

Evaluation of a Modified QuEChERS Extraction of Multiple Classes of Pesticides from a Rice Paddy Soil by LC-APCI-MS/MS

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ABSTRACT: A new method for the determination of clomazone, fipronil, tebuconazole, propiconazole, and azoxystrobin in samples of rice paddy soil is presented. The extraction of the pesticides from soil samples was performed by using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. Some extraction conditions such as salt addition, sample acidification, use of buffer, and cleanup step were evaluated. The optimized method dealt with a single extraction of the compounds under study with acidified acetonitrile, followed by the addition of MgSO₄ and NaCl prior to the final determination by liquid chromatography–atmospheric chemical pressure ionization–tandem mass spectrometry. Validation studies were carried out in soil samples. Recoveries of the spiked samples ranged between 70.3 and 120% with relative standard deviation lower than 18.2%. The limits of quantification were between 10 and 50 μg kg⁻¹. The method was applied to the analysis of real samples of soils where rice is cultivated.

KEYWORDS: pesticides, rice paddy soils, QuEChERS, liquid chromatography, atmospheric pressure chemical ionization

INTRODUCTION

The significant increase in the worldwide need for food demands higher agricultural productivity, which can be achieved only by an extensive use of pesticides.^{1,2} Pesticides are one of the most hazardous contaminants of the environment. Through overuse and misuse there is considerable waste contributing to the adverse environmental and health consequences. Inappropriate application of pesticides affects the whole ecosystem by entering the residues in the food chain and polluting the soil, air, and ground and surface waters.³

Rice is widely planted in Brazil. The country's production in 2009/2010 reached 11.36 million tons, and the southern region produced 72%; Rio Grande do Sul state alone has yielded 61% of the total.⁴ Because rice crops are affected by various diseases and the demand for food has increased, the use of pesticides is needed. Unfortunately, their continued, intensive, and sometimes inappropriate use increases the possibility that their residues will be found in the environment.⁵

The analysis of pesticide residues in soil and water has become indispensable to assess the quality of the environment. For the determination of pesticides, due to their low concentration levels, the different chemical properties of the analytes, and the complexity of the matrices, a sample preparation step is needed.⁶

The analysis of pesticide residue in food and environmental samples has been carried out by using many methods with different analytical characteristics.^{7–9} The introduction of new methods, capable of extracting multiple classes pesticides is, undoubtedly, the most efficient approach. The first multiresidue method for pesticide extraction was developed in 1960¹⁰ and deals with the determination of organochlorides in nonfatty samples. With regard to more polar compounds, such as organophosphorus

and organonitrogen, the Luke method was developed in 1975.¹¹ In the 1990s, increased urgency to further reduce solvent use and manual labor in analytical laboratories led to the commercial introduction of several alternative extraction approaches.¹² For the extraction of pesticides from soil samples, different sample preparations have been used, including Soxhlet extraction,¹³ pressurized liquid extraction (PLE),¹⁴ ultrasound-assisted extraction (UAE),¹⁵ dispersive liquid–liquid microextraction (DLLME),¹⁶ solid-phase extraction (SPE),¹⁷ solid-phase microextraction (SPME),¹⁸ and QuEChERS.¹⁹

The acronymic name, QuEChERS, reflects its major advantages (quick, easy, cheap, effective, rugged, and safe). This method was introduced in 2003¹² and, besides having several advantages, it explores the possibilities offered by modern instrumentation.²⁰ This method and several modified versions have been applied to the extraction of different types of pesticides, mainly of fruits and vegetables.²¹ Although this method has provided good results for the extraction of pesticides with different polarities in nonfatty foods, it is worth investigating its application to the analysis of these compounds in other nonfatty matrices such as soils, where pesticides tend to occur.²² The QuEChERS method had been applied to the extraction of pesticides from soils on few occasions. The first study found in the literature was developed in 2008.¹ Aiming at analyzing multiple residues of pesticides in soil samples, the authors compared ultrasonic solvent extraction, European Norm DIN 12393, the QuEChERS method, and PLE.

