

QuEChERS Extraction for High Performance Liquid Chromatographic Determination of pyrazosulfuron-ethyl in Soils

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Summary: This paper described a simplified quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for determination of pyrazosulfuron-ethyl (PE) from soil samples by high performance liquid chromatography/ultraviolet (HPLC/UV) detector. Optimized results were obtained dispersing soil in water, followed by the addition of 1% acetic acid (HOAc) in acetonitrile (MeCN), anhydrous magnesium sulfate (MgSO₄) and sodium acetate tri-hydrate (NaOAc·3H₂O), which was a modification of QuEChERS method without primary and secondary amine (PSA) and C₁₈ sorbent. The results showed the recovery ranged from 70.8% to 99.0% and 83.5% to 86.4% with the relative standard deviations of 2.1% to 7.9% and 7.0% to 10.0% in Changsha and Nanning soil samples, respectively. The limit of detection (LOD) of the method was 0.05 mg/Kg. The limit of quantification (LOQ) was 0.1 mg/Kg.

Introduction

Sulfonylureas, a modern class of herbicides, are first introduced in 1982 by DuPont Agricultural Products and have high herbicidal activity at low application rates (<100 g of active ingredient per hectare) [1]. They are extensively used to control a wide range of weeds in many crops, such as wheat, barely, rice, corn, soybean etc.

PE(ethyl-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate, structure shown in Fig. 1),

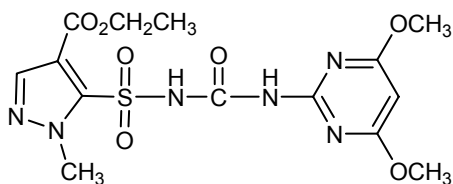


Fig. 1: The molecular structure of PE.

That is a herbicide of sulfonylurea group used widely in China [2]. It is active against annual and perennial grasses and broad-leaved weeds in cereals [3]. The use of PE is increasing steadily in China due to extremely low acute and chronic mammalian toxicities in comparison with most other herbicides. However, the widespread use of them is a

potential soil pollutant and presents environmental risk. Previous some reports indicated sulfonylureas have potential threat to human [4]. Residues of sulfonylureas have frequently been detected in surface water and groundwater due to runoff and leaching after their application [2, 3]. Due to their high herbicidal activity, some crops (e.g., legumes and pastures) are highly sensitive to trace-level residues of sulfonylurea herbicides in soils [3]. The residues of sulfonylureas in soil can significantly damage rotation or substitution crops [5]. Recently, some articles have appeared in this literature regarding the degradation and determination of the PE, which suggesting residues in soil may act as potential environmental hazards [6]. Therefore, it is necessary to develop analytical methodologies to monitor PE in the soil.

Currently, analytical methods such as high performance liquid chromatography [7, 8], liquid chromatography/mass spectrometry [9], gas chromatography [10], capillary electrophoresis [11], supercritical fluid chromatography [12], bioassay [13] and enzyme immunoassay [14] have been successfully used for the qualitative and quantitative determination of sulfonylurea herbicides. Concerning the development of different analytical methodologies, effective sample treatments are very important in soil matrixes in order to avoid interferences and improve

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the sensitivity of the methods, especially when using liquid chromatography. Modern analytical strategies tend towards automatization and integration of sample pretreatment in the chromatographic systems as far as possible. Development of solventless (or at least with low solvent consumption) sample preparation techniques constitutes a pillar of green analytical chemistry and has taken a rapid development during last years. A sample pretreatment process is indispensable to achieve reliable analysis based on the comprehensive consideration of these factors. Through the sample treatment step, the target analytes can be effectively enriched and obtained and the sample is cleaned up at the same time. Therefore, sample pretreatment has been an important step in trace analysis nowadays.

QuEChERS sample preparation is introduced by Anastassiades *et al* in 2003 and it has mainly been used for different food matrices with high water content [15, 16]. The QuEChERS approach is very flexible and it serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the lab. To our knowledge, the use of QuEChERS in soils is very limited [17] but with very good results. Pinto [18] reported a simplified version of the QuEChERS method for the extraction of chlorinated pollutant compounds from soil samples. Niell [19] compared two extraction solvents and conditions for three sulfonylurea herbicides residues in milled rice with liquid chromatography/diode array detection analysis.

In this paper, we describe a simple, fast and cheap sample preparation method to analyze PE in soil. In order to prove the suitability, soil samples of different physical and chemical properties for the extraction of PE have been chosen. The sample preparation method is suitable for analyses performed using HPLC/UV system.

