

Development and Validation of a HPLC-UV Method for Simultaneous Determination of Five Sulfonylurea Herbicide Residues in Soybean Oil followed by Matrix Solid-Phase Dispersion

T. Nageswara Rao^{1*}, A. Venkata Ramasubbih² and T. Parvathi¹

¹Andhra University, Visakhapatnam, AP, India.

²S.K University, Anaparthi, AP, India.

*E-mail: raochem@rediffmail.com

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ABSTRACT

A simple, sensitive and inexpensive method was developed using matrix solid-phase dispersion (MSPD), together with high performance liquid chromatographic method for determination of Sulfonylurea herbicide residues (Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron) in Soybean oil. The evaluated parameters included the type and amount of sorbent (silica gel, C18 and Florisil) and the nature of eluent (ethyl acetate, dichloromethane and acetonitrile). The best results were obtained using 1.0 g of Soybean oil sample, 1.0 g of C18 as sorbent and 20ml of ethyl acetate-dichloromethane (1:1, (v/v)). The method was validated using Soybean oil samples spiked with Sulfonylurea herbicides at different concentration levels (0.03 and 0.3 µg/mL). Average recoveries (using each concentration six replicates) ranged 90-97%, with relative standard deviations less than 3%, calibration solutions concentration in the range 0.01-2.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg/mL and 0.03 µg/mL respectively.

Key words: matrix solid-phase dispersion, Sulfonylurea herbicides, HPLC-UV.

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INTRODUCTION

Sulfonylureas are a family of environmentally compatible herbicides that were discovered by crop protection in 1975 and first commercialized for wheat and barley crops in 1982. They have now been developed and commercialized worldwide in all major agronomic crops and for many specialty uses.

Sulfonylurea herbicide represents a major advance global crop protection technology and has revolutionized weed control by introducing a unique mode of action. Specifically, these compounds interfere with a key enzyme required for weed cell growth – Acetolactate synthetases. Furthermore sulfonylureas are compatible with the global trend toward post emergence weed control and integrated pest management.

Various methods have been described for the determination of these Sulfonylurea herbicides, using solid-phase extraction (SPE), solid-phase micro extraction (SPME), supercritical fluid extraction (SFE) and matrix solid-phase dispersion (MSPD). However, none of the published researches to date have reported the simultaneous analysis of chemical classes such as Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron in Soybean oil [1-5]. The matrix solid-phase dispersion (MSPD) technique was developed by Barker in 1989. It has advantages over conventional techniques because it employs small amounts of sample and solvent, and the extraction procedure consists of only a few experimental steps. MSPD evolved from the solid-phase extraction (SPE) technique, modified for application to solid and semi-solid matrices. The MSPD procedure is based on the use of a sorbent, which acts as an abrasive in order to produce a modified “opening” of the solid matrix, facilitating the extraction process when using a suitable solvent for eluting the analytes. The use of MSPD for Sulfonylurea herbicides recovery depends on the solubility of the Sulfonylurea herbicides in the eluting solvent, as well as the interactions between the matrix components, sorbent and eluent.

Due to the lack of literature reports concerning the use of MSPD as an extraction technique for Sulfonylurea herbicides belonging to different chemical classes from plants, this paper presents an MSPD method for determination of residue of Sulfonylurea herbicides in Soybean oil. So, the present research considered five different chemical classes, namely Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron which analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV) [6-8].

MATERIALS AND METHODS

Standards, Reagents and Samples

Certificated analytical standards of Rimsulfuron (99.2%), Azimsulfuron (99.6%), Bensulfuron (99.6%) Pyrazosulfuron (99.1%) and Halosulfuron (99.9%) were obtained from Sigma Aldrich., Acetonitrile was purchased

from Rankem, New Delhi, Analytical grade solvents, dichloromethane and ethyl acetate, were supplied from Merck Limited, Mumbai, C18-bonded silica (50 μm) from phenomenex (Torrance, CA, USA), Florisil (60-100 mesh) from fluka chemie GmbH CH-9471 Buchs, AR grade sodium sulphate from Merck Limited, Mumbai and Soybean oil was purchased from local market. They were brought to the laboratory and stored in plastic bag at refrigerator condition until they were processed in the laboratory.

Standard Stock Solutions

The Sulfonylurea herbicide standard stock solutions were individually prepared in acetonitrile at a concentration level 100 $\mu\text{g/mL}$ and stored in a freezer at -18°C . The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample Preparation

Representative 1.0 g portions of Soybean oil fortified with 1000 μL of working standard solution. The mixture was then gently blended in the mortar for 30 min, to assess the homogeneity of the sample. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction procedure

1.0 g of Soybean oil sample was weighed out and homogenized with 1.0 g of C18 -bonded silica for 5 min. The homogenized sample was transferred to an MSPD column consisting of a 20mL capacity polyethylene syringe containing 1.0 g flurosil and 1.0 g of anhydrous sodium sulfate. The elution was performed under vacuum with 20 mL of ethyl acetate-dichloromethane (1:1).The eluent was collected into a round bottom flask and evaporated to near dryness. Finally make up with 5mL of acetonitrile and analysed by HPLC-UV system.