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Pesticides were analyzed by both GC-MS and HPLC-MS/MS. In comparison with other extraction methods that were tested, the QuEChERS method employing 20 mL of acetonitrile, 4 g of MgSO_4 , 1 g of NaCl, citrate salts, and a cleanup step with PSA and MgSO_4 was the most efficient one, with recoveries ($R\%$) from 27.3 to 120.9%. The limits of quantification (LOQs) were between 0.08 and $292 \mu\text{g kg}^{-1}$. In 2009, Drodzynsky and Kowalska²³ developed a modified QuEChERS method for the analysis of three ecological insecticides in soil samples with the determination by UPLC-MS/MS. The extraction conditions deal with 10 mL of acetonitrile, 100 μL of acetic acid, 4 g of MgSO_4 , 1 g of NaCl, citrate salts, and cleanup with MgSO_4 , PSA, and C18. Recoveries of the spiked samples ranged from 83 to 104% with relative standard deviation (RSD) below 9%. The LOQs were lower than $10 \mu\text{g kg}^{-1}$. The number of studies increased in 2010. Asensio-Ramos et al.²² presented a modified version of the QuEChERS method employing 20 mL of acetonitrile, 4 g of MgSO_4 , 1 g of NaCl, citrate salts, and cleanup with MgSO_4 and PSA for the determination of a group of 10 organophosphorus pesticides and 1 thiaziazine pesticide in three different types of soils. Recovery values were between 45 and 96% for most pesticides and soils, and the limits of detection (LODs) of the whole method ranged between 0.48 and $7.78 \mu\text{g kg}^{-1}$. Shi et al.²⁴ developed a simple and fast method with high rates of throughput by employing QuEChERS and gas chromatography coupled to electron capture detector (GC-ECD) to determine residues of the herbicide oxadiargyl in soil samples using 15 mL of acetonitrile, 4 g of MgSO_4 , and 1 g of NaCl. No purifying step was necessary, and $R\%$ ranged from 95.5 to 112.0%, with RSD values lower than 2.8% at all concentration levels. The LOQ was $10 \mu\text{g kg}^{-1}$. Dong et al.²⁵ presented a simple confirmatory method for the determination of metaflumizone in soil samples using UPLC-ESI-MS/MS. Using 10 mL of acetonitrile, 4 g of MgSO_4 , 1 g of NaCl, and cleanup with MgSO_4 and PSA, recoveries at four different levels were between 77.6 and 87.9%, with RSD lower than 7.9%. The LOQ was $4.0 \mu\text{g kg}^{-1}$. Chen et al.²⁶ proposed a modified QuEChERS method using 10 mL of acetonitrile, 2 g of NaCl, and cleanup with MgSO_4 and PSA for the extraction of residues of the fungicide procymidone in soil and determination by GC-MS. The recoveries ranged from 82.5 to 92.5% with RSDs from 3.7 to 7.0%. The LOQ was $5.52 \mu\text{g kg}^{-1}$. Rashid et al.²⁷ developed a procedure based on QuEChERS extraction using acetonitrile with acetic acid, 4 g of MgSO_4 , 1.7 g of sodium acetate, and a simultaneous liquid-liquid partition cleanup into *n*-hexane for the extraction of 19 organochlorine pesticides. The resulting hexane extracts were clean and suitable for determination using GC-MS/MS. The recovery values were generally between 70 and 100%, and the RSDs were at or below 20%. The method achieved LOQ, typically $1.0 \mu\text{g kg}^{-1}$.

Another method based on QuEChERS extraction has been proposed, developed and validated by Yang et al.²⁸ for the simultaneous determination of 38 pesticides in agricultural soils. The target pesticides were extracted with 20 mL of acetonitrile, 8 g of MgSO_4 , 2 g of NaCl, citrate salts, and cleanup with MgSO_4 and PSA with determination by using GC-MS. The proposed method enabled a simultaneous determination and confirmation of a large number of pesticides in soils with good reproducibility and LOQs between 4.0 and $31.2 \mu\text{g kg}^{-1}$.

This paper describes the optimization and validation of a new method employing an acetonitrile-based extraction (QuEChERS) without a dispersive solid-phase extraction (D-SPE) cleanup step

to extract pesticides from soil samples. In addition, LC coupled with a triple quadrupole mass analyzer in tandem with an atmospheric pressure chemical ionization (LC-APCI-MS/MS) for the sensitive and reliable determination of multiple classes of pesticides in soil samples was evaluated. The matrix effect related to the QuEChERS extraction from soils using an APCI source was also assessed. The selected pesticides (Table 1) belong to four different classes and have been widely used all over the world in different kinds of crops. Besides, studies of the extraction of azoxystrobin, clomazone, and tebuconazole from soil samples using the QuEChERS method have not been published yet.

MATERIALS AND METHODS

Chemicals. The analytical standards (purity > 99%) azoxystrobin (methyl (Z)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate), clomazone (2-[(2-chlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one), fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile), propiconazole (1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole), and tebuconazole (1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol) were supplied by Sigma-Aldrich (São Paulo, Brazil). The pesticides under investigation and their characteristics are listed in Table 1.

Magnesium sulfate anhydrous, sodium chloride, and sodium acetate were purchased from J. T. Baker (Phillipsburg, NJ); Bondesil-PSA (primary secondary amine) 40 μm was bought from Varian (Palo Alto, CA), and C18 was acquired from Chromabond (Duran, Germany).

