

Determination of pesticide residues and related compounds in water and industrial effluent by solid-phase extraction and gas chromatography coupled to triple quadrupole mass spectrometry

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Abstract Pollution of drinking water supplies from industrial waste is a result of several industrial processes and disposal practices, and the establishment of analytical methods for monitoring organic compounds related to environmental and health problems is very important. In this work, a method using solid-phase extraction (SPE) and gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS) was developed and validated for the simultaneous determination of pesticide residues and related compounds in drinking and surface water as well as in industrial effluent. Optimization of the method was achieved by using a central composite design approach on parameters such as the sample pH and SPE eluent composition. A single SPE consisting of the loading on a polymeric sorbent of 100 mL of sample adjusted to pH 3 and elution with methanol/methylene chloride (10:90, v/v) permitted the obtaining of acceptable recoveries in most cases. The concentration factor associated with sensitivity of the chromatographic analysis permitted the achievement of the method limit of detection values between 0.01 and 0.25 $\mu\text{g L}^{-1}$. Recovery assays presented mean recoveries between 70 and 120 % for most of the compounds with very good precision, despite the different chemical nature of the compounds analyzed. The selectivity of the method, evaluated through the relative intensity of quantification and qualification

ions obtained by GC-QqQ-MS/MS, was considered adequate. The developed method was finally applied to the determination of target analytes in real samples. River water and treated industrial effluent samples presented residues of some compounds, but no detectable residues were found in the drinking water samples evaluated.

Keywords GC · Sample preparation · Organic compounds · Water · Effluent

Introduction

Industries that use large amounts of water in different processes have the potential to pollute waterways through the discharge of their waste into streams and rivers or by runoff and infiltration of stored wastes into nearby water sources. Plants that manufacture pesticides use diverse manufacturing processes, including synthesis, separation, recovery, purification, and product finishing such as drying. Chemical synthesis can include chlorination, alkylation, nitration, and many other substitution reactions. Separation processes include filtration, decantation, extraction, and centrifugation. Recovery and purification are used to reclaim solvents or excess reactants as well as to purify intermediates and final products. Evaporation and distillation are common recovery and purification processes. Product finishing may involve blending, dilution, pelletizing, packaging, and canning [1]. These industries use large amounts of water in different processes and have high potential to pollute waterways through the discharge of their waste into the environment or by runoff and/or infiltration of stored wastes nearby water sources. These industrial wastes include pesticide residues, solvents, and a large variety of

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substances arising from chemical reactions and purification processes [2]. Many organic compounds used as feedstock for pesticide production have major environmental relevance and may have more adverse effects than the own pesticides to humans and the environment [3, 4]. The use of pesticides in food production, to improve agricultural productivity, has been a common practice around the world, raising great concern about the risks of food poisoning and environmental pollution [5].

The current concern about pesticide interactions with the environment demands effective control of these analytes in different compartments (e.g., soil, air, water, etc.). Because of this, the analytical methods needed to be rapid, cheap, and reliable. Due to the large number of compounds with different chemical properties, the analytical methods should allow multiclass determination. To meet these requirements, many methods for sample preparation and quantification of analytes have been proposed in the literature [6–9].

Several methods have been proposed to monitor the presence of a growing number of contaminants on the environment to meet the parameters set by legislation to protect human health [10]. Actually, various sample preparation techniques are available for determination of organic contaminants in water samples. During the last years, solid-phase extraction (SPE) has gradually replaced liquid–liquid extraction (LLE), especially because it prevents some problems which the LLE is subject, such as emulsion formation, high solvent consumption, and high extraction time [11, 12]. Moreover, SPE has other advantages such as low solvent demand, reduction of labor costs, high recovery efficiency, and precision [11]. SPE was applied to organic contaminant analysis in surface water [13–17], underground water [18, 19], drinking water [3, 8, 20], and industrial effluents [21–23]. SPE, combined with highly selective analytical techniques such as gas chromatography coupled to tandem mass spectrometry (GC-MS/MS), allows simultaneous determination of multiclass contaminants [11, 24–27].

Gómez et al. [28] performed a monitoring study for a year to evaluate the occurrence, persistence, and fate of a group of 14 organic compounds in a sewage treatment plant. SPE combined with multiresidue GC-MS method was developed and validated. This method provided recoveries higher than 75 % (relative standard deviation (RSD) ≤ 14 %). The method detection limit (LOD) was between 1 and 100 ng L⁻¹ with RSD that ranged from 1.8 to 11.2 %. The application of the proposed method has allowed the identification of all the target compounds at mean concentrations which ranged from 0.12 to 134 $\mu\text{g L}^{-1}$ in the influent and from 0.09 to 18.0 $\mu\text{g L}^{-1}$ in the effluent.

A multiresidue method has been developed for the determination of various classes of selected endocrine disruptors. This method allows the simultaneous extraction and quantification of different estrogens, pesticides, and bisphenol A in natural

waters. In this method, 500 mL of water was percolated on SPE cartridges. The analysis is carried out by LC-MS using atmospheric pressure chemical ionization in the positive ion mode for determination of pesticides and electrospray in the negative ionization mode for determination of estrogens and bisphenol A. Recoveries for most compounds were between 90 and 119 %, except for bisphenol A (81 %) and diethylstilbestrol (70 %), with RSD below 20 %. LODs ranged between 2 and 15 ng L⁻¹. Water samples from the river and aquifer, and after different treatment stages, were evaluated. The compounds atrazine, simazine, diuron, and bisphenol A were detected in river water and were relatively frequent at concentrations below 0.1 $\mu\text{g L}^{-1}$. Lower levels, below 0.02 $\mu\text{g L}^{-1}$, were usual for isoproturon. Estrone-3-sulfate and estrone were detected occasionally in the river [29].

In the last years, different sorbent materials for SPE technique were developed. A method based on SPE with magnetic multiwalled carbon nanotubes as adsorbent was developed by Fu et al. [30] for the determination of 13 phthalate acid esters (PAEs) in water samples by GC-MS. The extraction efficiencies ranged between 89.7 and 100.5 %. The method was sensitive with detection limits between 0.08 and 0.47 $\mu\text{g L}^{-1}$ for all the compounds. The method was successfully applied for the analysis of tap water, bottle drinking water, and lake water, and none of the 13 PAEs was detected. The recoveries ranged from 84.5 to 107.5 % with RSD between 1.9 and 12.8 %.

A variety of substance classes like industrial chemicals, analgesics, anticonvulsants, antihypertensives, psychoactive substances, flame retardants, and neutral and acidic pesticides were analyzed by Wode et al. [31]. The method was developed for the simultaneous quantification of 72 micropollutants in aqueous samples by ultrahigh-performance liquid chromatography–high-resolution mass spectrometry. A sample volume of 1 mL was enriched by online SPE, separated on a 2.6- μm core-shell column, and detected with a high-resolution mass spectrometer. The method was validated in the matrices drinking water (DW), diluted surface water (dSW), and diluted wastewater treatment plant effluent (dWW) by analyzing ten replicates spiked at two concentration levels. Limits of quantification (LOQs) ranged between 0.01 and 0.06 $\mu\text{g L}^{-1}$ in DW, 0.03 and 0.38 $\mu\text{g L}^{-1}$ in dSW, and 0.06 and 0.38 $\mu\text{g L}^{-1}$ in dWW.

