron-methyl, carbaryl, imazalil, metalaxyl, isoproturon, diuron, thiophanate, linuron, azoxystrobin, propanil, methiocarb, molinate, dimethomorph, myclobutanil, fenarimol, prochloraz, fipronil, kresoxim-methyl, haloxyfop, pyraclostrobin, triflumuron, buprofezin, haloxyfop-p-methyl, fluazifop-p-butyl, teflubenzuron and pendimethalin were added to the previous mixture at a concentration of 200 µg/kg each. None of these additional 52 molecules (excluded from the validation) showed interference signals on the optimized triazine, triazole and organophosphate transitions, thus demonstrating the method is selective.

The specificity of the method was studied with five blank samples and five spiked samples; their comparison confirmed the absence of false positives for the studied molecules, thus suggesting it is a specific method. In the case of ametryn, atrazine, cyromazine, cyproconazole, tebuconazole, triadimenol, chlorpyrifos and fenamiphos, slight signals were identified in their respective transitions, which implies a slight interference for subsequent detections. The detected signals showed S/N ratios > 10, which was the criterion to assign a positive signal. The S/N ratios and the ion ratio of the spiked samples were higher than the LOD, which does not affect their selectivity when applying the methodology (Table S3, Supplementary Material).

The ion ratio criterion was also used to evaluate both parameters, by comparing the ion ratio for each compound with the ion ratio of calibration curves. All the compounds showed good results in the selectivity test (spiked samples), as the ion ratio of the sample extracts were ± 30% of the average for calibration standards. In the case of specificity, all the compounds exhibited ion ratios out of the selection criterion.

3.3.2. Limit of determination (LOD)

Since the method is intended to be applied in biomixtures used for pesticide treatment, the expected concentrations in this matrix are quite high, in the order of more than 10 mg/kg (particularly at the moment of the disposal of pesticide-containing wastewater). The LOD was determined based on the lowest residue concentration that can be quantified for each pesticide, with a S/N > 10 for the quantified and confirmation ions.

The LOD is identified as the lowest level of spiked sample with acceptable recovery and precision; in some cases, it can be equated to the maximum limit of residues (MRL); however, there are no MRL for this type of matrix. The criteria to accept the LOD was RSD ≤ 20%. The acceptance criterion was the detection of the two transitions and the ion response ratios from the sample, and the average of the calibration standards lower than ± 30% [34]. The results for these experiments are presented in Table 4.

Cyromazine was the only molecule in the LOD study that presented a RSD greater than 20%, which is justified as it is a basic ionic molecule, that may be adsorbed to soil due to the known sorption of triazines to the humic groups of soil [43]; however, during the extraction process, the sample reached an approximate pH value of 4.5, due to the acetate buffer that was made in situ [43,44], which favors the extraction of other triazines, but not the cyromazine that has been shown to require a greater amount of acid for better extraction [45]. Some organophosphates such as cadusafos, ethoprophos, fenthion and malathion had an RSD value close to 15%. For these molecules, the greater variability at lower concentrations is not due to pH or p*K*a, but rather to the hydrogen bonds that are formed between pesticides and ionic compounds in the soil [43].

Table 4
Results of average RSD calculated for the precision parameter at three spiked levels (n = 7) and intermediate precision (n = 21); average recovery (%) calculated for trueness (n = 7), and LOD (n = 7) for the method proposed to determine triazines, triazoles and organophosphates in a biomixture composed by soil/compost/coconut fiber.

