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Article

Use of Solid Phase Extraction with Hydrophilic-Lipophilic Balance (HLB) Cartridge as the Appropriate Option for Metribuzin Extraction from Contaminated Soils

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Visando analizar resíduos de metribuzin em amostras de solo, precisamos usar métodos de extração especiais e adequados com alta eficiência. Cinco métodos de extração simples e rápidos (extração em fase sólida (SPE) com balanço hidrofílico-lipofílico (HLB), SPE com nanotubos de carbono de paredes múltiplas (MWCNTs), ultrassom, método *quick, easy, cheap, effective, rugged and safe* (QuECheRS) e extração líquido-sólido) acoplado a cromatografia gasosa foram usados na análise de resíduos do herbicida metribuzin em solos. Valores médios de recuperação do analito foram > 80%. Os extratos foram analizados por um sistema de cromatografia gasosa (GC) equipado com um detector de captura de elétrons (ECD). A ordem de valores médios de recuperação de metribuzin pelos cinco métodos de extração é: SPE com HLB > SPE com MWCNTs > ultrassom > QuECheRS > extração líquido-sólido. A recuperação média do analito depende do tipo de solo. Os resultados deste estudo mostram que o método de extração SPE com HLB é a melhor opção para extrair metribuzin de solos selecionados.

With a view to analyze metribuzin residues in soil samples, we need to use special and suitable extraction methods with high efficiency. Five simple and rapid extraction methods (solid phase extraction (SPE) with hydrophilic-lipophilic balance (HLB), SPE with multi-walled carbon nanotubes (MWCNTs), ultrasonic, quick, easy, cheap, effective, rugged and safe (QuECheRS) method, and liquid-solid extraction) coupled to gas chromatography were used for the analysis of metribuzin herbicide residues in soils. Mean recovery values of analyte were > 80%. Extracts were analyzed by a gas chromatographic (GC) system equipped with an electron capture detector (ECD). The order of mean recovery values of metribuzin for the five extraction methods is: SPE with HLB > SPE with MWCNTs > ultrasonic > QuECheRS > liquid-solid extraction. Mean recovery of analyte depends on the type of soil. The results of this study show that SPE with HLB extraction method is the best option for extracting metribuzin in selected soils.

Keywords: metribuzin, multi-walled carbon nanotubes, hydrophilic-lipophilic balance, QuECheRS, ultrasonic

Introduction

The application of pesticides is a usual practice in modern agriculture. However, owing to intensive use of these compounds, a fraction of the amounts used reach the soil and become an unavoidable part of the environment.[1,2] Therefore, it is important to monitor their residues in all environmental segments and their monitoring has been frequently performed throughout the world.[3-5]

The fate of pesticides in soil is controlled by the chemical, biological and physical dynamics of this matrix.[6-8] Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactions such as photolysis, hydrolysis, oxidation and reduction.[9,10] Biological degradation takes place when soil microorganisms consume or break down pesticides.[11-13] Triazine and organophosphorus pesticides are detected in the environment and their environmental behavior is of great concern, although several members of these classes have been banned for years.[13-17]

Metribuzin is an s-triazine herbicide with water solubility of 1.2 g L^{-1} at 20 °C widely used pre- and postemergence for broadleaf weed control in potato, sugarcane, soybean, and other crops.[18,19] Tiazine herbicides are weakly basic in nature and can be sorbed to both soil organic carbon and clay minerals[4,20] with sorption

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increasing slightly as the soil pH is decreased.[21,22] The extent to which metribuzin leaches to ground water is the inverse function of the organic matter content of soil.[23,24]

Most pesticides have strong binding to the soil matrix, and they have low concentration in soil solution. Therefore, the most important and critical step within the total scheme of soil analysis for reliable determination of pesticides is using special and suitable extraction methods with high efficiency.[25] The traditional extraction methods are laborious, time-consuming, expensive, require large amounts of organic solvents and usually involve many steps, leading to loss of some analyte quantity and can be reduced by using other extraction methods developed recently.[26] Ideally, sample preparation should be rapid, simple, cheap, and provide clean extracts. It is usual to develop an environmentally friendly procedure with a suitable organic solvent including carbon nanotubes (CNTs), quick, easy, cheap, effective, rugged and safe (QuECheRS) method, sonication[27,28] and in some cases prior to determination it is followed by a clean-up step with solid phase extraction (SPE) cartridges.[29] On the whole, the analytical procedure for each case should be chosen in order to reduce problems related to analysis duration, consumption of solvents, and also to minimize the number of involved analytical steps for minimizing potential sources of errors.