A multiresidue method consisting of online SPE step coupled to LC-MS/MS was developed for the determination of 88 polar micropollutants with different physicochemical properties. A single multilayer cartridge containing four different extraction materials was composed for the enrichment of water samples. This method allowed the simultaneous analysis of pesticides, biocides, pharmaceuticals, corrosion inhibitors, and many of their transformation products in three matrices, groundwater, surface water, and wastewater. LOQs were in the environmentally relevant concentration range of 0.1 to 87 ng L⁻¹ for groundwater and surface water and from

1.5 to 206 ng L⁻¹ for wastewater. Relative recoveries were largely between 80 and 120 %. The applicability of this method has shown that 36 substances of all compound classes could be found in concentrations between 0.1 and 600 ng L⁻¹. The results revealed the persistence of carbamazepine and sucralose in the groundwater aquifer as well as degradation of the metazolol metabolite 4-acetamidoantipyrine [32].

The combination of SPE and GC-MS/MS requires optimization of operational parameters for each compound, which increases the complexity of the development of the instrumental analysis, but also increases the selectivity and sensitivity of the analytical method allowing the determination of analytes in complex samples at very low concentrations [27, 33].

The aim of this study was to develop and validate a rapid and efficient method employing SPE and gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS) for the simultaneous determination of pesticide residues and related compounds in samples of drinking water, river water, and treated industrial effluent from the pesticide production industry. The studied compounds include several classes of pesticides frequently used, like pyrethroids, organophosphorus, organochlorines, strobins, triazines, pyrimidine, xylylalanine, pyrazole, dinitroanilines, carbamate, anilide, polychlorinated aromatic, substituted urea, dicarboximide and sulfenyl derivative, as well as related compounds such raw materials, reaction intermediates, and metabolites. To confirm the applicability of the method, it was applied to the determination of pesticide residues and related compounds in samples from the treatment plant effluent and from the environmental monitoring.

Experimental

Chemicals and reagents

The analytical standards with a minimum purity of 96.2 % were purchased from ChemService (West Chester, USA) and Dr. Ehrenstorfer (Augsburg, Germany). The analyzed compounds include pesticides like pyrethroids (lambda cyhalothrin, cyfluthrin-alpha + beta, cypermethrin, permethrin, deltamethrin, esfenvalerate, fenvalerate), triazoles (propiconazole, tebuconazole, epoxiconazole, myclobutanil, tetradifon, triadimenol), organophosphorus (chlorpyrifos methyl, chlorpropham, dichlorvos (DDVP), parathion methyl, pirimiphos methyl, parathion ethyl, pyrazophos, dimethoate, ethion, fenitrothion, methidathion, fenthion, malathion, monocrotophos, trichlorfon), strobins (azoxystrobin, kresoxim methyl, trifloxystrobin), triazines (atrazine, simazine), pyrimidine (fenarimol), xylylalanine (metalaxyl), pyrazole (fipronil), dinitroanilines (pendimethalin, trifluralin), carbamate (pirimicarb), anilide (propanil), polychlorinated aromatic (chlorothalonil), substituted urea (diuron), dicarboximide

(procymidone) and sulfenyl derivative (dichlofluanid), and organochlorines (alachlor, aldrin, DDT, dieldrin, endosulfan alpha, endosulfan beta, endrin, HCH-alpha, HCH-beta, HCH-delta, heptachlor, hexachlorobenzene, lindane, mirex, dicofol), as well raw materials, reaction intermediates, and metabolites, like 1,2-dichlorobenzene, 2,4-DDD, 3,4-dichloroaniline, 3,4-dichloro- α,α,α -benzotrifluoride (3,4 DCBTF), 2-bromomethyl-2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolane (bromoketal), 3,4-dichlorophenyl isocyanate (DCPI), 4-chloro-3,5-dinitro- α,α,α -trifluorotoluene (dinitro), 1-(4-chlorophenyl)-4,4-dimethyl-3-pentanone (ketone), 4-chloro-3-nitro- α,α,α -trifluorotoluene (mononitro), 4-chloro- α,α,α -benzotrifluoride (PCBTF), endosulfan sulfate, heptachlor endo-epoxide, and 2-[2-(4-chlorophenyl)ethyl]-2-(1,1-dimethyl-ethyl)-oxirane (oxirane).

Mixtures of all compounds at appropriate concentrations were used for the quantification by GC-QqQ-MS/MS and to spike blank samples for recovery experiments. The surrogate standard nitrobenzene was used to monitor the entire analytical process from sample preparation to instrument performance. Triphenylphosphate was used as internal standard. Dichloromethane, acetone, and methanol, all of pesticide residue grade, and anhydrous sodium sulfate were purchased from Merck (Darmstadt, Germany); helium 99.999 % and argon 99.9998 % were supplied by Air Products (São Paulo, Brazil), and water was purified with a Direct-Q UV3[®] system (resistivity 18.2 M Ω cm) from Millipore (Bedford, MA, USA).

For the preconcentration of the compounds, four types of SPE cartridges were evaluated: Strata C18-E 200 mg/3 mL and the polymeric materials Strata SDB-L 500 mg/3 mL and Strata X 200 mg/6 mL, both from Phenomenex (Torrance, CA, USA), and Bond Elut Nexus 60 mg/3 mL (Agilent Technologies, Santa Clara, CA, USA).

GC-QqQ-MS/MS analysis

The separation and quantification of the compounds was performed using a Varian gas chromatograph model 3800 with injector 1079 and insert of silanized quartz (inner diameter of 2 mm) filled with a CarboFrit plug (Restek, Bellefonte, USA), equipped with an autosampler AS8400 coupled to a triple quadrupole mass spectrometer Varian model 1200 (Palo Alto, CA, USA), and the software Varian MS Workstation version 6.9 was used for data processing.

Because this method was aimed at single runs of multiclass compounds, a programmed temperature vaporization (PTV) injector was chosen in order to help compound transference to the chromatographic column at a lower temperature (100 °C), avoiding losses by thermal degradation. The PTV allows greater carrier gas speed, reducing the permanence time of the compounds in the heated liner. Another additional benefit is obtained by reducing the contact surface of the active sites of the liner with the analytes, minimizing the matrix effect.

After the injection of 5 μL , in splitless mode, the injector temperature was held at 100 $^{\circ}\text{C}$ for 0.1 min and then increased at 200 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$ which was held for 28 min. After that, the injector heating was turned off, and the temperature decreased to 100 $^{\circ}\text{C}$ for the next injection, with no auxiliary cooling. The split vent was maintained closed for 2.0 min to ensure complete sample transfer, and then, a split ratio of 20:1 was used to clean the liner for the next injection.