Compound	Precision (as repeatability) (RSD < 20%)			Intermediate precision (RSD < 20%)			Trueness (Recovery % n = 7)	LOD 10 μg/kg (RSD)
	50 μg/kg	150 μg/kg	350 μg/kg	50 μg/kg	150 μg/kg	350 μg/kg	50 μg/kg	
Acephate*	5.06	6.16	8.72	12.25	6.07	6.89	92.1	11.4
Amethryn	3.27	7.77	2.80	4.44	5.04	10.74	106.0	4.2
Anilophos	4.17	4.63	3.47	6.77	5.27	8.39	90.0	6.4
Atrazine	3.83	7.06	3.30	5.72	4.60	7.75	111.5	5.7
Azinphos-methyl	3.25	7.58	2.35	5.90	5.15	6.85	107.6	5.5
Bitertanol	4.70	7.88	3.14	5.07	5.10	13.47	108.0	4.8
Cadusafos	6.92	7.48	5.06	21.77	8.34	12.42	99.9	17.6
Chlorpyrifos	7.81	10.66	3.99	12.76	7.53	10.97	103.2	9.7
Coumaphos	4.50	8.81	5.12	8.43	7.01	12.92	110.2	7.9
Cyanazine	5.19	7.32	3.16	5.38	4.73	10.01	112.1	5.3
Cyproconazole	6.27	8.59	5.25	7.67	6.10	12.24	116.4	7.4
Cyromazine	23.66	17.38	28.57	61.15	40.95	54.86	49.4	38
Dichlorvos	43.14	20.31	19.09	40.19	25.56	25.80	47.1	12.4
Difenoconazole	3.34	7.38	4.53	4.94	4.76	15.64	112.5	4.6
Dimethoate	3.76	7.78	4.65	5.45	5.18	5.57	110.4	5.6
Edifenphos	4.19	4.47	4.40	4.33	6.08	8.30	114.7	4.1
Epoxiconazole	2.75	7.62	2.88	4.19	5.13	13.67	108.2	4.1
Ethoprophos	7.32	10.09	2.78	11.05	9.29	9.93	97.0	14.5
Fenamiphos	4.72	6.47	3.88	8.15	6.39	8.78	105.3	8.1
Fenbuconazole	4.60	7.78	4.40	5.95	4.96	12.78	107.0	5.6
Fenthion	11.60	6.72	2.94	14.47	11.60	14.25	101.3	13.1
Flusilazole	4.01	6.77	3.84	5.33	4.76	13.23	108.1	5.2
Hexaconazole	3.63	8.09	3.67	4.29	5.39	12.80	102.1	4.2
Heptenophos	10.24	5.21	3.43	9.83	6.92	6.73	89.3	7.2
Isazofos	3.03	6.79	2.65	5.39	4.69	8.84	112.1	5.2
Malathion	8.28	4.76	3.41	19.41	9.18	8.54	93.7	13.4
Methamidophos	5.68	7.63	8.19	10.83	8.18	14.30	78.2	9.8
Monocrotophos	4.54	6.96	4.68	6.77	4.84	6.99	107.7	6.8
Myclobutanil	3.35	7.26	3.09	5.62	5.03	11.73	108.1	5.5
Paclobutrazol	3.94	7.89	3.24	4.16	6.08	10.06	106.7	4.0
Phoxim	3.35	5.52	5.14	8.63	5.86	8.73	114.2	8.1
Pirimiphos-methyl	2.74	7.78	3.32	6.74	5.14	13.36	113.0	6.3
Prometon	3.40	7.98	3.15	4.71	5.10	10.04	105.2	4.6
Prometryn	3.32	7.66	2.95	4.50	4.77	10.40	108.5	4.4
Propiconazole	4.16	7.55	3.38	5.43	5.17	14.10	103.7	5.1
Simazine	3.53	7.42	2.50	5.24	4.87	9.52	109.5	5.1
Simetryn	2.72	7.59	1.77	5.13	5.13	11.03	103.7	4.6
Tebuconazole	11.70	6.74	2.78	15.66	6.65	16.54	107.7	13.9
Terbuthylazine	3.28	7.09	3.26	5.07	4.48	10.14	109.3	4.9
Terbutryn	3.10	8.15	2.90	4.36	5.31	10.88	108.1	4.2
Triadimefon	2.52	7.56	2.64	4.35	5.21	12.86	109.9	4.2
Triadimenol	4.84	7.46	4.04	7.90	6.68	10.63	105.5	7.6
Triazophos	4.61	7.40	2.82	6.55	4.75	8.06	113.4	6.4

LOD: Limit of determination.
* Acephate had a LOD of 50 μg/kg.

3.3.3. Precision as repeatability and intermediate precision

After detecting the concentrations for LOD, it was necessary to work at three higher concentrations (50, 150 and 350 μg/kg) to establish the precision of the methodology. The critical criterion was an RSD < 20%. The precision as repeatability was determined with the results of one of the analysts (n = 7); intermediate precision was determined with three analysts who performed the methodology at different days (n = 21). The results are shown in Table 4.

