

ORIGINAL ARTICLE

Determination of Extraction efficiency of Sulfonylurea Herbicide residues in soil and Paddy leaf by with QuEChERS Extraction and column Separation methods

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ABSTRACT

A high performance liquid chromatographic (HPLC) method with Quenchers extraction kit and column separation methods were used to study the sulfonyl urea herbicides in soil and leaf samples. The quantification was performed on a Supelco C₁₈ column (4.6 mm×150 mm) with a linear gradient elution (Acetonitrile: Water: (2:1 v/v) as mobile phase) and the wavelength of an ultraviolet detector was set to 254 nm for the detection. Recoveries in two extraction methods from soil and leaf samples spiked with sulfonylurea herbicides at spiking levels of 2 and 4 mg/kg were 66.06 and 74.97 respectively in column separation and 86.09 and 85.04% respectively in Quenchers extraction kit method in soil samples. Where as in plant samples the recoveries were 55.47 and 65.15% in column separation and 84.49 and 83.81% in quencher's extraction kit method at fortification level of 2 and 4 mg/kg respectively. The results indicate that the Quenchers extraction kit method is easier, faster, sensitive and has better purification effect.

Keywords: extraction, herbicide, HPLC, QuEChERS, sulfonylureas.

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INTRODUCTION

Sulfonyl ureas (SUs) are herbicides mainly used in paddy fields. They show high activity at low application levels and low mammalian toxicity. Because of the poor thermal stability of SUs, gas chromatography (GC) requires prior derivatization, which is time-consuming. Therefore, liquid chromatography (LC) with photoconductivity detection [1] UV detection and diode array detection (DAD) [2-3], and capillary electrophoresis [4], have been developed. LC coupled to mass spectrometry (LC/MS) was also demonstrated to be suitable for SU analysis. The high polarity and chemical instability of SUs make them difficult to extract, cleanup and determine simultaneously, and little work has been reported on the analysis of multiple SUs in food. In this study, we selected rice as the target because it has high inspection demand, and we investigated analytical conditions of LC and a simple extraction and cleanup by Quenchers Extraction kit and column separation, which would be applicable to LC analysis in the future.

MATERIALS AND METHODS

Reagents

The herbicide Pyrazosulfuron-ethyl was obtained from M/s SIGMA-ALDRICH LABOCHEMIKALIEN GMBH Chemicals (Technical grade with 99.7% purity). Methanol (AR grade) was used extraction of pyrazosulfuron ethyl from the soil, and plant samples. Dichloromethane (AR grade) for partitioning and ethyl acetate (AR grade) for clean up. In quenchers kit method extraction was done by using the RESTEK Q-sep™ extraction kit (AOAC 2007.01 Method) and clean up kit (AOAC 2007.01 Method). For estimation using HPLC Acetonitrile (HPLC grade) and water (HPLC grade) were used.

Recovery test for pyrazosulfuron-ethyl

20 g of the soil and grinded paddy leaf samples were transferred in 50 ml of screw tight tube, for which 2 and 4 mg kg⁻¹ of pyrazosulfuron-ethyl fortification was made with standard. The fortified soil samples were analyzed for pyrazosulfuron-ethyl.

Column separation method**Extraction**

Soil or paddy leaf sample (20 g) of containing pyrazosulfuron-ethyl residue were placed in tightly stoppered test tubes and extracted twice with 50 and 25 ml methonal by horizontal mechanical shaker for 1 hour. The content was centrifuged and filtered through Whatmen filter paper No. 1 after the soil solids setted down. The twice extracts were than combined.

Partitioning

The combined methonal extract was transferred to 500 ml of separating funnel and diluted with 250 ml of de-ionised water and 50 ml saturated sodium chloride solution and 1 ml of 6 N HCl. pyrazosulfuron-ethyl residue was quantitatively re- extracted twice with 50 ml and 25 ml of dichloromethane by shaking. The combined dichloromethane extracts were than dissolved in 5 ml n-hexane.

Cleanup

Chromatographic glass column (15 mm X 50cm length) were prepared for cleanup of pyrazosulfuron-ethyl residue from other co extractives with a small plug of cotton and 2.5 g anhydrous sodium sulphate overlaid 10g of silica gel (grade V) as the adsorbant. The column were pre washed with 50 ml of n-hexane. pyrazosulfuron-ethyl residue in n-hexane and ethyl acetate (3:1). the elute was discarded. Finally the column was eluted with 75 ml solvent mixture of n-hexane and ethyl acetate (1:1). The elute was evaporated to dryness in rotary evaporater. The residues were then dissolved in acetonitrile. This solvent was evaporated to dryness and finally the volume was made up to 5 ml by repeated washing with acetonitrile. A suitable aliquot of the cleaned up sample was injected into HPLC with the help of micro-litre syringe for quantitative residue analysis.