The conditions for chromatographic separation were FactorFour analytical column VF-5MS low bleed (Agilent Technologies, Santa Clara, CA, USA) of 30 m length \times 0.25 mm inner diameter and 0.25 μm of film thickness, with helium as carrier gas at 1.0 mL min^{-1} . Column oven temperature programming: 50 $^{\circ}\text{C}$ (1.0 min), heating at 10 $^{\circ}\text{C min}^{-1}$ to 65 $^{\circ}\text{C}$ (0 min), heating at 25 $^{\circ}\text{C min}^{-1}$ to 180 $^{\circ}\text{C}$ (0 min), heating at 5 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$, and holding for 7.9 min.

The GC system was interfaced with a model 1200 triple quadrupole mass spectrometer (Varian, Middelburg, the Netherlands), operating in MS/MS mode, and electron ionization mode (70 eV). For the MS/MS experiments, argon was used as collision gas with the collision cell pressure set at 2.0 mTorr. The temperatures of the transfer line, ion source, and manifold were set at 290, 210, and 40 $^{\circ}\text{C}$, respectively. Selected reaction monitoring (SRM) method was used in MS/MS acquisition, which an ion of a particular mass is selected in the first stage and an ion product of a fragmentation reaction of the precursor ion is selected in the second stage for detection.

The triple quadrupole MS/MS operation conditions were carefully optimized during the development of this method, in order to achieve a compromise between detectability, adequate peak shapes, enough data points per peak for an adequate area measurement, and simultaneous determination of as many compounds as possible in a single run [34]. To optimize the analysis conditions of the GC-QqQ-MS/MS system, a solution containing all compounds in study at 1 mg L^{-1} , prepared in dichloromethane, was injected to evaluate the separation by the acquisition of the mass spectra in full scan mode scanning between 50 and 800 m/z . The retention times were identified by mass spectra and, if necessary, confirmed by injection of individual analytical solutions for each compound. To avoid eluting of a lot of compounds in a small time window and, consequently, for a lot of transitions to be monitored in a single segment, the GC temperature was adjusted to obtain adequate separation during the chromatographic run.

Two or three ions for each compound were selected from their respective acquired mass spectra, and several runs were made in the selected ion monitoring mode for time segment setting. Another sequence of runs was made to optimize the collisional energy. The ions for MS/MS were selected based on higher abundance, higher m/z , and fragmentation profile. The mass spectrometer operates in product ion scan mode varying the collisional energy for each ion. This process was

limited by scan rate fixed at 1,250 amu s^{-1} , and only one or two transitions can be made simultaneously in each segment. Hence, two product ions were selected, and the mass spectrometer was programmed for monitoring the reactions in the MS/MS mode with collisional energy optimized to obtain higher signal and response stability. In the last step, all transitions were programmed in the same method, and another sequence of runs was made to optimize the scan time and dwell time. The dwell time is automatically calculated by the software as function of scan time and the number of transitions monitored in each segment. The chromatographic run was divided in 17 segments to achieve best MS sensitivity and satisfactory peak shapes. The selected operational conditions are listed in Table 1.

Sample preparation by SPE

In the preliminary experiments, four different sorbent phases were tested (Strata C18-E, Strata SDB-L, Strata X, and Bond Elut Nexus) following by a general SPE procedure: precondition with acetone and water, percolation of 100 mL of sample at 3 mL min^{-1} , and elution with 1 mL of methylene chloride. For this study, blank water samples spiked at 5 $\mu\text{g L}^{-1}$ with a mixture of the selected compounds. Extracts were injected in the GC-QqQ-MS/MS system for evaluation.

Central composite design (CCD) was used to optimize the SPE extraction significant variables such as pH of the sample and eluent composition. An experimental CCD design was built for the evaluation of the main variables affecting the extraction recovery. The methodology of CCD design is a powerful and useful tool in rapidly searching key variables from a multivariable system. A CCD combines a 2^f factorial design with additional points (star points) and one point at the center of the experimental region to obtain properties such as rotatability or orthogonality, in order to fit quadratic polynomials. The star points are located at $+\alpha$ and $-\alpha$ from the center of the experimental domain (Table 2). This method can provide important information about each variable by relatively few experiments.

Validation of analytical method

To evaluate the accuracy and precision of the method, in terms of recovery, repeatability, and intermediate precision, treated industrial effluent and river and drinking water blank samples were spiked with a mixture of pesticides and related compounds followed by SPE extraction and GC-QqQ-MS/MS determination.

Intermediate precision was evaluated in order to simulate a more real routine analysis situation and was determined another day performing the full procedure using the spiked samples of treated industrial effluent and river and drinking water matrices.