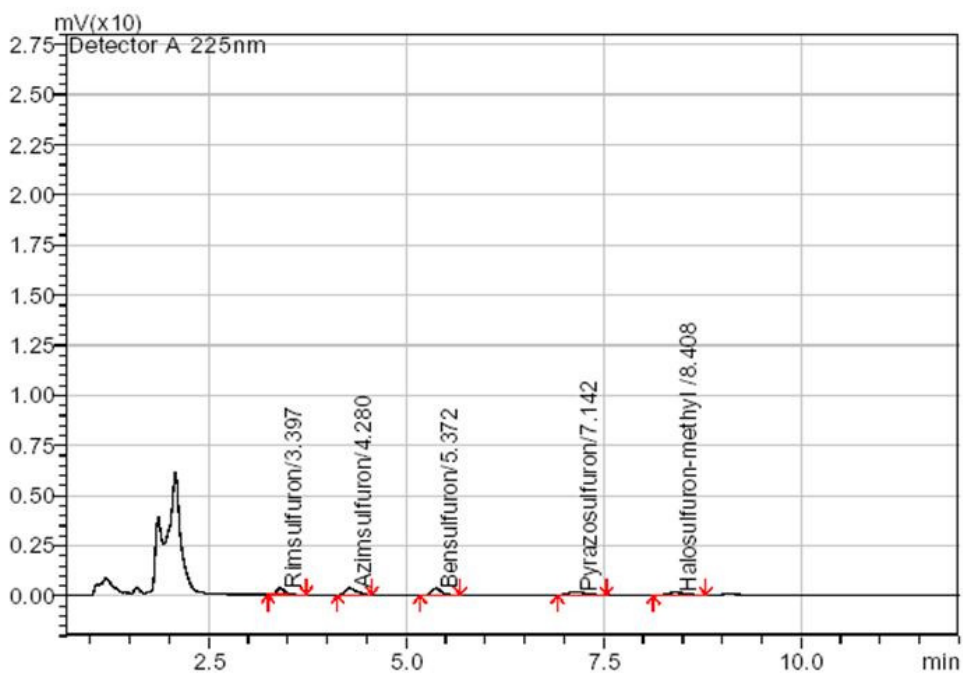


Fig.-1: Representative Chromatogram at fortification level of $0.03\mu\text{g/mL}$

Chromatographic Separation Parameters

A HPLC shimadzu prominence system (Japan) consisting of LC-20 AT pump, CT0-20A column oven, SIL-20A auto sampler and UV detector was used for this experiment. The auto sampler was set at 10°C . the absorbance was measured at 225 nm. An on Phenomenex (150mm length x 4.6mm id x $5\mu\text{m}$) was used as the analysis column. The column was maintained at a temperature 35°C . The chromatographic data were collected by LC-Solutions software.

The mobile phase consisted of Acetonitrile and milliQwater 50:50 v/v (pH-3.5 adjusted with ortho phosphoric acid). The flow rate was 1.0 ml/min.

Method Validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.03 and 0.3 mg/kg. Linearity was determined by different known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/ml) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given herbicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSIONS

Specificity

Specificity was confirmed by injecting the Soybean oil control. There were no matrix peaks in the chromatograms to interfere with the analysis of herbicide residues shown in Fig.1. Furthermore, the retention times of Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron were constant at 3.4±0.2, 4.3±0.2, 5.4±0.2, 7.1±0.2 and 8.4 ±0.2 min.

Linearity

Different known concentrations of Sulfonylurea herbicides (0.01, 0.05, 0.1, 0.5, 1.0, 2.0 µg/mL) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of Sulfonylurea herbicides were used to calculate linear regression equations. These were $Y= 105469.4X+65.61$, $Y=128014.8X+73.47$, $Y=119609.9X+49.91$, $Y=107626.26X+4.98$ and $102227.9+33.48$ with correlation coefficients of 0.9999, 1.0000, 0.9999, 0.9999 and 0.9998 for Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron respectively. A calibration curve showed in Fig. 2.

Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron in Soybean oil. The recovery data and relative standard deviation values obtained by this method are summarized in Table-1.

These numbers were calculated from five (6) replicate analyses of given sample (Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<3 %).