After the evaluation of 43 molecules, cyromazine and dichlorvos showed the greatest dispersion data along the three concentration levels of the intermediate precision parameter (> 40% and > 25%, respectively). The precision as repeatability and intermediate precision had an RSD higher than 20%, which exceeds the acceptance criterion of SANTE [34]. The results for these molecules suggest the need to consider the behavior of other parameters to determine whether the methodology is appropriate for their analysis. Thus, it is considered that the multiresidue method proposed is not precise for these molecules, or in the worst-case scenario, it would be better to test other methodologies for their analysis.

The remaining 41 molecules exhibited higher coefficients of variation at low concentrations, which decreased at higher concentrations. All organophosphates but dichlorvos presented results that met the acceptance criteria. From the remaining 19 molecules, chlorpyrifos showed the highest RSD value. Acephate and methamidophos exhibited a different behavior, as their RSD increased with the concentration. The polarity of both molecules and the possibility to have greater hydrogen bonding between the molecules and the biomixture, may favor their greatest data dispersion; nonetheless, the dispersion was not greater than that allowed by the validation criterion in this case. For the case of cadusafos, no precision was considered at low concentration (RSD = 21.77% at 50 μg/kg). All the triazoles presented acceptable values of standard deviation, as the other nine triazines.

The RSD values were in general higher for intermediate precision than repeatability. This is expected, as data executed by three different analysts on different days was employed, which consequently gives a greater variability to the method.

3.3.4. Trueness

The trueness of the method is applied as a validation parameter in

the absence of an interlaboratory test for the biomixture matrix. The trueness is understood as the average recovery of the concentration levels evaluated with a recovery percentage between 70 and 120 % [34]. The trueness of the method was determined using a low concentration, that is, the recovery value of 50 μg/kg.

When comparing these results with those obtained in Table 4, it was observed that dichlorvos (47.1%) and cyromazine (49.4%) presented results below the acceptance criteria (70–120%). For acephate and methamidophos, the recoveries were quite good compared to other methodologies applied to fruits, vegetables, meats and soils, since they showed values of 92% and 78% respectively [46–49]. The organophosphates anilophos (90%) and heptenophos (89%) also exhibited relatively low recoveries, thus, these molecules should be carefully revised in a control chart to verify that this average is maintained. The recovery values obtained for the triazoles were within the acceptance criterion.

More than 80% of the molecules showed recoveries > 100%, a finding that could be ascribed to the ionization technique (electrospray system) applied in the LC-MS/MS, which exerts a signal improvement effect. This behavior is classified by some authors as a cause of matrix effect [50–52]; nonetheless in this case, the matrix effect was negligible in most of the molecules and the ion ratio values were acceptable and less than 30% deviated from the reference value in the calibration curve [34].

3.3.5. Linearity

Three independent (not consecutive) calibration curves were prepared with 10 concentration levels each in the blank extract sample, including concentrations from the LOD value (10 μg/kg) to 1000 μg/kg. Three acceptance criteria were considered: (i) correlation coefficient greater than 0.99; (ii) the percentage of residuals for each level must be less than 20%; and (iii) the slope ratio between the three calibration curves should be higher than 80%. Table 5 shows the results of the calibration curves of the study molecules.

Every single molecule yielded acceptable results for each criterion. However, it is important to consider that, for acephate, azinphos-methyl, coumaphos, cyromazine, dichlorvos, fenamiphos, fenbuconazole, methamidophos, and pirimiphos-methyl, one of the work levels reached residual values close to or greater than 10%, which implies that care must be taken in estimating the curve, as this could lead to an increase of LOD if lower levels need to be eliminated to ensure linearity range.

3.3.6. Matrix effect

The matrix effect (ME) is the comparison between the response of the calibration levels in the organic solvent and the matrix [34,53–56]. The acceptance criterion was ME lower than 20%. The curve that was prepared in the matrix presented the same characteristics as the final extract (a phase of water-acetonitrile (1:1), acidified with formic acid 0.1%). The results are shown in Table 5.

Cyromazine (17%), methamidophos (9.2%) and phoxim (7.6%) presented the greatest matrix effect; throughout the validation these molecules have shown the least favorable performance in the multiresidue methodology. The other molecules evaluated in the methodology presented ME less than 4%. However, none of the molecules failed to meet the acceptance criterion of ME < 20%.