Table 1 Acquisition parameters of the system GC-QqQ-MS/MS

	Pesticide	Retention time (min)	Time window (s)	Scan time (s)	Dwell time (s)	SRM transitions, <i>m/z</i> (CE, <i>V</i>)	
						Quantification	Confirmation
1	PCBTf	4.0	1	0.2	0.1	180>130(20)	180>145(20)
2	3,4 DCBTf	5.1	2	0.2	0.025	179>143(20)	179>144(20)
3	1,2-Dichlorobenzene	5.5	2	0.2	0.025	146>111(20)	146>75(20)
4	Nitrobenzene (surrogate)	5.9	2	0.2	0.025	77>51(18)	77>77(18)
5	Mononitro	6.2	3	0.12	0.015	179>143(18)	179>144(18)
6	DDVP+trichlorfon ^a	6.9	3	0.12	0.015	185>63(15)	185>93(10)
7	DCPI+diuron ^a	7.2	3	0.12	0.015	143>93(20)	143>123(20)
8	Dinitro	7.3	3	0.12	0.015	159>73(8)	159>124(8)
9	3,4-Dichloroaniline	8.1	4	0.2	0.1	161>99(16)	161>126(16)
10	Ketone	9.6	5	0.105	0.013	139>103(15)	139>77(15)
11	Trifluralin	9.8	5	0.105	0.013	306>264(10)	306>206(15)
12	Chlorpropham	9.9	5	0.105	0.013	213>171(10)	213>127(20)
13	Monocrotophos	10.1	5	0.105	0.013	127>109(15)	127>95(18)
14	HCH-alpha	10.5	6	0.2	0.08	219>109(25)	219>147(20)
15	Hexachlorobenzene	10.6	6	0.2	0.08	284>214(35)	284>249(30)
16	Dimethoate	10.7	6	0.2	0.08	125>93(40)	125>79(10)
17	HCH-beta	11.0	6	0.2	0.08	219>109(25)	219>147(20)
18	Oxirane	11.1	6	0.2	0.08	138>77(12)	138>103(12)
19	Ethion	11.2	6	0.2	0.08	231>129(25)	231>175(10)
20	Lindane	11.3	6	0.2	0.08	219>109(25)	219>147(20)
21	Chlorpyrifos methyl	10.6	6	0.2	0.08	288>93(20)	288>286(10)
22	Simazine	10.8	6	0.2	0.08	201>138(15)	201>173(15)
23	Atrazine	10.9	6	0.2	0.08	215>200(10)	215>173(10)
24	Chlorothalonil	11.6	6	0.2	0.08	266>133(35)	266>168(35)
25	Parathion methyl	11.6	6	0.2	0.08	263>109(25)	263>136(10)
26	Pirimicarb	11.9	7	0.1	0.025	166>83(18)	166>96(15)
27	HCH-delta	11.9	7	0.1	0.025	219>109(25)	219>147(20)
28	Propanil	12.5	8	0.2	0.012	161>126(20)	161>99(20)
29	Alachlor	12.7	8	0.2	0.012	188>160(10)	188>130(40)
30	Metalaxyl	12.9	8	0.2	0.012	206>132(20)	206>162(10)
31	Heptachlor	13.0	8	0.2	0.012	274>237(20)	274>239(20)
32	Pirimiphos methyl	13.2	8	0.2	0.012	290>151(15)	290>180(10)
33	Fenitrothion	13.4	8	0.2	0.012	277>260(10)	277>109(25)
34	Malathion	13.5	8	0.2	0.012	173>99(15)	173>127(10)
35	Dichlofluanid	13.6	9	0.2	0.014	224>123(10)	224>224(5)
36	Aldrin	14.0	9	0.2	0.014	263>191(30)	263>193(30)
37	Fenthion	14.0	9	0.2	0.014	278>109(20)	278>125(18)
38	Parathion ethyl	14.1	9	0.2	0.014	291>109(20)	291>81(25)
39	Dicofol	14.3	9	0.2	0.014	139>111(10)	139>75(25)
40	Fipronil	14.8	10	0.2	0.01	367>178(40)	367>213(30)
41	Bromoketal	14.9	10	0.2	0.01	259>173(8)	259>69(8)
42	Heptachloro endo-epoxide	15.0	10	0.2	0.01	353>263(15)	353>282(15)
43	Pendimethalin	15.1	10	0.2	0.01	252>161(15)	252>191(10)
44	Triadimenol	15.3	10	0.2	0.01	168>70(10)	168>168(5)
45	Procymidone	15.4	10	0.2	0.01	283>96(10)	283>67(20)
46	Methidathion	15.7	10	0.2	0.01	145>85(10)	145>58(15)
47	Endosulfan alpha	16.3	11	0.15	0.012	241>206(10)	241>170(20)

Table 1 (continued)

	Pesticide	Retention time (min)	Time window (s)	Scan time (s)	Dwell time (s)	SRM transitions, <i>m/z</i> (CE, <i>V</i>)	
						Quantification	Confirmation
48	Myclobutanil	16.8	11	0.15	0.012	179>125(10)	179>90(25)
49	Dieldrin	17.0	11	0.15	0.012	277>241(10)	277>206(15)
50	Kresoxim methyl	17.1	11	0.15	0.012	206>116(10)	206>130(18)
51	2,4-DDD	17.2	11	0.15	0.012	235>165(20)	235>199(20)
52	Endrin	17.8	12	0.2	0.025	263>191(30)	263>193(30)
53	Endosulfan beta	18.2	12	0.2	0.025	241>206(10)	241>170(20)
54	Trifloxystrobin	19.1	13	0.15	0.015	222>190(5)	222>162(10)
55	Propiconazole	19.2	13	0.15	0.015	259>69(10)	259>173(15)
56	Endosulfan sulfate	19.4	13	0.15	0.015	272>237(15)	272>235(10)
57	DDT	19.4	12	0.2	0.025	235>165(20)	235>199(20)
58	Tebuconazole	19.9	14	0.15	0.015	250>125(15)	250>163(10)
59	Epoxiconazole	20.4	12	0.2	0.025	192>138(15)	192>111(25)
60	Triphenylphosphate (IS)	20.5	14	0.15	0.015	325>169(18)	325>226(18)
61	Tetradifon	22.2	14	0.15	0.015	229>201(15)	229>199(15)
62	Lambda cyhalothrin	22.7	15	0.15	0.015	197>141(10)	197>161(5)
63	Mirex	23.1	15	0.15	0.015	272>237(10)	272>143(40)
64	Pyrazophos	23.3	15	0.15	0.015	221>193(10)	221>198(20)
65	Fenarimol	23.4	15	0.15	0.015	251>139(20)	251>111(35)
66	Permethrin	24.8	15	0.15	0.015	165>91(10)	165>127(5)
67	Cyfluthrin-alpha+beta ^a	26.0	16	0.1	0.05	164>127(5)	164>91(15)
68	Cypermethrin	26.3	16	0.1	0.05	164>127(5)	164>91(15)
69	Fenvalerate	28.1	17	0.2	0.018	225>197(5)	225>147(10)
70	Esfenvalerate	28.9	17	0.2	0.018	225>91(25)	225>147(10)
71	Deltamethrin	30.2	17	0.2	0.018	253>174(10)	253>172(5)
72	Azoxystrobin	30.7	17	0.2	0.018	344>329(10)	344>156(30)

SRM selected reaction monitoring, CE collision energy, IS internal standard

^a Compounds determined simultaneously (sum), since the chromatographic and mass spectrometric conditions used did not allow the separation. In both cases, the toxicity and environmental behavior are similar

The instrumental linearity was evaluated by injections of analytical solutions of a mixture of compounds in methylene chloride in eight concentration levels from instrumental LOQ to 1 mg L⁻¹.

The first criterion for LOD and LOQ estimation was the signal-to-noise ratio (*S/N*) calculated by the software of the equipment, considering a peak with a minimum of eight data points and *S/N* of 3 as the LOD and with *S/N* of 9 as the LOQ. Based on this initial estimation, the LOD and LOQ values provided by the whole proposed method (SPE and GC-QqQ-MS/MS determination) were established from experimental determinations. Nine aliquots of blank sample were spiked with a mixture of compounds and analyzed. The obtained results in concentration were used for the determination of method LOD and LOQ values for each compound.

Matrix effects (generally recognized as a suppression or enhancement of the analytical signal due to co-eluting matrix

components) have been widely studied and recognized as a source of error in chromatographic quantitative analysis of food samples [35]. The matrix effect was evaluated by injection of matrix-matched standards and solvent analytic solutions in three concentrations along the method linear range. Samples of treated industrial effluent and river and drinking water were submitted to the proposed SPE procedure, and the obtained extracts were spiked with a mixture analytical solution of compound in study, just before injection in GC-QqQ-MS/MS system. The regression equations were calculated, and matrix effect matrix effects were investigated by comparing the slopes of the calibration curves [36].