Although some of the reference methods for plant and water samples are rarely used for extraction of soil samples, it is interesting to study whether these methods could be applied to soil analysis. This paper describes some of new extraction techniques and their ability and usage for metribuzin residue determination in soil samples. Taking into account the above considerations, the main objective of this research was to compare the extraction efficiency of five different analytical methods (SPE with hydrophilic-lipophilic balance (HLB), SPE with multi-walled carbon nanotubes (MWCNTs), QuECheRS, ultrasonic, liquid-solid extraction) for metribuzin residues in soil and its determination by gas chromatography with electron capture detector (GC-ECD) in different soil samples.

Experimental

Sampling and soil sample preparation

In this research, five kinds of soils were selected from different agricultural lands. The soil samples contained no detectable amount of metribuzin residues. Samples were collected from surface soil (0-20 cm), and prior to use, soil samples were carefully homogenized, sieved (2 mm mesh), air-dried at room temperature and stored at 4 °C until analysis. The main physicochemical characteristics of soils are given in Table 1. Soil pH was determined in 1:2.5 soil-water suspension with a glass pH electrode, the soil organic matter (Walkley and Black method) and cation exchange capacity (CEC) were measured according to the standard methods.[30] Soil particle fractions were determined by the Gee and Bauder[31] proposed method.

Reagents and chemicals

Metribuzin (4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one) standard with purity of 99% was used for this study. All organic solvents (methanol, acetonitrile, dichloromethane and ethyl acetate), were high-performance liquid chromatography (HPLC) grade and other chemicals, such as anhydrous magnesium sulfate, sodium chloride, sodium sulfate, acetic acid, hydrochloric acid and sodium hydroxide were analytical grade. Bulk quantities of anhydrous $MgSO_4$ were heated to 500 °C for more than 5 h to remove phthalates and any residual water prior to their use in the laboratory. Stock solution of metribuzin was prepared dissolving 10 mg of the standard in 10 mL acetonitrile and further diluted with acetonitrile to 10 μg mL^{-1} and stored in the dark at –20 °C. From the stock solution, working standard sets for metribuzin (0.02, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.0, 2.0, and 3.0 μg mL^{-1}) were prepared by appropriate dilutions with acetonitrile to encompass the entire linear range of the method, then stored at 4 °C. They were kept for 2 h at ambient temperature prior to their use.

Table 1. Physical and chemical properties of the tested soils

Soil No.	Textural class	pH	OC[a]	Clay	Sand	Silt	CEC[b] / (cmol(+) kg^{-1})	SS[c] / (m^2 g^{-1})
			/ (g kg^{-1})					
1	Loam	7.73	23.3	254	409.6	336.4	23.15	52.45
3	Clay loam	7.82	6.2	374	369.6	256.4	30.35	103.32
5	Silty clay	7.78	7.6	454	149.6	396.4	34.97	119.41
7	Loam	7.6	4.75	134	509.6	356.4	12.77	22.55
8	Sandy loam	7.97	6.2	194	589.6	216.4	20.62	40.38

[a]OC: organic carbon; [b]CEC: cation exchange capacity; [c]SS: specific surface.

Spiking of soil samples

Ten grams of soil were weighed in a 50-mL centrifuge tube and fortified by adding the appropriate volume of the working standard solutions, so that metribuzin concentration was achieved as 0.1, 0.4, 0.7 and 1.0 μg g^{-1} soil. The sample was slightly shaken inside the tube to ensure a homogeneous mixture of metribuzin with the whole quantity of the soil. After the bulk of the solvent was evaporated, the materials were finally dried for at least 24 h at room temperature, kept away from light and then they were analyzed. Extractions of blank samples were done in parallel to extractions of the spiked ones.