The fact that no considerable matrix effect was observed in the biomixture was due to the change in solvent, since there was a change in polarity of the sample and this caused a decrease in the amount of co-extracts before injection [52]. This finding was also supported by the direct observation of less particles remaining in the sample container and the filter used.

The decrease in co-extracts favors the formation of ions [51,53,54,56], since it reduces the ionic suppression effect normally achieved in the ionization technique by electrospray (which decreases the signal), compared to the chemical ionization technique at atmospheric pressure (which increases the signal) [50–52,56]. This factor avoids the production of high concentration of other compounds, which may increase the surface tension, the viscosity of the droplets in the nebulizer, and the proton affinity between the analytes and the co-extracts [51].

3.3.7. Application range

When an unknown sample is processed, there is a risk that the concentration of the analyte surpasses that of the highest level in the calibration curve; this implies that the sample must be diluted so that the analyte concentration remains within the validated parameters. In order to corroborate the method properly extracts and detects higher concentrations than those validated with satisfactory recovery, a two-part experiment was performed. For the first one, the methodology was applied to five spiked samples with a concentration of 2000 μg/kg. The final extracts were diluted to an intermediate value of the calibration curve, and then quantified. The criteria for this parameter was the recovery (> 70%, < 120%) and RSD (< 20%).

Only cyromazine and dichlorvos presented recoveries below 70% after dilution (Table 5). The remaining 41 molecules were extracted from the matrix at a concentration of 2000 μg/kg and still had a recovery between 70% and 120%, with an RSD less than 20%. This implies that, although there is no linear relationship between concentration and response at high concentrations of the analyte, the method allows to work at such concentrations using proper dilutions. Moreover, the sample dilution provides the desired effect of an additional decrease in the matrix effect.

3.3.8. Robustness

The robustness test was carried out to demonstrate that the methodology is still reliable and accurate, after the variation of several extraction conditions (factors) (Table S4, Supplementary Material). Several experimental designs are used to evaluate the robustness of analytical methods [57–61]; the Youden-Steiner test was performed in this work. This test consists of a fractional factorial design of resolution III, which is represented by the mathematical model 2_{III}^{7-4}. It works at two levels of effect, in which seven factors or conditions that can result in significantly different results are considered (but not their interactions), for a total of eight experiments [35,58,62,63].

The robustness was calculated by comparing the difference of the values of each factor, according to Tables 1 and 2, in relation to the value calculated as critical, which depends on the total standard deviation of the experiment [35,58,59]. The results are shown in Table S4 of the Supplementary Material.

Forty molecules met the robustness criterion. In particular, for amethryn, simazine, dimethoate, bitertanol and hexaconazole the seven proposed factors did not statistically affect the behavior of the molecules; on the other hand, four analytes (chlorpyrifos, coumaphos, cyromazine and methamidophos) showed high critical values (> 20) that represented their high variability; nonetheless they passed the robustness test. This finding represents a drawback of the statistical method; as the critical factor increases with RSD > 20%, this method may mask the effect of conditions in the case of molecules with poor precision. Other two molecules, dichlorvos and fenamiphos, showed high critical values of 14 and 16, but they are still considered as acceptable.

Only cyproconazole, dichlorvos, fenamiphos and methamidophos failed the robustness test for one condition. In the case of cyproconazole, the condition was the temperature of the water bath; as the temperature increased, a lower recovery of the molecule was obtained, which implies that temperature control must be used during sample concentration with nitrogen. In the case of fenamiphos, the critical factor was the agitation time with acetonitrile, as a decrease greater than 20% in the percentage of recovery was obtained with less agitation. For methamidophos, a greater variability was obtained as a cause of a shorter agitation time in the centrifuge, which does not allow an adequate phase separation. On the other hand, dichlorvos was affected by the time it was in contact with water; however, this molecule

Table 5
Results of validation parameters: linearity, application range and matrix effect, for the method proposed to determine triazines, triazoles and organophosphates in a biomixture composed by soil/compost/coconut fiber.