Application in real sample

The developed method was applied to the determination of the studied compound in real samples of the studied matrices. The

Table 2 Design matrix for 2² central composite design

	Levels				
	Low (-1)	Central (0)	High (+1)	- α	+ α
(X ₁) pH of sample	3.5	5.5	7.5	2.7	8.3
(X ₂) eluent MeOH/DCM, v/v	15/85	50/50	85/15	0/100	100/0
Runs	X ₁		X ₂		
1	-1		-1		
2	1		-1		
3	-1		1		
4	1		1		
5	- $\sqrt{2}$		0		
6	0		$\sqrt{2}$		
7	$\sqrt{2}$		0		
8	0		- $\sqrt{2}$		
9	0		0		
10	0		0		
11	0		0		

DCM methylene chloride, MeOH metanol

samples of river water, collected 200 m downstream from the point of release of the treated industrial effluent, showed the following average characteristics: pH=7.0, chloride content of 3.7 mg L⁻¹, and chemical oxygen demand (COD) <10 mg O₂L⁻¹. The samples of treated effluent had the following average characteristics: pH=7.0, chloride content of 1,420 mg L⁻¹, and COD of 63.2 mg O₂L⁻¹. Drinking water samples were from the public water supply sources. Samples were stored in amber glass bottles and if necessary kept refrigerated at 3±3 °C until the analysis within 7 days.

Results and discussion

Preliminary tests

For the concentration of the compounds, SPE cartridges containing polymeric Bond Elut Nexus sorbent were employed, because during the preliminary tests conducted in this work, only this sorbent allowed the efficient simultaneous extraction of the compounds of interest. Mixed mode polymeric SPE Nexus is an ultraclean spherical polymer that consists of hydrophilic commercial sorbent based on the copolymer of methacrylate-divinylbenzene which has bimodal porosity and a high surface area. The sorbent Nexus offers a nonpolar retention mechanism with no preconditioning required [37].

This SPE sorbent has been used to isolate acidic, neutral, and basic drugs in one analytical run from highly viscous samples, like biological fluids [38, 39], and also for the extraction of sulfonamides from honey samples [37]. To the best of

our knowledge, no publication used Bond Elut Nexus for the preconcentration of pesticide residues or related compounds in water samples. According to the supplier information, the Nexus Bond Elut cartridge does not need the conditioning step, but as xylene contamination was observed during the tests, a step of cleaning with two portions (3 mL) of acetone and two portions of ultrapure water was performed before use.

For elution with dichloromethane, the cartridge should be free of water to avoid formation of emulsion in the final extract and injection of water into the chromatographic system, so that the cartridges were dried with N₂ gas for 5 min until there was no more presence of moisture in the sorbent. This procedure allowed the extraction of 70 added to the sample, but an additional optimization is needed to achieve the required accuracy.

Method optimization using CCD

After the initial tests, the main goal of method optimization was to increase the accuracy for selected compounds in study and increase the peak area and consequently the LOD and LOQ. To evaluate the mean peak area, all pesticides and number of compounds with accuracy between 70 and 120 % of (Y), 11 experiments were conducted according to the CCD method. The spiked samples were processed under conditions described in Table 2, and the final extracts were injected in the GC-QqQ-MS/MS system. The parameters of factorial design were evaluated by ANOVA to verify the adequacy and significance of the quadratic model. This model was used to plot two response surfaces for each dependent variable studied: one for mean peak area and another for the number of compounds with accuracy between 70 and 120 %.

The composition of elution solvent, expressed in terms of proportion (in percent) of methanol (MeOH) by methylene chloride, shows a negative significant effect, i.e., when the proportion of methanol decreases, both mean peak and number of compounds with a good accuracy increase. Although the variable pH had no significance at 95 % confidence level, the two-way interaction with the variable %MeOH shows significant effect ($p > 0.05$). This effect can be observed in the surface response presented in Fig. 1a. For mean peak area, the CCD parameters indicate very similar behavior, except that the effect of pH is less visible in the surface response at Fig. 1b. The regression model indicates elution without or with a low fraction (<15 %) of methanol at any pH level tested (ranged between 3.5 and 8.3) as the best condition for the proposed method; then, the final choice was the use of MeOH/methylene chloride (10:90, v/v) and the sample pH adjusted to 3.0. The importance of the sample pH adjustment and the use of methanol together with methylene chloride as SPE elution solvent indicated by the CCD method can be explained by the physicochemical characteristics, like high polarity and acidity of some of the selected compounds.

Extraction procedure

The following was the final proposed methodology: SPE cartridges were conditioned with 6 mL of acetone followed by 3 mL of ultrapure water, and the sample volume used was 100 mL for each sample with pH adjusted to 3.0 with H_3PO_4 (1:1, v/v). For the preparation of the spiked samples, 80 mL of blank sample (industrial effluent, drink water, river water) was transferred to a 100-mL volumetric flask; the required amount of analytical solution for spiking was added, and the volume was completed with the blank sample. Before sample percolation, purified water was added to fill the cartridge, and the samples were then percolated at a flow rate of around 3 mL min^{-1} . After percolation of the sample 5 mL of purified

water was added to wash the cartridge, and this was subjected to a low flow of N_2 for 5 min to remove moisture. Then, the elution was effected with portions of 0.5 mL of elution solution, and the extract was collected in a graduated tube for volume measurement. The final volume was 1 mL by addition of small volumes of elution solution over the cartridge, and the extracts were analyzed by GC-QqQ-MS/MS.

The low amount of sorbent phase in the selected cartridge required a small volume of elution solvent, but requires low sample flow to prevent earlier elution of polar compounds, although the time of percolation was 33 min, comparable with 25 min of the EPA Method 523 [40] and 532 [41] and lower than EPA method 526 [42]. Considering that the proposed method does not require the step of concentration of the extracts, the time required will still be lower than the EPA official methods as shown in Table 3. Another advantage of the proposed method is that a smaller quantity of organic solvents is required. Comparison between the LOD of the different methods indicated that they are very similar.

The proposed method, when compared with traditional EPA methods, uses less sample volume, can be performed in reasonable time, avoids a concentration step, and allows achievement of similar results in terms of method LOD for a higher number of pesticides from different classes together with several related compounds.

The profile of a typical chromatogram of the compounds at $100 \mu\text{g L}^{-1}$, obtained with the optimized conditions, is shown in Fig. 2 were a suitable chromatographic behavior can be observed. In detail, five SRM transitions of co-eluted and or near-eluted compound are shown.

A comparison of the chromatogram obtained in the range of retention time (t_R) of trifluralin and the mass spectrum of this compound in either full scan and MS/MS mode can be seen in Fig. 3, which also shows the signal-to-noise ratio for both cases with a significant increase in sensitivity when using the mode MS/MS selected in this work.