The LC-grade methanol and acetonitrile were purchased from Mallinckrodt (Phillipsburg, NJ). Water was purified with a Direct-Q UV3 (resistivity 18.2 $\text{M}\Omega \text{ cm}$) water purification system (Millipore, Bedford, MA). Formic (98–100%) and glacial acetic acid were purchased from Merck (Darmstadt, Germany).

Preparation of Standard Solutions. Individual stock solutions were prepared by dissolving 10 mg of standard in 10 mL of acetonitrile. Multicomponent stock standard solutions were prepared by diluting 1 mL of each standard in 10 mL of acetonitrile, reaching $100 \mu\text{g mL}^{-1}$. They were later diluted with acetonitrile to achieve concentrations of 0.01, 0.038, 0.05, 0.1, 0.38, 0.5, and $1.0 \mu\text{g mL}^{-1}$. The solution that was used to spike the samples was prepared by diluting 0.1 mL of multicomponent stock standard with 100 mL of acetonitrile, reaching $0.1 \mu\text{g mL}^{-1}$. All of the standard solutions were stored at 4 °C away from light.

Sampling and Soil Sample Preparation. The soil samples, both for the method optimization and for the method application, were collected in a rice field. However, the former were collected in areas where pesticides had not been used.

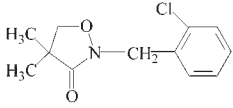
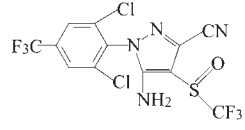
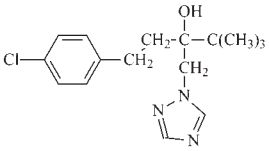
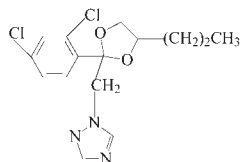
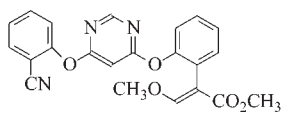
An amount of the rice soil (0.5 kg) that was collected at a depth between 0 and 20 cm was transported to the laboratory. Samples were cooled to 4 °C (or below) in the field and kept refrigerated at 4 °C, away from light prior to extraction, which was done within 48 h.

Soils were homogenized, sieved (2 mm mesh), and air-dried at room temperature before their use. The main physicochemical properties of the soil are pH (in KCl) 5.2, organic matter 0.6%, and clay (w/w) 16%.

Spiked soil samples for the method optimization were prepared by adding 10 mL of a $0.1 \mu\text{g mL}^{-1}$ standard mixture of pesticides to 10 g of soil. This volume enabled the solvent to thoroughly cover the soil particles. The bulk of the solvent was slowly evaporated at room temperature for at least 24 h.²²

Apparatus and Software. Liquid chromatography with mass spectrometric detection was performed in a Waters Alliance 2695 Separations Module (Waters, Milford, MA) fitted with an autosampler,

Table 1. Physicochemical Properties (M_w , Molecular Weight; Water Solubility; K_{OW} , Octanol/Water Coefficient; K_{OC} , Organic Carbon Sorption Constant; pK_a , Acid Ionization Constant) of the Selected Pesticides²⁹

Pesticide	M_w (g mol ⁻¹)	Chemical Structure	Chemical class	Water Solubility (mg L ⁻¹)	K_{OC} (cm ³ g ⁻¹)	K_{OW}	pK_a
clomazone	239.7		isoxazolidinone	1100	150-562	2.5	n.a.
fipronil	437.2		pyrazole	1.9 (pH 5) 2.4 (pH 9)	427	4.0	n.a.
tebuconazole	307.8		triazole	36 (20 °C) pH (5-9)	1000 ^a	3.7	n.a. ^b
propiconazole	342.2		triazole	100 (20 °C)	950	3.7	1.1
azoxystrobin	403.4		strobilurin	6 (20 °C)	500	2.5	n.a.

^a From ref 30 ^b na, not available.

a membrane degasser, and a quaternary pump. Mass spectrometry was performed on a Micromass Quattro Micro API (Waters) with an APCI interface. The LC column was an XTerra 3.5 μ m particle size (50 \times 3 mm i.d.) (Waters).

Analytical instrument control, data acquisition, and treatment were performed by Masslynx software version 4.1, 2005 (Micromass, Waters, Milford, MA).

A sample volume of 10 μ L was injected by an autosampler. The mobile phase was methanol/water acidified with 0.1% formic acid (70:30, v/v), at a constant flow of 0.2 mL min⁻¹. Run time was 7 min. Typical interface conditions were optimized for maximum intensity of the precursor ions as follows: corona current was 0.5 μ A; desolvation and cone gas flows were set at 350 and 100 L h⁻¹, respectively; source block and desolvation temperatures were 120 and 500 °C, respectively. Nitrogen was used

as nebulizing, desolvation, and cone gas, whereas argon was used as collision gas.