Results and discussion

Extraction and Cleanup Evaluation

MeCN and MeOH were tested as possible extraction solvents and it was demonstrated that MeCN was better than methanol (Table-1, Fig. 2).

This result is like Niell [19], in which MeOH and MeCN were evaluated and compared for extraction of PE residues in milled rice.

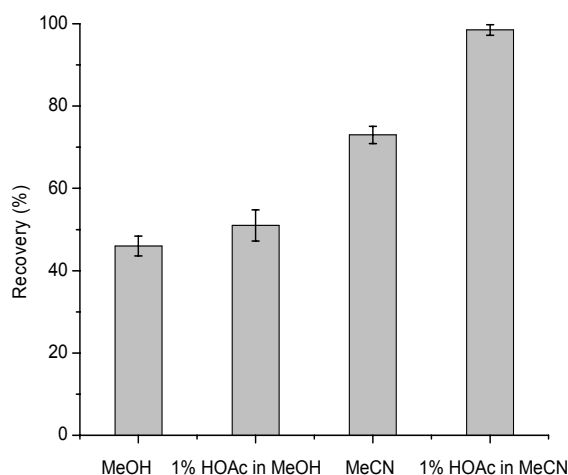


Fig. 2: Comparison different solvent (MeOH Sonication, 1% HOAc in MeOH hand shake, MeCN hand shake and 1% HOAc in MeCN hand shake) extraction for HPLC determination of PE in fortified 0.5 mg/Kg. Error bars signify standard deviation (n=5).

Table-1: Differences and similarities of the four sample preparation methods compared.

Method	MeOH	H ₂ O+1% HOAc in MeOH	H ₂ O+1% HOAc in MeCN	H ₂ O+1% HOAc in MeCN
Sample size	5g	5 g	5g	10g
Amount of water added	0	2mL	2mL	4mL
Amount of solvent	10	10 mL	10mL	20mL
Agitation method	Sonication, 15 min	Hand shake, 2 min, twice	Hand shake, 2 min, twice	Hand shake, 2 min, twice

Soil samples, in contrast with rice, do not have high contents of lipid materials. Soil samples in different area have different physical and chemical properties including types, pH, organic matter fraction (10–15%) and cation exchange capacity (Table-2). Therefore, the main disadvantage of MeCN (co-extraction of non-polar compounds such as lipids) may not be significant here, and MeCN could be suitable for the extraction of PE from soil matrices with HPLC method.

Table-2: Physical and chemical properties of soils

Area	Soil type	pH value	Organic material (%)	Cation exchange Capacity (cmol/kg)
Changsha	Alluvial soil	5.83	9.2	9.43
Nanning	Purple soil	7.13	13.4	33.46

The modifications to the original QuEChERS consisted in using 1% HOAc is added to the MeCN for extraction, NaOAc (and MgSO₄) instead of NaCl (and MgSO₄) to yield consistent pH of the procedure independent of the pH of the original sample [20-22]. In this study, PSA and C₁₈ sorbents were evaluated for cleanup of soil samples. Fig. 3 shows the results from an experiment in which different sorbents were used for cleanup of soil extracts. As the figure showed, low recoveries were obtained for PE, when PSA and C₁₈ sorbents was added to cleanup. Because herbicides react with the sorbent (PSA and C₁₈) due to their chemical nature [19]. The buffered QuEChERS modification has advantages with respect to higher recoveries and greater stability of pH-sensitive pesticides [20], thus, it was used in all other experiments.

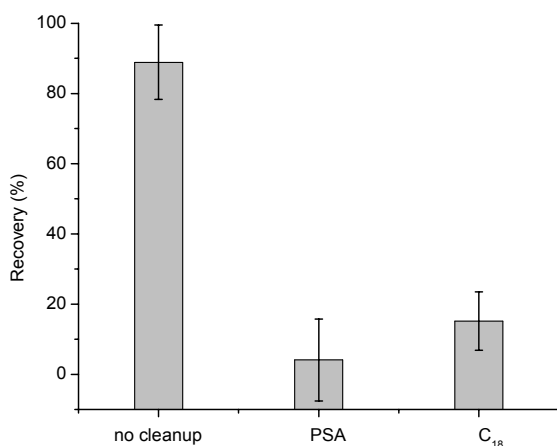


Fig. 3: Comparison of the modified QuEChERS approach extraction PE for cleanup: no cleanup, PSA and C₁₈ in fortified 0.5 mg/Kg. Error bars signify standard deviation (n=5).