Fig. 1 Response surface obtained for the factorial experimental design considering (a) the number of compounds with accuracy between 70 and 120 % and (b) the mean peak area

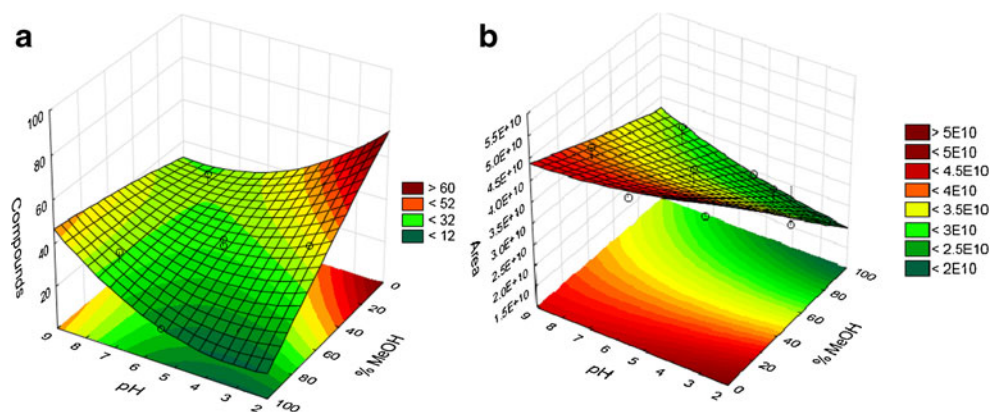


Table 3 Comparison of EPA methods with the proposed method

	EPA 526 (2000)	EPA 523 (2011)	EPA 532 (2000)	This method
Compounds	SVOCs (11)	Triazines	Phenylureas	Multiclass
Sorbent	SDVB	Graphitized carbon	C18	Polymeric
Phase amount (mg)	500	250	500	60
Sample amount (mL)	1,000	250	500	100
Flow rate (mL min ⁻¹)	20	10	20	3
Extraction time (min)	50	25	25	33
Elution solvent	EtAc + DCM	EtAc + DCM + MeOH	MeOH	MeOH + DCM
Eluent volume (mL)	13	14	6	1
Concentration step	Yes	Yes	Yes	No
Final volume (mL)	1	2	1	1
Concentration factor	1,000×	125×	500×	100×
Method LOD (μg L ⁻¹)	0.03–0.14	0.10–0.69	0.03–0.09	0.01–0.25

EtAc ethyl acetate, DCM methylene chloride, MeOH methanol, SDVB styrene divinyl benzene, LOD limit of detection, MDL method detection limit, DL detection limit, SVOC semivolatile organic compounds

Validation of analytical method

Table 4 presents the linear range and coefficients of determination (r^2) for each compound. The r^2 values were >0.99 for all studied compounds, being appropriate for analysis of pesticide residues. In cases where the linear calibration model was not accepted in the F test, the quadratic equation was calculated, and the model was subjected again to analysis of variance using the F test [43]. The repeatability of the instrument was evaluated by injection, with six replicates each, of eight analytical solutions used to obtain the calibration curves, obtaining suitable RSD values (<15 %). Table 4 also presents the mean values of accuracy (recovery) and overall precision (RSD%), in terms of repeatability, for spiked samples of

treated industrial effluent, river water, and drinking water blank samples, at concentrations 0.5, 2.5, and 5.0 μg L⁻¹ for all three matrices. Most of the compounds presented satisfactory results of recovery and RSD because they are within the accepted values for the determination of pesticide residues, which should be between 70 and 120 %, with RSD < 20 % in all spiked levels [44]. However, the compounds 1,2-dichlorobenzene, 3,4-dichloroaniline, chlorothalonil, dimethoate, malathion, methidathion, parathion methyl, pyrazophos, and propanil showed unsatisfactory recovery on one of the matrices; however, RSD < 20 % can be considered within the acceptable criteria for the concentration levels and the goal of the proposed method [45]. These values of recovery are satisfactory when dealing with complex environmental

Fig. 2 GC-QqQ-MS/MS SRM chromatogram of a solution containing 100 μg L⁻¹ of all compounds of interest employing the chromatographic conditions described in Table 1

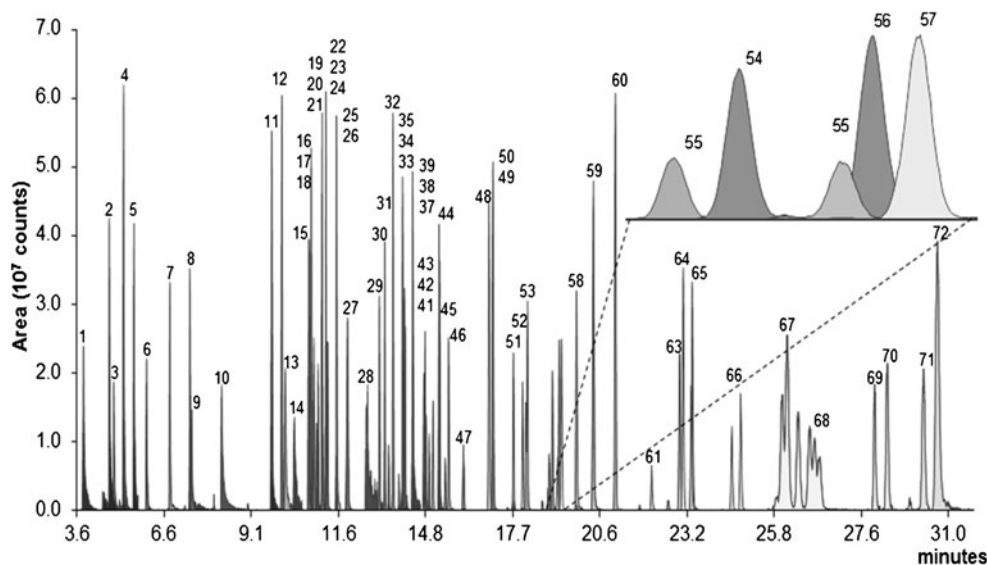
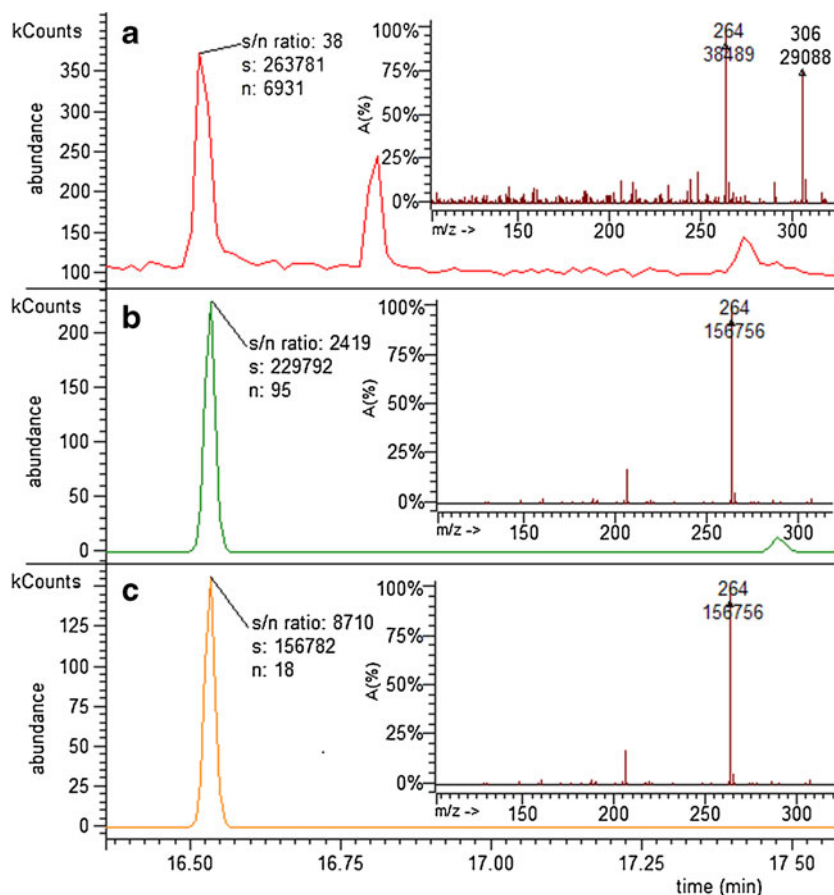