The optimization of the MS/MS conditions, that is, the choice of the ionization mode, identification of the parent and product ions, and selection of the cone and collision voltages, more favorable for the analysis of the target analytes was performed by the injection of their individual standard solutions. Both modes of ionization, negative and positive, were tested.

Sample Extraction. A 10 g dried soil subsample was placed into a polypropylene centrifuge tube (50 mL), and then 100 μ L of acetic acid and 10 mL of acetonitrile were added and the mixture was hand-shaken for 15 s; after that, it was shaken vigorously in a laboratory shaker for 1 min. Four grams of anhydrous magnesium sulfate and 1 g of sodium chloride were added, and the mixture was immediately hand-shaken for

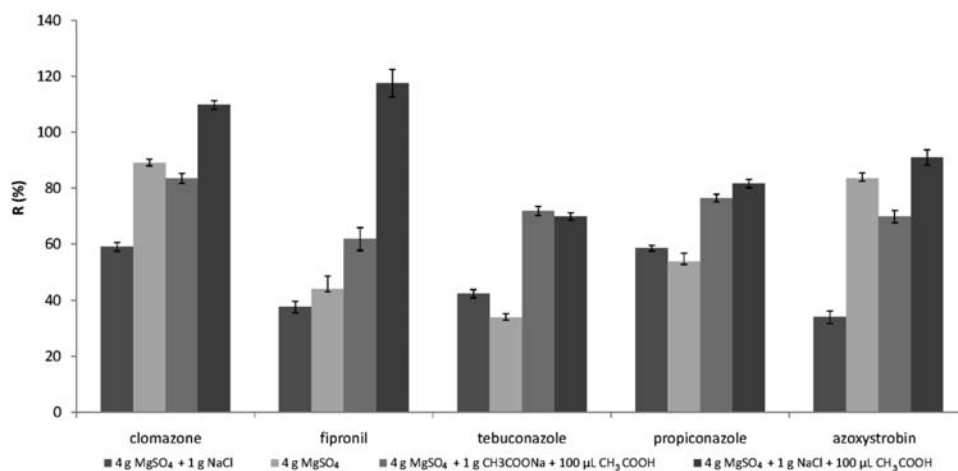


Figure 1. Recovery (%) of the comparison among different salts and acidification in the extraction of a mixture of pesticides from soil without a dispersive-SPE cleanup step (conditions: different salts and acidification, 10 mL of acetonitrile, 5 min 5000 rpm) ($n = 9$). Errors bars represent the RSD.

15 s. After that, it was shaken vigorously in a laboratory shaker for 1 min and then centrifuged at 5000 rpm for 5 min.

Validation Experiments and Internal Quality Control Criteria. The validation of the analytical method under study was performed by using spiked control samples of soil and was assessed according to SANCO.³¹ The recovery was determined for three replicates in the spiking concentrations of 10, 50, 100, and 500 $\mu\text{g kg}^{-1}$ for clomazone and azoxystrobin, and in the spiking concentrations of 50, 100, and 500 $\mu\text{g kg}^{-1}$ for fipronil, tebuconazole, and propiconazole. Calculations of recoveries were done by using the peak areas. The precision was calculated as RSD% for each concentration level. The linearity of the calibration curves was evaluated at a concentration range between 0.01 and 1.0 $\mu\text{g mL}^{-1}$ using seven calibration solutions prepared in blank control sample extracts and in the solvent. The first calibration level was always the concentration that was equivalent to the LOQ. LODs and LOQs were determined as the lowest injected pesticide concentrations that yielded signal-to-noise (S/N) ratios of 3 and 10 (when the quantification ion was monitored), respectively. The resulting values were also checked experimentally.

To ensure the quality of the results, some internal quality criteria have been applied.³² The set of samples under analysis each day was processed together with a blank extract that eliminates a false positive by contamination in the extraction process, instrument, or chemicals and with a blank extract spiked at the concentration of 100 $\mu\text{g kg}^{-1}$ to assess the extraction efficiency and calibration curves prepared daily in blank matrix extracts to check both sensitivity and linearity in the working range of concentrations. Thus, quantification mistakes caused by possible matrix effects of instrumental fluctuations could be avoided.

The matrix effect was also investigated. The degree of ion suppression/enhancement not only varies from sample to sample and from compound to compound but may also depend on the analyte concentration as well as the matrix to analyte concentration ratio; therefore, the matrix effect (ME) was investigated according to eq 1, by comparing the slopes in calibration solutions prepared in matrix (blank soil extract) and in solvent.³³ The extent of effects due to the matrix components was rated according to the percentage of signal enhancement (+) or suppression (−).

$$\text{ME (\%)} = 100 \times \left(1 - \frac{S_m}{S_s} \right) \quad (1)$$

S_s is the slope in solvent, and S_m is the slope in matrix. No matrix effect is observed when ME is equal to 100%. Values above 100% indicate ionization enhancement, and values below 100% show ionization suppression.