QuEChERS extraction method**Sample extraction**

Homogenized fortified sample was generated using pestle and mortar. From which 0.2 g of homogenized product was transferred into clean 1 ml tube. To which 0.5 ml of 1% acetic acid in acetonitrile (v/v) was added thoroughly mixed and then 0.5 g of AOAC 2007.01 extraction buffer was added followed by shaking vigorously by hand for 1 minute and centrifuge for 1 min at 13600 rpm to separate solid material.

Clean up

Using centrifuged extracts resulting from earlier stage of sample extraction was transferred to the dSPE tube and immediately vertexed or shaken vigorously by hand for 30 seconds. Afterwards centrifuged for 1 min at 13600 rpm to separate solid material then supernatant was collected and used for the analysis.

Sample analysis

The HPLC UV system used, consisted of Shimadzu high performance liquid chromatography with LC-20AT pump and SPD- 20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 150 mm × 4.6 mm and particle size 3.5 µm, column temperature was maintained at 40°C. The injected sample volume was 20 µL. Mobile phases acetonitrile: Water: (2:1 v/v). The flow-rate used was kept at 0.6 mL/min. The detector wavelength was 254 nm. The external standard method was used for this analysis.

RESULT AND DISCUSSION

Pyrazosulfuron-ethyl standards were injected to the HPLC at different concentrations with Shimadzu equipped with UV detector and C₁₈ column. The standard chromatogram and table are presented in Table 1 and Fig 1. Recovery studies for pyrazosulfuron-ethyl in soil and plant samples were carried out using column separation and QuEChERS extraction kit. Sulfonylurea group of herbicide are currently analyzed by analytical methods such as high performance liquid chromatography [5] liquid chromatography/mass spectrometry [6], gas chromatography [7], capillary electrophoresis [8] Supercritical fluid chromatography [9]. Bio assay [10] and enzyme immune assay [11]. The methods (both physic-chemical and bioassays) have been successfully used for the qualitative and quantitative determination of sulfonylurea herbicides.

In the present study, column separation method and QuEChERS kit method was employed to estimate the herbicide residue in the fortified soil and plant samples. It was found that in column separation method the mean recovery percent was 66.06 and 74.97 per cent from the soil fortified with 2 and 4 mg kg⁻¹ of pyrazosulfuron ethyl respectively. On the other hand in the QuEChERS method the recovery percent was 86.09 and 85.04 per cent from soil fortified with 2 and 4 mg kg⁻¹ of pyrazosulfuron ethyl respectively (Table 2).

Similarly in leaf samples, the recovery percent in column separation was 55.47 and 65.15 per cent at fortification level of 2 and 4 mg kg⁻¹ of pyrazosulfuron ethyl respectively and in QuEChERS method the recovery percent was 84.49 and 83.81 per cent (Table 2).. The result indicates that the recovery percent was more in QuEChERS method than column separation method and the solvent required in column

separation is more. The matrix interferences were also observed in the column separation that decreases the qualitative estimation efficiency of the herbicide (Fig. 2). The time required for extraction of herbicide in column separation is more compared to QuEChERS method. Earlier reports suggests that the 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 [12]. 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. The use of QuEChERS in soils is very limited [13] but with very good results. [14] reported a simplified version of the QuEChERS method for the extraction of chlorinated pollutant compounds from soil samples.