Fig. 3 Chromatograms and mass spectra. (a) Total ion chromatogram (full scan), (b) total ion chromatogram in MS/MS mode, and (c) chromatogram of the ion 264, in MS/MS mode, with their mass spectra obtained from a solution containing 1 mg L^{-1} of trifluralin



samples like the ones analyzed in this work. The variations in the extraction efficiencies can be attributed to the distinct chemical properties of the substances, which undergo different interactions with the sorbent in the extraction process.

Results of intermediate precision evaluated by full analysis sequence made in different days in spiked drinking water, river water, and treated industrial effluent blank samples presented RSD values from 3.2 to 19.7 %, and the recovery results were similar to the repeatability test, considered satisfactory for the proposed method.

Mean recoveries for 1,2-dichlorobenzene in river water (61 %) at different spike levels are probably because this compound is very volatile, and losses from extracts can occur during analysis. Low recoveries in drinking water for chlorothalonil (39 %), dimethoate (40 %), malathion (54 %), methidathion (38 %), parathion methyl (45 %), and pyrazophos (59 %) and in river water for dimethoate (31 %) are probably due the high levels of matrix effect observed for these compounds.

Figure 4 shows the matrix effect, calculated as the difference (in percent) from the slope of the calibration curves obtained with analytical solutions in solvent and in the matrix of spiked blank samples, both at the concentrations 0.5 , 2.5 , and $5.0 \text{ } \mu\text{g L}^{-1}$. Most of the compounds presented matrix effect below 25 % for potable water and treated industrial effluent, corresponding to 54 and 67 compounds,

respectively. For river water, only 34 compounds presented matrix effect below 25 %. For potable water, 11 compounds showed matrix effects higher than 50 %, and in river water, seven compounds had similar behavior, probably due to the presence of humic and fulvic acids in river water and their residuals after basic treatment for potable water. For industrial treated effluent, none of the studied compounds presented matrix effects higher than 50 %, because this effluent, in spite of having been generated in an industrial plant for production of pesticides, was treated in an advanced wastewater treatment plant by flocculation, sedimentation, activated biological treatment, oxidation with UV light in presence of hydrogen peroxide, and finally adsorption of organic compounds in activated charcoal, resulting in a very low residual content of organic compounds.

Method applicability in real samples

Application of the method with five real samples of each of the studied matrices showed excellent performance in terms of method suitability. No chromatographic interferences were observed for the different types of water investigated. No pesticide or related compounds were detected in the five drinking water samples evaluated. River water samples presented residues of metalaxyl, myclobutanil, azoxystrobin,

Table 4 Linearity, method LOD and LOQ, recovery, and precision values presented by the proposed method

Compound	Linearity r^2	Method		Mean recovery (%)			Repeatability RSD ^a	Intermediate precision RSD ^a
		LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Drinking water	River water	Treated effluent		
1,2-Dichlorobenzene	0.9928	0.07	0.29	107	61	125	6.1	19.7
2,4-DDD	0.9939	0.07	0.44	99	86	87	7.6	7.4
3,4 DCBTF	0.9904	0.07	0.25	103	101	86	8.8	12.6
3,4-Dichloroaniline	0.9998	0.09	0.63	131	84	94	5.9	5.8
Alachlor	0.9942	0.06	0.47	79	81	85	7.9	6.7
Aldrin	0.9938	0.07	0.40	102	81	85	5.9	8.3
Atrazine	0.9979	0.07	0.46	87	77	85	6.5	5.6
Azoxystrobin	0.9999	0.20	1.16	99	81	105	9.5	18.5
Bromoketal	0.9918	0.08	0.53	94	93	83	9.6	7.0
Lambda cyhalothrin	0.9999	0.11	0.57	119	95	101	11.8	8.4
Cyfluthrin-alpha+beta	0.9999	0.23	0.67	117	100	85	12.2	8.8
Cypermethrin	0.9999	0.15	0.69	119	104	92	12.9	6.4
Chlorothalonil	0.9878	0.02	0.52	39	101	77	13.4	7.7
Chlorpyrifos methyl	0.9953	0.14	0.39	87	83	76	6.2	14.0
Chlorpropham	0.9999	0.10	0.72	107	90	89	8.2	6.0
Kresoxim methyl	0.9986	0.25	0.63	103	78	88	5.9	7.2
DCPI+diuron	0.9994	0.07	0.37	72	75	83	6.6	8.8
DDT	0.9999	0.05	0.36	75	82	88	9.2	6.0
DDVP + trichlorfon	0.9999	0.03	0.36	86	73	75	12.3	8.6
Deltamethrin	0.9999	0.16	0.74	112	94	112	11.3	14.2
Dichlofluanid	0.9840	0.11	0.76	78	106	80	9.1	5.9
Dicofol	0.9714	0.05	0.78	116	104	76	9.5	7.4
Dieldrin	0.9956	0.09	0.41	94	80	83	9.1	6.0
Dimethoate	0.9999	0.07	0.21	40	31	90	16.5	5.5
Dinitro	0.9996	0.06	0.49	105	85	70	6.6	6.1
Endosulfan alpha	0.9964	0.17	0.97	94	83	86	8.8	6.1
Endosulfan beta	0.9946	0.20	0.99	101	79	90	7.7	7.8
Endosulfan sulfate	0.9907	0.07	0.94	76	95	114	12.1	13.5
Endrin	0.9998	0.09	0.49	108	101	91	9.1	9.3
Epoxiconazole	0.9999	0.18	1.23	99	73	87	6.2	6.1
Esfenvalerate	0.9999	0.15	0.66	119	103	118	12.7	13.4
Ethion	0.9914	0.05	0.53	83	113	87	11.8	8.1
Fenarimol	0.9999	0.08	0.59	106	75	97	6.0	7.0
Fenitrothion	0.9993	0.04	0.43	73	119	94	13.5	11.2
Fenthiona	0.9851	0.05	0.57	76	111	80	11.3	7.3
Fenvalerate	0.9997	0.17	0.61	118	105	94	14.4	8.4
Fipronil	0.9998	0.18	0.75	120	83	86	6.6	7.3
HCH-delta	0.9919	0.06	0.44	70	114	89	13.3	6.9
HCH-alpha	0.9943	0.08	0.46	79	115	86	11.4	7.3
HCH-beta	0.9974	0.08	0.51	90	112	87	12.6	11.7
Heptachlor	0.9937	0.06	0.46	85	86	83	6.7	6.8
Heptachlor endo-epoxide	0.9932	0.07	0.51	101	94	80	10.1	6.1
Hexachlorobenzene	0.9878	0.06	0.46	99	72	82	5.9	6.3
Ketone	0.9999	0.12	0.50	103	104	91	7.5	8.6
Lindane	0.9996	0.09	0.36	81	83	87	10.4	8.8
Malathion	0.9997	0.04	0.43	54	103	84	14.7	7.9