RESULTS AND DISCUSSION

Optimized QuEChERS Conditions. In this study, a modified QuEChERS method combined with LC-APCI-MS/MS was used for the determination of selected pesticides in soil samples. Some parameters that affect the extraction performance and efficiency were investigated and, then, the optimal conditions were selected.

Sample Size. Most studies that employ QuEChERS to extract pesticides use 10 g size sample.^{1,25,26} On the basis of the evidence found in the literature and on our experience, we chose a sub-sample size of 10 g.

Comparison of the Recoveries Using Buffer, Salt Addition, and Acidification. Figure 1 shows the results of experiments designed to determine the effect of salt addition, buffer, and/or acidification on the partitioning of the pesticides on the upper layer. Combinations of salts (MgSO_4 and NaCl) were added to induce phase separation. The salting-out effect resulting from the addition of NaCl depends on the nature of the solvents involved in the partitioning step.¹² The addition of the proper amounts and combination of salts can be used to control the percentage of water in the organic phase (and vice versa for organic solvent in the water phase), thus enabling a certain degree of adjustment in the polarity of the phases.

Combinations of MgSO_4 and NaCl were more effective for tebuconazole and propiconazole, but for the more polar compounds (clomazone and azoxystrobin), the recoveries decreased more than 20% when 1 g of NaCl was added. Fipronil showed better recoveries with the use of MgSO_4 alone than in combination with NaCl.

Anastassiades et al.¹² stated that the more NaCl is added to the system, the more complete the phase separation becomes. Therefore, less water remains in the acetonitrile phase. It becomes less polar and less receptive to polar compounds such as clomazone and azoxystrobin.

The use of buffers in QuEChERS is common,²³ and it was tested in this study for soil samples. The buffer used was composed of acetic acid and acetate salt. The recoveries increased for three compounds when the buffer used, whereas the recoveries decreased for two others in comparison with the experiment without buffer.

As shown in Figure 1, 4 g of MgSO_4 , 0.1% acetic acid, and 1 g of sodium chloride enabled the highest recoveries of all compounds.

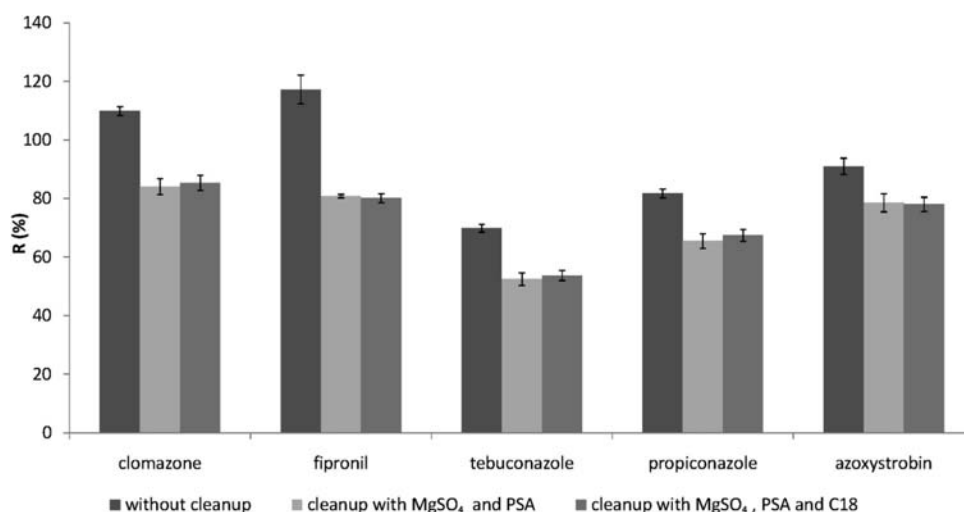


Figure 2. Recovery (%) of the comparison among different dispersive-SPE types in the extraction of a mixture of pesticides in soil (conditions: 4 g of MgSO₄, 1 g of NaCl, 10 mL of acetonitrile, 1% acetic acid, 5 min 5000 rpm) ($n = 9$). Errors bars represent the RSD.