Soil sample extraction techniques

Five extraction methods were assessed and compared in this study: (*i*) SPE with HLB; (*ii*) SPE with MWCNTs; (*iii*) ultrasonic; (*iv*) QuECheRS; and (*v*) liquid-solid extraction

Solid phase extraction with HLB method

This method has been described by Belmonte Vega *et al.*[32] before and we used it with some changes. The method is as follows:

Ten grams of air-dried soil was extracted with 15 mL of methanol/water (4:1 v/v) in an ultrasonic bath for 30 min. First, the suspension was centrifuged for 10 min at 4000 rpm and then filtered through a 0.45 μm filter, then the organic solvent was evaporated in a rotatory evaporator at 35 °C and the residual water was made up to 30 mL with milli-Q water. The sample was acidified to pH 3.5 by HCl/NaCl and then extracted using HLB (200 mg) cartridges pre-conditioned with 4 mL dichloromethane, 4 mL methanol and 5 mL water, consecutively. Extracts were loaded on cartridges at a rate of 8 mL min^{-1} by using a vacuum-operated pumping system. Afterwards the cartridges were dried in an air current during 30 min. The elution of analyte was achieved with 5 mL methanol, 5 mL dichloromethane and the organic solvent was concentrated to approximately 0.5 mL in a rotary evaporator at a temperature of 35 °C. The final volume was made up to 2 mL with methanol before being analyzed by GC.

Solid phase extraction with multi-walled carbon nanotubes method

Solid-phase extraction cartridge: packed cartridges were prepared with empty polypropylene cartridges (0.5 g, 3 mL). An aliquot of 0.1 g MWCNTs were weighted into the cartridge after a polypropylene frit was set at the cartridge bottom. At the front of the cartridge, another polypropylene frit was set. Each MWCNT cartridge was used only once.

SPE procedure for soil: this method has been described by Min *et al.*[33] before and it was used with some changes and optimizing the addition of a second solvent to methanol. Ten grams air-dried soil was weighed into a clean centrifugal tube, and then 20 mL methanol/acetonitrile solution (1:1) was added. After the mixture was shaken on a rotational shaker for 5 min at 300 rpm, the mixture was separated in a centrifuge at 4000 rpm for 15 min. The supernatant was carefully transferred into a clean beaker. The residues were then rinsed with 10 mL of methanol/acetonitrile solution (1:1) and the supernatants were combined. Then the soil final extract was evaporated to remove methanol in a rotary evaporator at 35 °C. The soil extract was diluted with milli-Q water to 30 mL and then passed through the multi-walled carbon nanotube-packed cartridge (which was washed with 3 mL methanol and 5 mL purified water before use) at a flow rate of 4 mL min^{-1} by a vacuum pump. After the sample was applied, the cartridge was kept under vacuum for 5 min to remove any residual water. The objects retained on the cartridge were eluted by 4 mL ethyl acetate at a flow rate of 1 mL min^{-1}. The effluents were collected into a test tube and condensed to dryness under a gentle flow of nitrogen at room temperature and re-dissolved with 1 mL acetone before analysis by GC.[33]

Soil extraction method using ultrasound

This method has been described by Fenoll *et al.*[34] before and this method was carried out with some changes, with 10 g of soil sample in a centrifuge tube. Samples were extracted with 20 mL acetonitrile/water (1:1, v/v) solution by sonication followed by a salting-out step with 2 g NaCl. Sonication (Hielscher ultrasonic processor model UP400S, sonic dismembrator 400 W generator equipped with standard titanium probe) took place for 15 min at 0.5 cycles and the vibration amplitude was 30%. The tube was shaken for 1 min and centrifuged for 15 min at 4500 rpm. The supernatant extract was filtered through a 0.45 μm filter, transferred into a vial, and analyzed by GC.[34]

Soil extraction method using QuECheRS

The QuECheRS method described by Caldas *et al.*[35] is based on the extraction of 10 g of soil sample with 100 μL of acetic acid (0.1%) and 10 mL of acetonitrile, followed by a salting-out step with 4 g $MgSO_4$, 1 g NaCl, and hand-shaking the mixture immediately for 15 s. After that, it was shaken vigorously in a laboratory shaker for 1 min and then centrifuged at 4500 rpm for 15 min. The supernatant was passed through a 0.45 μm filter and 1.5 mL of the extract was transferred into vials for GC analysis. We set up an

experiment to optimize using salts and acidification of the mixture.