Table 4 (continued)

Compound	Linearity r^2	Method		Mean recovery (%)			Repeatability RSD ^a	Intermediate precision RSD ^a
		LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Drinking water	River water	Treated effluent		
Methidathion	0.9998	0.03	0.48	38	97	88	14.3	7.2
Metalaxyl	0.9999	0.10	0.48	100	82	88	8.1	6.2
Myclobutanil	0.9999	0.07	0.58	105	79	85	5.7	6.1
Mirex	0.9936	0.04	0.36	95	76	83	8.2	7.0
Monocrotophos	0.9999	0.01	0.55	72	119	99	5.3	3.2
Mononitro	0.9998	0.09	0.44	103	75	75	3.9	8.4
Oxirane	0.9927	0.06	0.65	93	107	70	8.3	13.2
Parathion ethyl	0.9998	0.08	0.48	82	112	87	10.8	6.0
Parathion methyl	0.9997	0.09	0.38	45	123	78	10.9	11.7
PCBTF	0.9910	0.04	0.48	82	70	71	6.9	5.2
Pendimethalin	0.9997	0.09	0.46	106	94	86	10.3	6.6
Permethrin	0.9999	0.09	0.49	110	77	85	9.3	7.0
Pyrazophos	0.9999	0.08	0.60	59	120	92	15.6	9.6
Pirimicarb	0.9999	0.05	0.49	102	92	84	19.5	4.6
Pirimiphos methyl	0.9998	0.06	0.44	86	89	83	9.4	6.8
Procymidone	0.9961	0.06	0.58	92	92	80	7.4	6.5
Propanil	0.9999	0.14	1.04	131	101	92	6.9	6.2
Propiconazole	0.9999	0.12	0.64	107	73	98	5.9	6.5
Simazine	0.9959	0.12	0.65	83	85	84	11.2	9.0
Tebuconazole	0.9999	0.10	0.69	110	82	91	6.4	6.5
Tetradifon	0.9999	0.15	0.57	106	98	91	10.3	9.1
Triadimenol	0.9999	0.09	0.62	105	89	85	6.6	5.6
Trifloxystrobin	0.9997	0.14	0.49	99	107	85	13.7	7.2
Trifluralin	0.9911	0.07	0.50	87	86	80	6.7	5.4

^a Mean RSD for all evaluated spike levels and matrices

and tebuconazole, but below the method LOQ in all five samples. Treated industrial effluent presented residues of propiconazole at concentrations from LOQ to $0.83 \mu\text{g L}^{-1}$ and tebuconazole, mononitro, trifluralin, and propanil $<\text{LOQ}$ in all five samples. In two samples, bromoketal was detected at a concentration below the LOQ.

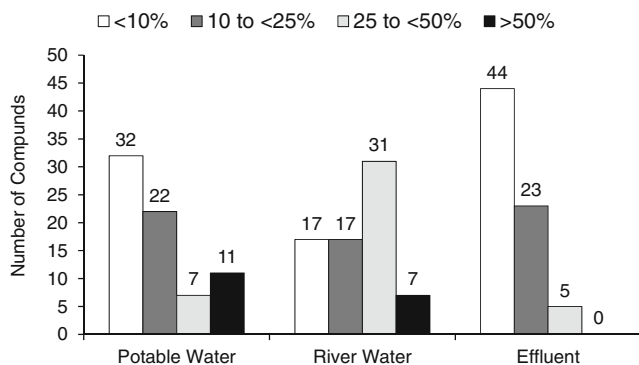


Fig. 4 Comparison of the matrix effect for the studied matrices

Conclusions

The results indicate that the proposed method is efficient and accurate for the determination of residues of 2,4-DDD, 3,4 DCBTF, alachlor, aldrin, atrazine, azoxystrobin, bromoketal, lambda cyhalothrin, cyfluthrin-alpha + beta, cypermethrin, chlorpyrifos methyl, chlorpropham, kresoxim methyl, DCPI + diuron, DDT, DDVP + trichlorfon, deltamethrin, dichlofluanid, dicofol, dieldrin, dinitro, endosulfan alpha, endosulfan beta, endosulfan sulfate, endrin, epoxiconazole, esfenvalerate, ethion, fenarimol, fenitrothion, fenthion, fenvalerate, fipronil, HCH-delta, HCH-alpha, HCH-beta, heptachlor, heptachlor endo-epoxide, hexachlorobenzene, ketone, lindane, metalaxyl, myclobutanil, mirex, monocrotophos, mononitro, oxirane, parathion ethyl, PCBTF, pendimethalin, permethrin, pirimicarb, pirimiphos methyl, procymidone, propiconazole, simazine, tebuconazole, tetradifon, triadimenol, trifloxystrobin, and trifluralin in samples of drinking water, river water, and treated industrial effluent with recoveries from 70 to 120 % and RSD <20 %. The method is

also suitable for 3,4-dichloroaniline, chlorothalonil, malathion, methidathion, pyrazophos, and propanil residue determination in river water and 1,2-dichlorobenzene in drinking water. The method is rapid and economical, allowing the simultaneous preparation of several samples for a multiclass pesticide determination and reducing time-consuming steps like water batch solvent evaporation, the amount of solvent and glassware compared to other methods.

Based on the validation, the proposed method is adequate for the monitoring of selected compounds in drinking water, treated industrial effluent, and surface water, emphasizing that the use of GC-QqQ-MS/MS gives information about the identity of the compound, and has an excellent selectivity and sensitivity, making the process very reliable, dispensing other analytical procedures for confirmation of the analytes. The concentration factor of the SPE procedure associated with sensitivity of the chromatographic analysis permitted the achievement of the method LOD values between 0.01 and 0.25 $\mu\text{g L}^{-1}$.

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