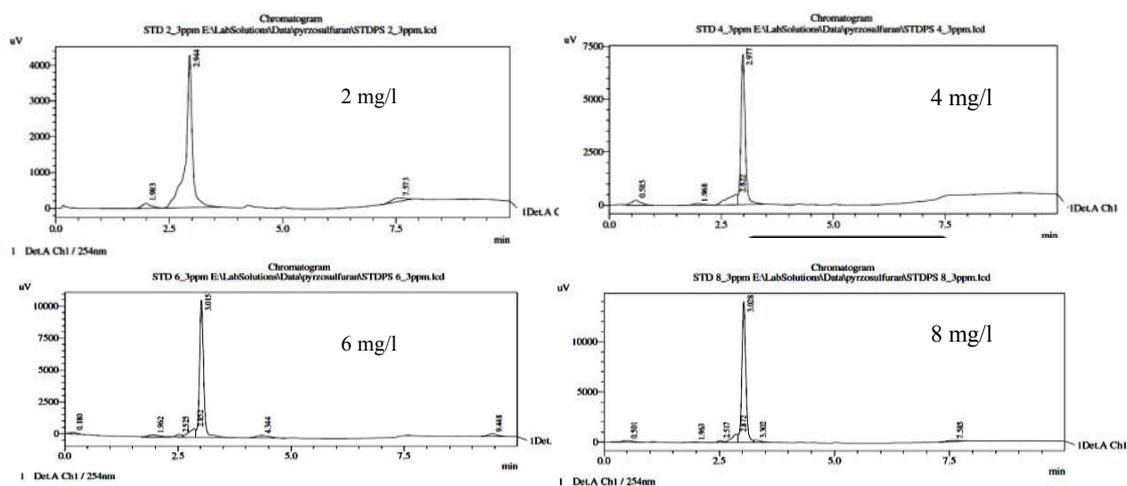


Figure 1: Pyrazosulfuron ethyl standards and its chromatogram run in HPLC

Table 1: Pyrazosulfuron ethyl standards and its peak area run in HPLC.

Concentration (ppm)	RT (min)	Peak area
2	2.944	34468
4	2.977	46797
6	3.015	67036
8	3.028	85106

Table 2: Recovery of pyrazosulfuron ethyl in the fortified soil and plant samples by column separation and QuEChERS extraction kit method

Amount added (mg kg ⁻¹)	Column Separation			QuEChERS		
	Mean recovery (mg kg ⁻¹)	Per cent recovery (%)	RSD (%)	Mean recovery (mg kg ⁻¹)	Per cent recovery (%)	rsd (%)
Soil sample						
2	1.32	66.06	2.03	1.72	86.09	5.18
4	3.00	74.97	7.50	3.40	85.04	5.99
Recovery factor	1.4			1.2		
Plant sample						
2	1.11	55.47	9.36	1.69	84.49	5.67
4	2.61	65.15	4.37	3.35	83.81	0.36
Recovery factor	1.7			1.2		

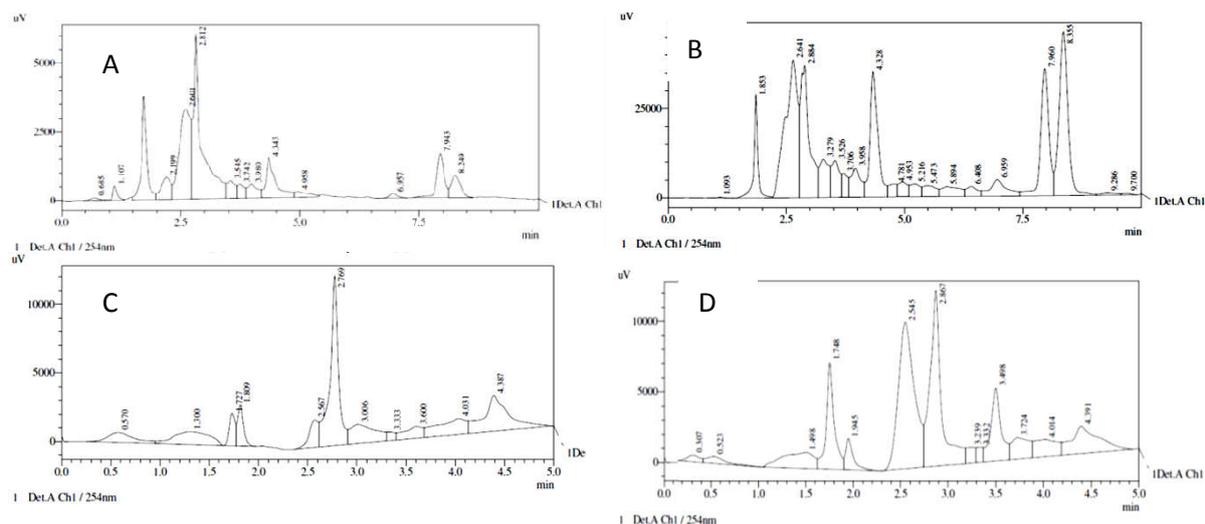


Figure 2: Chromatograms showing the matrix interferences after extraction of herbicide A) Extracted using QuEChERS kit from plant B) Extraction using column separation method from plant C) Extracted using QuEChERS kit from soil and D) A) Extracted using column separation method from soil.

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