Compound	Linearity (3 replicates; 10 calibration levels)			Application range; spiked at 2 mg/kg (RSD % with n = 21; diluted samples)	Matrix effect (Dif < 20%)
	r^2	Highest % of residuals detected < 20%	Calibration curve		
Acephate	0.994	9.99	y = 0.00043749 x – 0.00000017	102.5 (7.0)	0.24
Amethryn	0.999	5.38	y = 0.004057 x – 0.000042	106.6 (5.0)	1.57
Anilophos	0.996	7.88	y = 0.0002312 x – 0.0000019	108.4 (6.5)	0.22
Atrazine	0.992	6.49	y = 0.002688 x – 0.000031	108.2 (3.3)	0.48
Azinphos-methyl	0.996	10.0	y = 0.00006759 x – 0.00000063	106.2 (4.1)	2.20
Bitertanol	0.991	7.50	y = 0.0003322 x – 0.0000027	109.8 (4.9)	0.79
Cadusafos	0.995	7.89	y = 0.0006059 x – 0.0000046	94.1 (3.8)	1.22
Chlorpyrifos	0.994	6.31	y = 0.00009017 x – 0.00000088	103.8 (4.3)	1.19
Coumaphos	0.991	10.38	y = 0.0001379 x – 0.0000019	118.1 (7.8)	4.00
Cyanazine	0.998	8.41	y = 0.0004952 x – 0.0000066	115. 8 (4.7)	0.48
Cyproconazole	0.991	6.16	y = 0.0008256 x – 0.0000026	110.1 (4.4)	1.00
Cyromazine	0.997	14.33	y = 0.00043203 x + 0.00000018	29.4 (49)	17.29
Dichlorvos	0.995	9.85	y = 0.0002714 x – 0.0000019	61.7 (12.4)	1.64
Difenoconazole	0.990	6.90	y = 0.0009981 x – 0.0000095	99.4 (6.7)	2.06
Dimethoate	0.998	5.60	y = 0.00040682 x – 0.00000034	100.7 (3.9)	0.92
Edifenphos	0.997	5.56	y = 0.0002329 x – 0.0000021	113.3 (4.0)	1.05
Epoxiconazole	0.993	8.44	y = 0.000928 x – 0.000011	109.1 (3.8)	1.87
Ethoprophos	0.995	6.09	y = 0.0002974 x – 0.0000025	103.0 (3.7)	1.10
Fenamiphos	0.991	12.03	y = 0.0002977 x – 0.0000041	108.1 (5.9)	2.71
Fenbuconazole	0.992	9.70	y = 0.0005668 x – 0.0000077	111.4 (4.9)	0.10
Fenthion	0.995	8.65	y = 0.00005111 x – 0.000000095	102.7 (6.8)	1.32
Flusilazole	0.993	8.78	y = 0.001284 x – 0.000016	110.2 (4.4)	0.50
Hexaconazole	0.993	4.91	y = 0.001271 x – 0.000015	108.8 (4.3)	0.02
Heptenophos	0.996	7.48	y = 0.0002239 x – 0.0000016	100.2 (3.5)	2.85
Isazofos	0.995	7.89	y = 0.001438 x – 0.000011	109.4 (4.0)	0.66
Malathion	0.995	5.84	y = 0.000245 x – 0.000013	95.2 (4.3)	1.87
Methamidophos	0.997	10.44	y = 0.00017877 x – 0.00000098	102.8 (17.2)	9.15
Monocrotophos	0.996	5.62	y = 0.0003956 x – 0.0000032	109.1 (4.7)	2.57
Myclobutanil	0.994	4.74	y = 0.0009990 x – 0.0000084	111.0 (4.3)	0.62
Paclobutrazol	0.995	6.58	y = 0.002872 x – 0.000031	112.1 (5.7)	2.99
Phoxim	0.997	8.43	y = 0.00008963 x – 0.00000027	98.5 (4.5)	7.59
Pirimiphos-methyl	0.994	11.07	y = 0.003116 x – 0.000039	117.8 (4.2)	0.89
Prometon	0.993	5.11	y = 0.004279 x + 0.000054	105.1 (3.2)	0.65
Prometryn	0.994	5.97	y = 0.005715 x – 0.000071	107.5 (4.6)	0.13
Propiconazole	0.992	5.73	y = 0.0007152 x – 0.0000092	111.2 (4.1)	1.55
Simazine	0.993	5.07	y = 0.000963 x – 0.000012	108.3 (4.5)	0.56
Simetryn	0.994	4.89	y = 0.001779 x – 0.000021	101.7 (4.6)	0.83
Tebuconazole	0.994	6.09	y = 0.0015468 x – 0.00000088	107.5 (4.0)	1.66
Terbuthylazine	0.994	7.66	y = 0.006191 x – 0.000071	111.3 (4.0)	0.64
Terbutryn	0.993	7.24	y = 0.006781 x – 0.000088	106.3 (4.5)	0.20
Triadimefon	0.995	6.57	y = 0.0007691 x – 0.0000069	109.2 (4.1)	0.30
Triadimenol	0.992	5.28	y = 0.001368 x – 0.000011	103.2 (3.9)	1.24
Triazophos	0.996	5.68	y = 0.001367 x – 0.000011	105.4 (4.2)	0.02