The use of 0.1% acetic acid led to improvement in recoveries. It enabled the increase of the pesticide stability prior to analysis.³⁴

Dispersive-SPE Cleanup. Traditionally, a dispersive-SPE cleanup has been carried out in studies that employ QuEChERS. Normally, an aliquot of the sample extract is added to a vial containing a small amount of SPE sorbent (PSA and/or C18), and the mixture is briefly shaken or mixed in a vortex mixer to distribute the SPE material evenly and, thus, make the cleanup process easier. The sorbent is then separated by centrifugation, and an aliquot of the final extract is taken for analysis. With some exceptions,^{24,35,36} all studies that employ QuEChERS to extract pesticides from soil samples use a dispersive-SPE cleanup step.^{1,22,23,25–28,37} Dispersive-SPE enables the user to prepare whatever combination of sorbents in any amounts needed.³⁴ During the optimization of the QuEChERS method, a dispersive-SPE cleanup was carried out and the recoveries (the ones obtained with and without cleanup) are shown in Figure 2.

After the analytes were extracted by a water-soluble solvent (1% (v/v) acetic acid in acetonitrile) followed by the partitioning of the analyte molecules in organic solvent in the presence of a salt mixture (salting out effect), the acetonitrile phase was further cleaned up and dried by mixing with the SPE sorbents and anhydrous MgSO₄. The sorbents were chosen to retain the matrix components and to enable the analytes of interest to stay in the acetonitrile phase. The process of sample preparation showed that, for these soil samples, the different dispersive sorbents did not have a significant influence on the purification and recovery of these compounds from the extracts. In the QuEChERS method, by using the dispersive-SPE approach, PSA and C18 were compared. The procedure without the cleanup step got the highest recoveries.

PSA is a structure that has a high chelating effect due to the presence of the secondary amine, as well as the primary one. The result is the retention of fatty acids and other polar compounds in the matrix.²⁰ C18 is a reversed phase sorbent that is effective at trapping (binding) and removing starch and sugar from some samples.³⁸ This kind of coextractive may not be present in the soil extracts; therefore, the cleanup process does not improve the recoveries.

Table 2. Analytical Conditions Used for the Pesticides under Study, by MRM and LC-APCI-MS/MS (Dwell Time = 0.3 s)

pesticide	APCI	transition (m/z)	cone voltage (V)	collision energy (eV)
clomazone	+	240 → 125 ^a	25	20
		240 → 100	30	15
fipronil	–	435 → 330	30	15
		435 → 250 ^a	25	26
tebuconazole	+	308 → 70 ^a	40	20
		308 → 88	33	50
propiconazole	+	342 → 159 ^a	32	22
		342 → 69	30	20
azoxystrobin	+	404 → 372 ^a	20	20
		404 → 329	30	18

^a Transition used for the quantification.

MS/MS Optimization Parameters. Results of mass spectrometric conditions for the simultaneous analysis of pesticides are shown in Table 2. With the exception of fipronil, all selected pesticides showed more efficient ionization in the positive mode. For each compound, the optimum collision energies were selected to get two characteristic MRM transitions with the best signal intensity. The MRM transition with the best signal intensity was chosen for quantification, and the second best was used to confirm the pesticide.

Validation Experiments. Due to the complexity of soils, the sample matrix may affect the quantification of the target analytes.^{22,39} Employing QuEChERS as an extraction method allowed the matrix effect for some compounds to be evaluated by using the APCI interface.

Table 3 shows the LOQ_m, the data of linear regression for the curves prepared in the blank extract soils and in the solvent, and the matrix effect. To obtain the matrix-matched calibration

Table 3. Calibration Data, Matrix Effect (ME), and LOQ_m of the Pesticides under Study

pesticide	calibration data in the solvent			calibration data in the extract			ME (%)	LOQ _m (μg kg ⁻¹)
	equation (y = ax + b) ^a			equation (y = ax + b) ^a				
	a	b	r	a	b	r		
clomazone	29823.8	-59.0397	0.997	25973	-13.6607	0.999	-12.9	10.0
fipronil	1794.74	+35.5626	0.992	1020.14	-6.35569	0.994	-43.2	50.0
tebuconazole	25252.3	-193.633	0.998	23542.3	-106.068	0.996	-6.8	50.0
propiconazole	48651.2	-700.617	0.996	46711.2	-353.025	0.997	-4.0	50.0
azoxystrobin	28173.4	+9.35574	0.997	29652.1	+49.2844	0.997	+5.2	10.0

^a y = peak area, a = slope, x = concentration, and b = intercept.

Table 4. Recovery of the Determination of Pesticide Residues in Spiked Soil Samples^a

level of fortification (μg kg ⁻¹)	clomazone		fipronil		tebuconazole		propiconazole		azoxystrobin	
	R (%)	RSD	R (%)	RSD	R (%)	RSD	R (%)	RSD	R (%)	RSD
10	70.7	14.2	<LOQ		<LOQ		<LOQ		73.6	15.0
50	94.6	3.9	104.5	11	81.0	18.2	85.8	6.1	70.3	7.7
100	88.1	4.2	120.0	11	70.7	9.3	80.1	6.1	80.4	10.4
500	106.6	10.1	110.8	7.8	77.0	5.7	91.5	4.0	118.9	3.7

^a n = 9.

curves, first, the soil was extracted to verify whether there were pesticides or not. No pesticides were found in the soil extract.