Modified QuEChERS Method Validation

In the application of the method to dry matrices, it is very common to add a volume of water

to the samples, prior to the extraction step, to hydrate them and make the pores in the sample more accessible to the extraction solvent [17-19]. Sieved soil sample was weighed in a glass centrifuge tube with screw cap, which keeps the tube closed for most of the process of sample preparation to avoid as much as possible losses of volatile compounds during this stage. Two different soils were evaluated in this paper.

The standard calibration curve of PE during HPLC/UV analysis was constructed by plotting the analyte concentration versus peak area. The regression equation of the standard calibration curve was $y=89230x-19067$ ($R^2=0.9994$). Therefore, the calibration curve showed excellent linearity in the concentration range 0.05–20 mg/L.

The limit of detection (LOD) of PE was defined as the minimum concentration of PE that was detected with acceptable certainty. The LOD was estimated to be 0.05 mg/Kg for soil. The limits of detection (LODs) of the proposed method were determined at a signal-to-noise (S/N) ratio of 3 for the individual herbicides in soil by HPLC/UV, whereas the limits of quantitation (LOQs) were obtained as the lowest spiked level with acceptable recovery and RSD. The LOQ values were estimated to be 0.1 mg/Kg for soil, corresponding to the lowest spiking level used.

Based on the original QuEChERS method, some parameters were slightly modified. Good recoveries were obtained for PE using 10.0 g soil. Soil blanks were fortified at 0.1, 0.5 and 1.0 mg/Kg and processed as described above. The modified QuEChERS methods (as described in the Materials and Methods section) gave good results, showing high recoveries (70.8–99.0%) and low relative standard deviation (RSD) (10.0%) (Table-3).

Table-3: Percent recovery of PE extracted with the modified QuEChERS method from soils.

Sample	Spiked level/(mg/kg)	Recovery (%)					Average recovery (%)	RSD (%)
		1	2	3	4	5		
Changsha Soil	0.1	71.5	74.0	68.7	69.3	70.3	70.8	2.1
	0.5	81.7	101.9	96.4	88.1	88.3	91.3	7.9
	1	104.9	94.4	88.6	105.4	101.9	99.0	7.3
Nanning Soil	0.1	74.6	79.5	100.4	90.7	85.4	86.1	10.0
	0.5	80.7	77.9	92.5	76.9	89.4	83.5	7.0
	1	88.7	99.3	85.9	77.4	80.9	86.4	8.4

Experimental

Chemicals and Reagents

PE standard (96%) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Methanol (MeOH) and MeCN HPLC grade was purchased from TEDIA (USA). NaOAc·3H₂O, HOAc, MgSO₄ and sodium sulphate anhydrous (Na₂SO₄) were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The purity was not taken into account when making acid solutions, thus the % indicates the volume fractions of acid solutions (e.g. 1% HOAc in MeCN was prepared by mixing 10mL glacial HOAc with 990mL MeCN). The two different types of soils (0–15 cm) were collected from agricultural fields in Changsha and Nanning, China. No PE in soil samples was validated using traditional analysis method. Soil samples were ground to powder after air drying and passed through a 2 mm sieve, then stored in 4 °C until analysis.

Apparatus and Conditions

HPLC analysis of PE was conducted using a Waters 600 equipped with UV detector and autosampler. Chromatographic separation for the PE was performed using Diamonsil C₁₈ column (250×4.6 mm i.d., 5 µm particle size) at 30 °C. The mobile phase consisted of MeOH/water with 0.2% HOAc (75/25, v/v), the flow rate was 1 mL /min. The detections were performed at 241 nm and the injection volume was 20 µL. Peak areas were recorded and calculated using the Empower Software (Waters, USA) [23, 24].

QUEChERS sample preparation

For extraction, 10 g samples were transferred into a 50 mL polypropylene centrifuge tube. Approximately 4 mL of ultrapure water was added and mixed using a Vortex mixer for 1 min. Subsequently, 20 mL of MeCN (1% HOAc) were added, the mixture was shaken vigorously for 2 min. 6 g of MgSO₄ and 1.8 g of NaOAc·3H₂O were added, shaken as quick as possible to prevent formation of MgSO₄ conglomerates and centrifuged for 5 min at 5000 rpm. A 18 mL aliquot was filtered through a Na₂SO₄ column and dried under a stream of nitrogen, then redissolved in 1.0 mL of MeOH for HPLC/UV analysis.

Conclusions

A modified and simplified QuEChERS

approach has been evaluated for the determination of PE in soil matrices. LC/MS/MS systems are currently too expensive for most environmental laboratories worldwide, but the HPLC/UV method presented is a reliable tool for the determination of low-dose PE in soil.

Acknowledgements

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