Soil extraction method using liquid-solid extraction

This method was done based on a method described by Khoury *et al.*,[36] with some changes in the procedure. A 5 g dried soil sample was placed into a polypropylene centrifuge tube (50 mL), then 10 mL of HPLC-grade dichloromethane were added and the mixture was shaken with a rotary shaker for 30 min. After centrifugation at 4500 rpm for 15 min, 5 mL of the supernatant were evaporated under dry nitrogen and then diluted in 0.5 mL of HPLC-grade methanol. The soil extract was transferred into vials for analysis by using GC-ECD.

Validation experiments

The metribuzin standard solutions were used for the validation of the method (determination of limit of detection (LOD), limit of quantification (LOQ), the construction of the calibration standard curve and the preparation of the fortified soil samples for recovery experiment). In GC systems, quantification was made by external standard calibration curves made by use of standard working solutions (0.02 to 3.0 μg mL^{-1} for the GC-ECD system). The analytical methods were validated with the analysis of spiked soil samples. The recovery was determined for three replicates in the spiking concentrations of 0.1, 0.4, 0.7, and 1.0 μg g^{-1} for metribuzin. Calculations of recoveries were done by using the peak areas. The precision was calculated as relative standard deviation percentage (RSD%) for each concentration level. The linearity of the calibration curve was evaluated at a concentration range between 0.02 and 3.0 μg mL^{-1} using ten calibration solutions prepared in acetonitrile. 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. For the GC-ECD-based method, the limit of detection (μg kg^{-1}) of metribuzin was determined as the lowest concentration giving a response of three times the standard deviation of the baseline noise. The limit of quantification (μg kg^{-1}) was determined as the lowest concentration of metribuzin giving a response that could be quantified with an RSD lower than 20%.[37]

Apparatus and analytical conditions

GC analyses were performed on a Hewlett-Packard (Agilent Technologies) GC Model 7890A Series gas chromatograph equipped with ^{63}Ni electron capture detectors. An HP-5 (30 m × 0.32 mm i.d.) (Agilent Technologies) fused silica capillary column with a 0.25 μm film thickness was used with nitrogen (99.99% purity) as carrier gas at a flow rate of 5 mL min^{-1}. One microliter of the sample was injected in the splitless mode. Detector and injector temperatures were 300 °C. The GC oven was operated with the following temperature program: initial temperature 120 °C, ramped at 20 °C min^{-1} to 270 °C and held for 0.5 min. Under these conditions, retention time of metribuzin was 4.06 min. The Agilent ChemStation software was used for data analysis.

Data analysis

Statistical analysis was performed using the softwares MSTAT-C and SPSS 16.0. Relationships between soil properties and mean recoveries were tested by Pearson correlation. We used least significant difference (LSD) test at $p < 0.05$ to determine the differences between recoveries of metribuzin in different soils and methods and their interactions.

Results and Discussion

Soil sample characteristics

Some of the physicochemical properties of studied soils are shown in Table 1. The pH of soils is in the range of 7.60-7.97. The organic carbon content varied from 4.75 to 23.3 g kg^{-1} and clay content varied from 134 to 454 g kg^{-1}. The CEC ranged from 12.77 to 34.97 cmol(+) kg^{-1} and the specific surface (SS) is in the range of 22.55-119.41 m^2 g^{-1}. The organic carbon content was positively correlated with CEC, and was negatively correlated with pH, sand, SS and clay content. This result is according to the Ding *et al.*[38] findings. The clay content had significant and positive relationships with CEC, SS and was negatively related to the sand content (Table 2). Also, there was a positive relationship between CEC and SS ($p < 0.01$).