consistently exhibited lower recoveries and unsatisfactory RSD.

Summarizing, every analyte showed satisfactory results for the determination of LOD, linearity, specificity and selectivity. Besides these parameters, the proposed methodology exhibited low matrix effects; only cyromazine had a value out of the acceptance criterion. For the parameters of trueness and precision, cyromazine and dichlorvos were the only two molecules that showed unsatisfactory results, with values of recovery < 70% and RSD > 20%. The lack of precision and accuracy suggests the use of another methodology to work with these molecules. The parameter of robustness demonstrated that, for most of the molecules, slight changes do not affect the methodology performance. Only four molecules exhibited unsatisfactory results for one different methodology condition. Overall, the proposed methodology, except for dichlorvos and cyromazine, met the acceptance criteria for the determination of 41 of the evaluated molecules in the biomixture.

3.4. Removal of pesticide-containing wastewater in a BPS: application of the method

The methodology was applied to monitor the removal capacity of a 104 L BPS. The synthetic wastewater applied on the biomixture contained eight pesticides (ametryn, atrazine, chlorpyrifos, diazinon, malathion, tebuconazole, terbutryn and triadimenol), at similar concentrations to those expected after the disposal of wastewater residues from field application, according to the recommendation in the commercial formulations. Initial concentrations in the BPS ranged from 3.9 mg/kg to 51.1 mg/kg (malathion and tebuconazole, respectively). Triadimefon was not added to the wastewater, and was initially

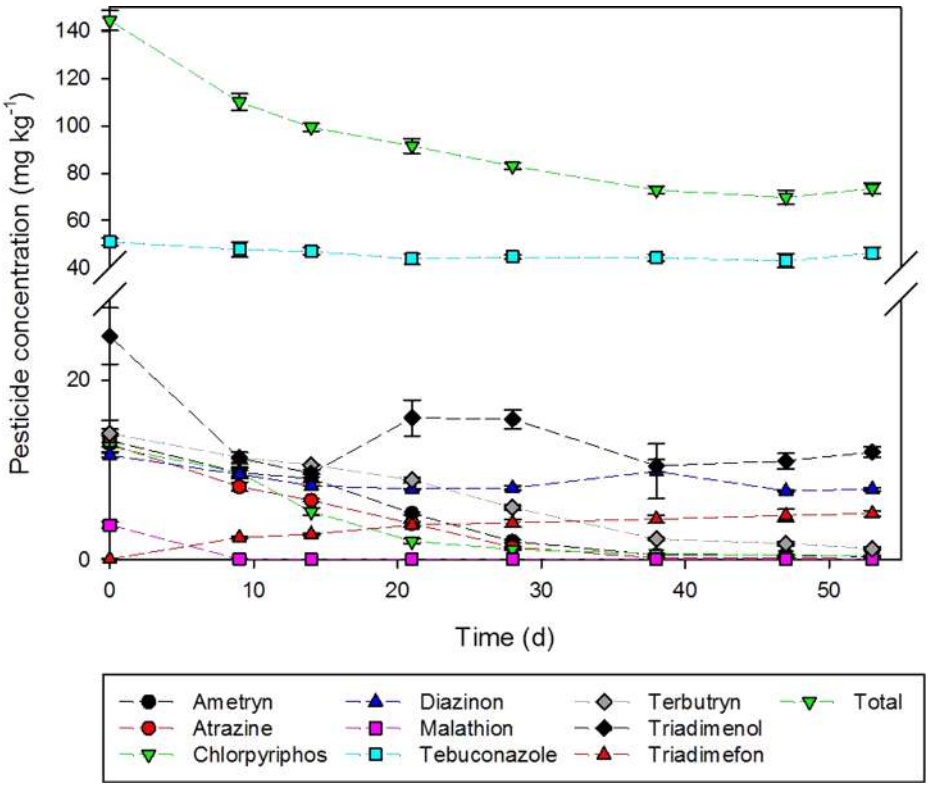

Fig. 3. Removal of eight pesticides from commercial formulations during their treatment in a BPS for 53 days. Plotted values are means ± SD.

detected at 0.09 mg/kg. Its origin is likely due to contamination in the formulation containing tebuconazole/triadimenol, as triadimenol is a known transformation product of triadimefon [64].