The slopes of standard curves constructed in acetonitrile and in the extracts may serve as an indicator of the matrix effect (ME (%)). The calibration curves both in the standard solutions and in the soil extract solutions were linear in the concentration range between 0.01 and 1.0 μg mL⁻¹. After a comparison among the slopes of the calibration equations obtained by standard solutions dissolved in pure solvent (acetonitrile) and the ones obtained by the standard solutions dissolved in the final sample extracts (acetonitrile, in this case), the ME (%) values for fipronil, clomazone, tebuconazole, and propiconazole were about -4.0 to -43.2%, showing lower slopes than those in the standard solutions in acetonitrile. Likewise, the ME (%) for azoxystrobin was about +5.2%, showing a higher slope than the ones in the standard solutions in acetonitrile (Table 3). A high suppression effect, >40%, was observed only for the compound fipronil. The results show that coeluting matrix substances may reduce the ion intensity of the target compounds and cause signal suppression. The use of a simple external standard calibration method may produce erroneous results on the quantification of fipronil in soil samples. In the quantitative analysis by LC-MS, several strategies are appropriate to diminish the signal suppression/enhancement of the analyte.³³ In this study, to solve this problem, a matrix-matched calibration was considered to improve the accuracy of the quantification.

The method was validated using the calibration curves at seven different concentration levels obtained by the extracted soil sample to avoid matrix effects. The linearity of the calibration curves was studied by using the peak area. The response of the detector was linear for each pesticide in the range, with correlation coefficients (r) higher than 0.992.

LOD values ranging from 4 to 17 μg kg⁻¹ were obtained for soil samples. LOQ values ranging from 10 to 50 μg kg⁻¹ were also obtained for soil samples (Table 3).

The results for the extraction recoveries are presented in Table 4. The recoveries of the method for the analytes are between 70.3 and 120% at concentration levels from 10 to 500 μg kg⁻¹, and the RSDs ranged from 3.7 to 15%, showing that the QuEChERS procedure can be applied prior to the chromatographic analysis, resulting in pesticide quantification at 10–50 μg kg⁻¹. Studies of the extraction of azoxystrobin, clomazone, and tebuconazole from soil samples using the QuEChERS method have not been published yet. For fipronil and propiconazole, the results found in this study were in agreement with the ones previously published.²⁸ LOQs, in the range of micrograms per kilogram, and recoveries in the same range, found in other studies, were obtained. Besides, the method developed in this research has the advantages of using less solvent and less salt. It does not require the cleanup step, either.

Other extraction methods that determine at least one of the pesticides under study showed similar results in the literature. Clomazone, propiconazole, and others were extracted from soil samples using pressurized liquid extraction.⁴⁰ Six grams of dried soil sample was extracted using ethyl acetate/methanol (3:1, v/v) with 2 min of preheat time and 85 °C as the extraction temperature. The extracts were concentrated by a vacuum rotary evaporator at 45 °C and the concentrated extracts were then adjusted to 1950 μL with ethyl acetate and 50 μL of internal standard solution. The recoveries for clomazone and propiconazole were between 70 and 96% with LOQs of 1.7 and 3.3 μg kg⁻¹.

Solid-phase microextraction was also employed for the extraction of tebuconazole and clomazone and others.⁴¹ The optimized conditions deal with a fiber of 100 μm PDMS, desorption for 7 min at 270 °C, extraction time of 30 min, and 5% NaCl content (m/v). The recoveries were between 67.8 and 90.1 for these two compounds and the LODs in the range of 0.07 and 3.82 μg kg⁻¹. In comparison with those studies, the modified QuEChERS developed in this research has proven to be simpler and faster. It also requires fewer laboratory apparatus in its execution. Besides,