Calibration curve

Under the chromatographic conditions described above, the linearity of the calibration curve was studied by using the peak area. Ten different concentrations (0.02, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.0, 2.0, and 3.0 μg mL^{-1}) for metribuzin were plotted *vs.* the peak area of herbicide (Table 3) and good linearity was achieved in the concentration range between 0.02 and 3.0 μg mL^{-1}. The correlation coefficient derived from the linear regression was higher than 0.999,

Table 2. Correlation coefficients between soil properties for the five soils studied (n = 5)

	OC[a]	pH	Clay	Sand	Silt	CEC[b]	SS[c]
OC	1	–	–	–	–	–	–
pH	–0.143	1	–	–	–	–	–
Clay	–0.006	0.213	1	–	–	–	–
Sand	–0.086	0.162	–0.904[d]	1	–	–	–
Silt	0.203	–0.742	0.273	–0.657	1	–	–
CEC	0.040	0.360	0.986[e]	–0.838[d]	0.147	1	–
SS	–0.094	0.228	0.993[e]	–0.872[d]	0.212	0.976[e]	1

[a]OC: organic carbon; [b]CEC: cation exchange capacity; [c]SS: specific surface; [d]correlation is significant at 0.05 probability level; [e]correlation is significant at 0.01 probability level.

Table 3. Calibration data of the metribuzin herbicide

Compound	Concentrations / (μg mL^{-1})	R[a]	Slope	Intercept	R^{2b}
Metribuzin	0.02, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.0, 2.0, 3.0	1	42592	–0.1184	0.999

[a]Correlation coefficient; [b]determination coefficient.

with significant correlation between concentration and peak area for the herbicide.

Optimization of salt addition and acidification (QuECheRS method)

To study the effect of salt addition and acidification, the QuECheRS extraction was performed by using three treatments: 4 g $MgSO_4$ + 1 g NaCl, 4 g $MgSO_4$, and 4 g $MgSO_4$ + 1 g NaCl + 100 μL acetic acid 0.1%. The metribuzin recovery results are shown in Figure 1. The experiment was performed in three soils (soils 1, 5, and 7) at a spiking level of 0.7 μg mg^{-1}. The addition of 1 g NaCl to 4 g $MgSO_4$ significantly increased the mean recovery of metribuzin in three soils (mean recovery increased 12-15%) (Figure 1).

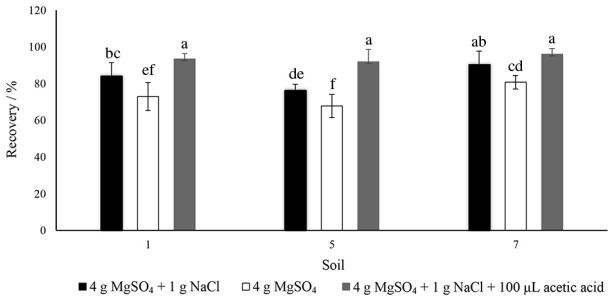

Figure 1. Effect of salt addition and acidification on the recoveries (%) of metribuzin in three soils with error bars representing the standard deviation (extraction conditions: 10 mL of acetonitrile, spike level of 0.7 μg g^{-1}).

Leon *et al.*[39] and Nakamura and Daishima[40] have described that the recovery for more hydrophilic analytes (log K_{ow}< 3.5) dramatically increased on increasing concentration of NaCl. However, Wu *et al.*[41] found that the addition of salt caused a decrease in the recovery of the pesticides studied. Combinations of salts ($MgSO_4$ and NaCl) were used to enhance phase separation. The salting-out effect resulting from the addition of NaCl depends on the nature of the solvents involved in the partitioning step. The treatment using $MgSO_4$ and NaCl together showed better recoveries than using $MgSO_4$ alone.

Durovic *et al.*[42] stated that the metribuzin recovery values increased by addition of up to 5% NaCl to the system. Buffer application in QuECheRS is usual, and it was studied for three soil samples. The metribuzin recoveries significantly increased when the buffer was used than in two other treatments without acetic acid (Figure 1). In general, by using 4 g $MgSO_4$ + 1 g NaCl + 0.1% acetic acid treatment, the highest recoveries of metribuzin herbicide were achieved. However, there is no significant difference between mean recoveries of treatments 4 g $MgSO_4$ + 1 g NaCl and 4 g $MgSO_4$ + 1 g NaCl + 0.1% acetic acid in soil 7 (it was significant for soils 1 and 5). Thus, the use of 0.1% acetic acid led to improvement in the recoveries. It enabled to increase the metribuzin stability prior to analysis.