Although most of the compounds were at least partially removed (Fig. 3), none of them was eliminated at levels below the LOD. The triazole tebuconazole and the organophosphate diazinon were not significantly removed. Previous works on BPS report unsuccessful elimination of triazoles, including tebuconazole and triadimenol [2,28,65]; nonetheless, in this case triadimenol was partly removed at the end of the treatment after 53 d (up to 51.8%). Many investigations indicate that triadimenol is a metabolite of triadimefon [64,66]; however, in this experiment triadimefon was not added and an increase in its levels was observed (up to 5.14 mg/kg). This finding could be due to an oxidation of the triadimenol in the biomixture, favored by the conditions of temperature and humidity, a scarcely studied reaction described by Deas & Clifford [67] in transformations with fungi. Other biomixtures have shown the ability to remove diazinon in peat-based biomixtures, with DT_{50} in the range of 4.9 to 10.8 d, with an accelerated effect after successive applications [68]. Contrary to diazinon, other organophosphates were removed from the biomixture at different rates; chlorpyrifos at an estimated DT_{50} of 10.5 d, while malathion concentration decreased to only 1.9% after nine days of treatment (DT_{50} = 1.6 d). The removal of chlorpyrifos was significantly faster than data from soil (DT_{50} 27–386 d) [69] and slightly faster than reported in other biomixtures, for which DT_{50} values are within the range 15–59 d [65,70–72]. The fast elimination of malathion in biomixtures was also described in a peat-based matrix (DT_{50} 3.8 d) [25] and a compost-based mixture (DT_{50} 7.1 d). From the three triazines tested, atrazine exhibited the faster removal (estimated DT_{50} 11.2 d), followed by ametryn (DT_{50} 13.4 d) and terbutryn (DT_{50} 19.4 d). The removal of atrazine has been widely described in biomixtures, with DT_{50} values ranging from as low as < 10 d (after single or repeated applications) [29,32,65] to more than 20 d [2,73]. Comparable removal patterns to those observed in this work have been achieved for ametryn [2] and terbutryn [30] in compost-based biomixtures.

4. Conclusions

The modified QuEChERS methodology was validated for the analysis of pesticides in a solid matrix (biomixture) made up of soil, compost and coconut fiber, aimed to remove pesticides from wastewater of agricultural origin. The method was proved under several validation parameters, where the results were satisfactory for most of the triazines, triazoles and organophosphates evaluated, except for dichlorvos and cyromazine, which did not meet the acceptance criteria for some parameters.

The developed LC-MS/MS methodology allows working with low and high pesticide concentrations, with good recovery percentages between 70% and 120%, coefficients of variation of less than 20%, with linearity conditions that exceed the coefficient of determination value of 0.99, and with matrix effects lower than 20%, for 41 out of the 43 evaluated molecules.

The validated methodology was successfully applied to determine the efficiency of a pilot scale biopurification system, employed for the removal of wastewater residues containing eight pesticides from commercial formulations.

CRediT authorship contribution statement

Mario Masís-Mora: Conceptualization, Methodology, Formal analysis, Investigation, Funding acquisition, Writing - original draft, Writing - review & editing. **Wilson Beita-Sandí:** Formal analysis, Writing - review & editing. **Javier Rodríguez-Yáñez:** Conceptualization, Writing - review & editing. **Carlos E. Rodríguez-Rodríguez:** Conceptualization, Project administration, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Vicerrectoría de Investigación, University of Costa Rica (Project 802-B4-503) and the Ministry of Science, Technology and Telecommunications of Costa Rica (Project FI-093-13). The authors would like to thank the cooperation of the master program "Management of Natural Resources with an emphasis in Environmental Management" of the Universidad Estatal a Distancia (UNED, Costa Rica).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2020.122296.

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