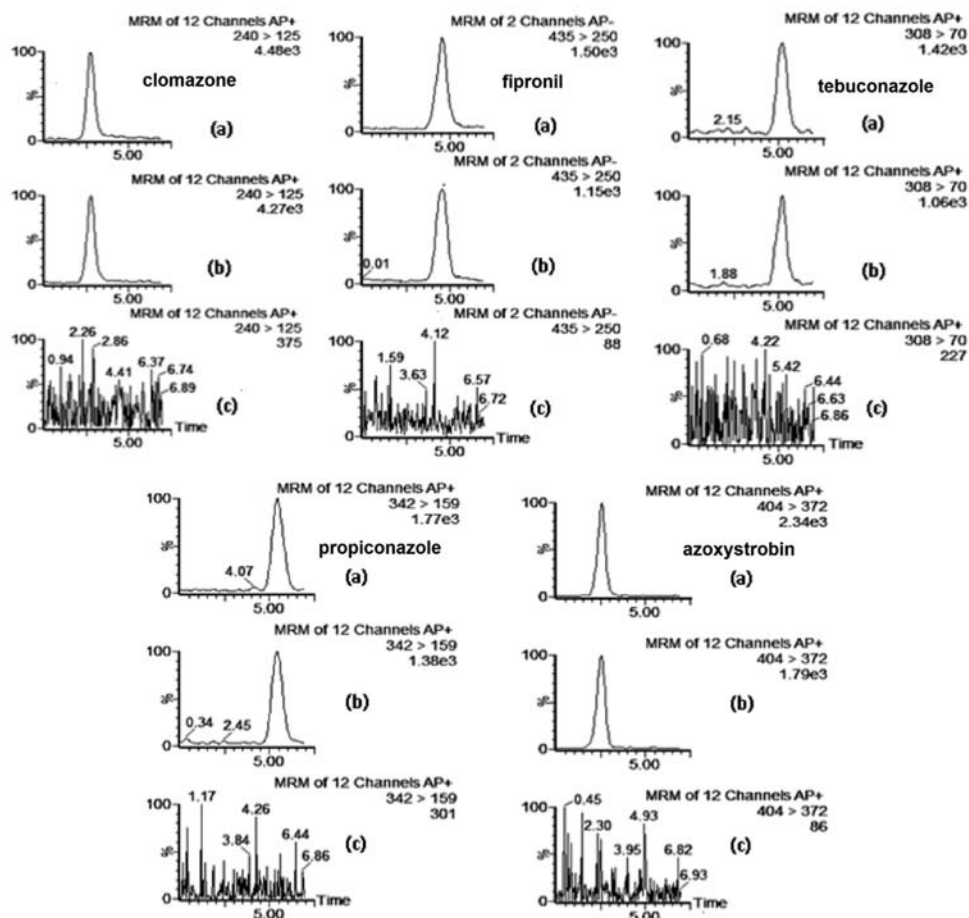


Figure 3. Chromatograms obtained by $100 \mu\text{g kg}^{-1}$ standard solution (a), soil extract spiked at the level of $100 \mu\text{g kg}^{-1}$ mixture of pesticides (b), and soil samples with no detectable pesticide in the MRM mode (c).

it shows good recovery and limits of detection in the range of micrograms per kilogram.

Selectivity, or the existence of potential interferences in the chromatograms from the agricultural samples, was monitored by running control blank samples in each calibration. The absence of any chromatographic components at the same retention times as target pesticides suggested that no chemical interferences were occurring (Figure 3), thus, avoiding quantification mistakes.

Application to Real Soil Samples. The validated method was applied to soils collected in an area where the compounds under study were used on rice fields. Tebuconazole and fipronil were found in concentrations between 10 and $182 \mu\text{g kg}^{-1}$. Figure 4 shows the overlap of the chromatograms concerning the two transitions monitored for fipronil (a) and tebuconazole (b) in a positive soil sample.

The new modified QuEChERS method proved to be simple and very efficient for the determination of fipronil, clomazone, propiconazole, tebuconazole, and azoxystrobin on rice paddy soils. This analytical methodology enables the determination of selected pesticide residues at trace levels with good analytical performance. The optimized method, involving LC-APCI-MS/MS, obtained satisfactory validation parameters such as linearity, repeatability, precision, and selectivity. The method shows high recovery and limits of detection in the range of micrograms per kilogram. The extraction method consumes a low amount

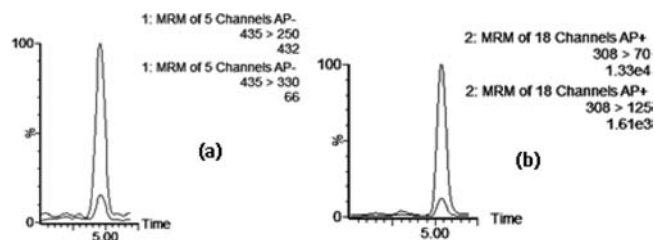


Figure 4. MRM chromatograms showing the two transitions used for the quantification and confirmation of the contaminated sample containing fipronil (a) and tebuconazole (b).

of organic solvent (10 mL), besides it is simple and fast. The study of the matrix effects revealed that matrix-matched calibration should be used, as some compounds show signal suppression higher than 40%. This method was successfully applied to the analysis of soil samples, thus showing that it is suitable for the determination of the selected pesticides in real samples.

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