Optimization of a second solvent addition to methanol (SPE with MWCNTs method)

We decided to study the effect of changing the second solvent with methanol on metribuzin recovery to achieve the highest recoveries possible. As it can be seen in Figure 2, the results of mean recovery of soils for methanol/hexane

(1:1) mixture were between 43.81-61.3%, for methanol/ acetonitrile (1:1) mixture were between 91.18-95.87%, for methanol/ water (1:1) mixture were between 68.86-87.91%, and for methanol/acetonitrile (3:1) mixture were between 80.36-91.12%. Among the different selected mixtures, the methanol/acetonitrile (1:1) combination achieved highest recovery values compared with the other different mixtures, although there is no significant difference between metribuzin recovery values for methanol/ acetonitrile (1:1) and methanol/acetonitrile (3:1). Since methanol is very toxic and based on decreasing solvent consumption, methanol/acetonitrile (1:1) was selected as the best extracting mixture for this method.

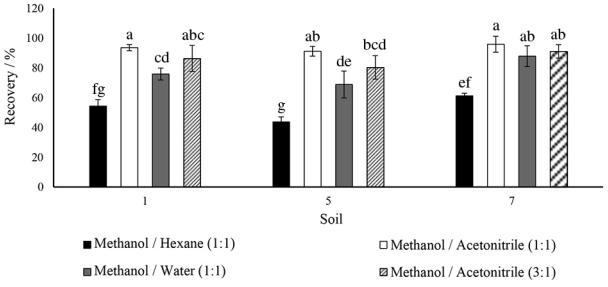

Figure 2. Effect of the different second solvent relative to methanol on the recoveries of the metribuzin herbicide in three soils (n = 3) with error bars representing the standard deviation (extraction conditions: 10 g of soil, 20 mL of solvent, 15 min shake, 15 min centrifugation (at 4000 rpm), spike level of 0.7 μg g^{-1}). Conditions for MWCNT-SPE procedure: analyte re-dissolved in 30 mL of water, and elution with 4 mL ethyl acetate.

An explanation can be obtained for the lower recovery in soil 5 (see Figure 2); the higher clay and OC contents in soil 5 (Table 1) imply a higher retention of analyte by the soil components[43] and this is what seems to takes place.

Recovery experiment

Five soils with different physical chemical properties were selected to validate the methods. The recoveries of the methods were determined by spiking soil samples free of metribuzin with four concentrations (0.1, 0.4, 0.7 and 1.0 μg mg^{-1}) of working standards. The recovery of metribuzin was calculated at each of concentration level by comparing the measured concentrations with the spiked concentrations. Five un-spiked soil samples and five reagent blanks served as the negative control for quality assurance purposes. The recoveries for metribuzin in five soils with different extraction methods were calculated. Figure 3 shows the recoveries of metribuzin in soil 5 with the used extraction methods.

The mean recovery values ranged between 78.17 and 94.19% for soil 1, between 62.39 and 89.18% for soil 3, between 60.93 and 88.54% for soil 5, between 89.11 and

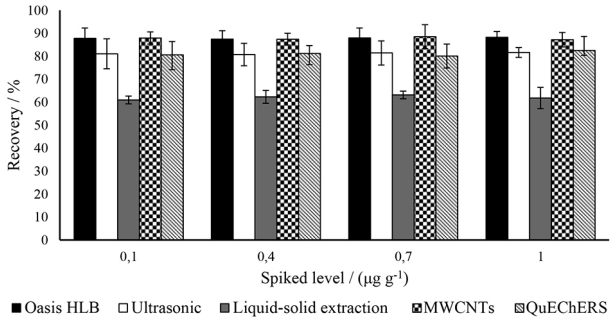

Figure 3. Recovery (%) of metribuzin for five different extraction methods at four spiking levels for soil 5 (n = 3). Error bars represent the standard deviation.