Table-1: Recoveries of the Sulfonylurea herbicides from fortified Soybean oil control sample (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)				
		Rimsulfuron	Azimsulfuron	Bensulfuron	Pyrazosulfuron	Halosulfuron
0.03	R1	88	87	90	89	91
	R2	90	90	91	88	90
	R3	91	89	89	89	94
	R4	89	88	93	90	93
	R5	89	88	90	91	91
	R6	90	90	90	93	90
	Mean	90	89	91	90	92
	RSD	1.17	1.36	1.52	1.99	1.80
0.3	R1	95	93	95	93	96
	R2	98	96	93	93	93
	R3	96	94	96	94	92

0.3	R4	95	94	92	92	95
	R5	95	93	94	93	93
	R6	93	93	92	95	92
	Mean	95	94	94	93	94
	RSD	1.71	1.25	1.74	1.11	1.76

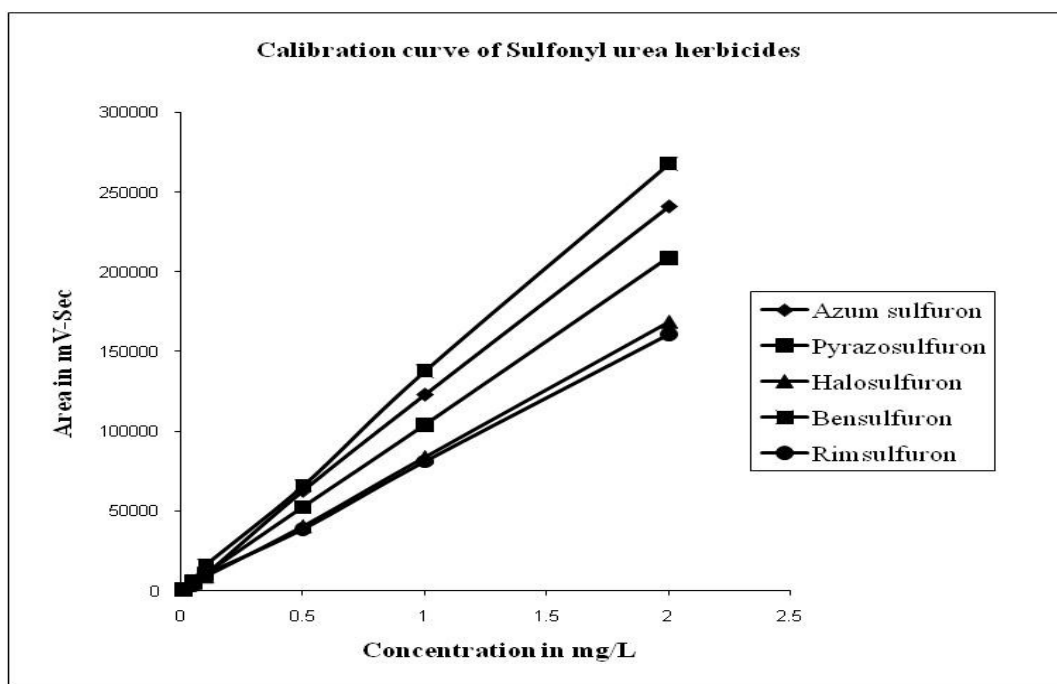


Fig.-2: Representative Calibration curve of Sulfonylurea herbicides.

Detection and Quantification Limits

The limit of quantification was determined to be 0.03 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (90-97%, RSD<3%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

Storage Stability

A storage stability study was conducted at -20±1°C with Soybean oil samples spiked with 0.1 µg/mL of Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron. Samples were stored for a period of 30 days at this temperature. Analysed for the content of Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only 2% for Rimsulfuron, Azimsulfuron, Bensulfuron, Pyrazosulfuron and Halosulfuron showing no significant loss of residues on storage. The results are presented in table 2.

CONCLUSIONS

This paper describes for the first time a fast, simple sensitive analytical method based on MSPD-HPLC-UV was developed and validated for the simultaneous determination of five Sulfonylurea herbicides residues in Soybean oil. The MSPD extraction procedure of the described method is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the matrix. Whole Soybean oil extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method. The mobile phase Acetonitrile and 0.1% formic acid yields good separation and resolution and the analysis time required for the

chromatographic determination of the five Sulfonylurea herbicides are very short (around 15 min for a chromatographic run). Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines. For all of the Sulfonylurea herbicides the sensitivity of the method was good enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of Sulfonylurea herbicides residues on a large number of oil samples.

Table-2: Storage stability Details (n=6)

Fortified Concentration in $\mu\text{g/mL}$	Storage Period in Days	Replication	Recovery in %				
			Rimsulfuron	Azimsulfuron	Bensulfuron	Pyrazosulfuron	Halosulfuron
0		R1	92	93	91	91	92
		R2	94	94	93	93	93
		R3	95	92	95	91	95
		R4	92	92	92	95	93
		R5	93	94	94	92	94
		R6	92	92	91	92	92
0.1		Mean	93	93	93	92	93
		RSD	1.36	1.25	1.76	1.63	1.25
		R1	90	91	90	89	91
30		R2	89	90	89	89	89
		R3	90	92	90	91	91
		R4	91	89	91	90	91
		R5		90	92	91	91
		R6	91	91	92	91	89
		Mean	91	91	91	91	90
	RSD	1.08	1.05	1.34	1.14	1.03	

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