105.61% for soil 7, and between 87.47 and 108.53% for soil 8 (an example is shown in Figure 3). In general and based on the different works previously published, the type of soil can affect recovery values.[44] As can be deduced from the results, the recovery percentages for soil 7 were higher than for the other soils. The reason might be the relatively low amount of organic matter that this soil contains compared with the rest of them. Soil 7 had a high content of sand and low percentage of OC and clay, as a result, it contained low CEC and SS, which can affect the extraction efficiency of metribuzin. As Weber[20] and Santos and Galceran[45] mentioned, the OC content is the most important soil property affecting the degree of adsorption.

The correlation result between soil properties and mean recoveries of metribuzin in soils show that the mean recovery of analyte negatively correlated with OC (–0.051), clay (–0.975), CEC (–0.943), and SS (–0.976) of soils and correlations are significant at the 0.05 probability level. The mean recovery values of metribuzin at all fortification levels were not significantly different from each other (data not shown). The differences between mean recovery values were evaluated by using the LSD test at $p < 0.05$ level. Analysis of data show that the differences between herbicide mean recoveries for five soils and also for five extraction methods were statistically significant (Table 4).

The highest metribuzin recovery values were achieved in soils 7 and 8 and were followed by soils 1, 3, and 5. The mean recovery values difference between soils 7 and 8 and also between soils 3 and 5 were not significant (Table 4). Soils 3 and 5 have higher clay and OC content compared with soil 7 and 8 (Table 1). The possible reason for the lower metribuzin recoveries in soils 3 and 5 than in others is the strong binding of metribuzin with OC and clay of these two soils. The order of mean recovery values of metribuzin for the five extraction methods is: SPE with HLB > SPE with MWCNTs > ultrasonic > QuECheRS > liquid-solid extraction (Table 4), but there is no significant difference between using SPE with MWCNTs, ultrasound and QuECheRS. Liquid-solid

Table 4. Mean recoveries of soils and extraction method (at 1.0 μg g^{-1} spiking level)

Soil No.	Mean recovery / %	Extraction method	Mean recovery / %
1	88.24ab	SPE with HLB	95.97a
3	81.13b	SPE with MWCNTs	90.67b
5	80.26b	QuECheRS	87.46b
7	95.41a	Ultrasonic	87.52b
8	96.50a	Liquid-solid extraction	75.90c

Results are expressed as a mean of three measurements. Means within a column followed by the same letter are not significantly different at $p \geq 0.05$ by LSD test.

extraction had the lowest recoveries in all soils (the mean recoveries of liquid-solid extraction method were between 60.93 to 90.71%). The difference between metribuzin recovery values for the different extraction method depends on the type of extractor solvent and the extraction steps.

The interaction of mean recovery values of analyte from soils and extraction methods (spiking level ranges between 0.1-1.0 μg g^{-1}) are shown in Table 5. All methods can be used for extraction of metribuzin from soils 7 and 8, even soil 1. Extraction of analyte from soils 3 and 5 can be done by using all methods except for liquid-solid extraction, because this method had relatively low recovery for these two soils. The results of this experiment show that the proposed methods to determine metribuzin residues in soils are simple, rapid, and uses low volumes of organic solvents in the sample extraction. High recoveries (> 80%) were obtained for the herbicide studied by using all extraction methods (except liquid-solid method).

As the SPE with HLB method had the highest recovery of metribuzin in all soils and spiking levels and there is no significant difference between SPE with MWCNTs, ultrasonic and QuECheRS methods (except for liquid-solid), thus for some of the same soils with properties in this range, the SPE with HLB method can be selected as the suitable option for metribuzin herbicide extraction. The possible reason for higher recovery of SPE with HLB is that the HLB sorbent is water wettable, it maintains its capability for higher retention and excellent recoveries even if the sorbent runs dry.

Accuracy and precision

The repeatability of the methods was determined by performing the analysis of spiked samples at 0.7 μg g^{-1}. The RSD values obtained for the retention times ranged from 0.01-0.05%, whereas values for mean recovery for extraction methods ranged from 2.87-6.32% (Table 6). Comparing the extraction techniques, QuECheRS and ultrasonic showed lower repeatability than liquid-solid extraction.

Table 5. Comparison of mean recovery of soils and extraction methods (n = 3)

Soil No.	Method				
	SPE with HLB	SPE with MWCNTs	QuECheRS	Ultrasonic	Liquid-solid extraction
1	94.11bcd	92.31cd	89.14de	87.43de	78.17f
3	88.88de	88.62de	82.73ef	83.03ef	62.39g
5	88.21de	87.19de	82.48ef	81.62ef	61.79g
7	100.13abc	101.42ab	92.48cd	93.32bcd	89.67de
8	108.53a	103.81a	90.48de	92.18cd	87.47de

Means within a column followed by the same letter are not significantly different at $p \geq 0.05$ by LSD test.

Table 6. Relative standard deviation (RSD) of metribuzin recoveries with five extraction methods

Soil No.	Method				
	SPE with HLB	SPE with MWCNTs	QuECheRS	Ultrasonic	Liquid-solid extraction
1	3.54	4.93	2.87	5.28	5.49
3	5.21	5.05	4.49	4.06	4.61
5	4.28	3.86	6.32	5.80	4.33
7	3.90	4.53	4.28	4.23	3.93
8	5.47	4.27	6.27	5.47	4.38

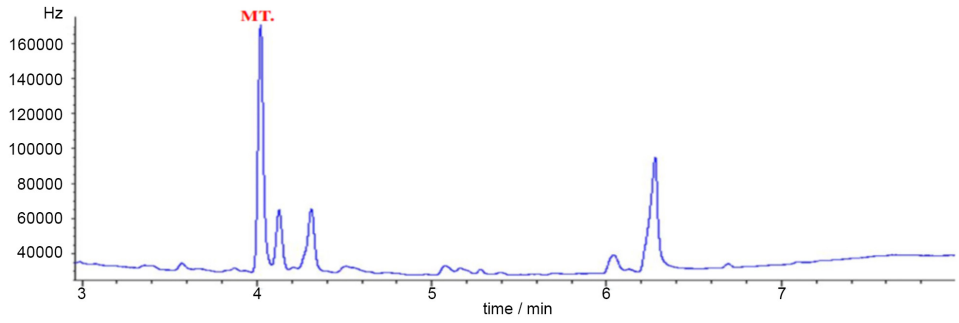

Figure 4. Chromatogram from GC-ECD analysis of metribuzin (MT.) with retention time 4.06 min.

Since metribuzin recovery values are > 80% (except for the liquid-solid method) and respective RSDs are < 20% at all fortification levels, under these conditions, the accuracy and precision of the methods are acceptable. The result showed that metribuzin recovery values and the RSDs in all extraction methods (except for the liquid-solid extraction method) were between 80.11-108.53% and 1.09-9.14%, respectively, that clearly demonstrate the accuracy, precision and suitability of the proposed methods.

The LOD value of the SPE with HLB method was determined at signal/noise of 3 for metribuzin in soil 5 by GC-ECD, whereas the LOQ values were obtained at signal/noise of 10. The LOD and LOQ values obtained for metribuzin were 0.0087 and 0.029 μg g^{-1}, respectively. Figure 4 shows a metribuzin chromatogram sample and its retention time was 4.06 min.

Conclusions

Sample extraction is time-consuming and it can be considered as the most important and critical step in the whole analytical procedure. Improvements in the sample preparation techniques for different samples have led to modifying the existing methods and development of new techniques, in order to save time and reduce use of solvents and thus improve the efficiency of the analytical process. The results presented in this work demonstrate that SPE with HLB, SPE with MWCNTs, QuECheRS, and ultrasonic extraction methods have high efficiency and are suitable for determination of metribuzin in soils with a wide range of OC and clay content. These proposed methods had satisfactory recoveries (> 80%), good repeatability (%RSD < 6.32) and with capability to provide accurate results, but SPE with HLB (with the highest recoveries) is the best method among the selected methods in this study. We plan to further explore different pesticide residue analysis in a wide range of soil samples. Additionally, we will apply more than one instrument for quantifying and comparing pesticide residue value.

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