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Determination of Ethoxysulfuron Residues in Sugarcane Juice followed by HPLC-PDA Detection and Confirmation of Residues by LC-MS/MS

Tentu Nageswara Rao[a*], T. Benarji Patrudu[b], M. V. Basaveswara Rao[a] and Karri. Apparao[a]

[a] Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India.
[b] Department of Chemistry, GITAM University, Hyderabad, Telangana, India.

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Abstract

A simple, sensitive and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with PDA detection for determination of ethoxysulfuron residues. The evaluated parameters include the extracts by silica gel column using methanol, dichloromethane and acetone solvents. The method was validated using sugarcane juice samples spiked with ethoxysulfuron at different fortification levels (0.03 and 0.3 µg mL^{-1}). Average recoveries (using each concentration six replicates) ranged 85-92%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01-10.0 µg mL^{-1} and limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg mL^{-1} and 0.03 µg mL^{-1} respectively. Finally, the sugarcane juice residue samples were re analyzed by LC-MS/MS for confirmation of ethoxysulfuron.

Keywords:

HPLC-PDA, LC-MS/MS, Ethoxysulfuron and Sugarcane juice

1. Introduction

Sulfonylurea herbicide [4],[5] a modern class of herbicides, are extensively used to control a wide range of weeds in many crops. These herbicides exhibit a simple but effective biological mode of action through inhibiting acetolactate synthase, a key enzyme that participate in the protein synthase of plants. Ethoxysulfuron is synthetic herbicide it has the unique characteristic of exhibiting herbicidal activity against weeds that show resistance to commercialized sulfonylurea herbicides.

Various methods have been described for the determination of these residues, using solid-phase micro extraction (SPME) Supercritical fluid extraction (SFE) and liquid – liquid extraction. However, none of the published researches to date have reported the simultaneous analysis of Ethoxysulfuron in sugarcane juice.

2. Experimental

2.1. Standards, Reagents and samples

The analytical standard of ethoxysulfuron (99.9%) was obtained from Sigma Aldrich. HPLC grade acetonitrile and water was purchased from Rankem, Analytical grade solvents i.e.,

[*] **Corresponding Author**
E-mail: tentu6581@rediffmail.com
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dichloromethane, acetone and methanol were procured from Merck Limited and sugarcane juice was purchased from local market.

2.2. Standard stock solutions

The Ethoxysulfuron stock solutions was individually prepared in acetonitrile at a concentration level 1000 μg mL^{-1} 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.

2.3. Sample preparation

Representative 50.0 mL portions of sugarcane juice fortified with 0.1 mL of working standard solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

2.4. Extraction procedure

1 mL methanol and 1 mL 0.2% HCl was added to the 50 mL of sugarcane juice and mixture was allowed to stand for 2-3 hours. Then the mixture was made alkaline by sodium hydroxide solution. The above solution was transferred into 1000 mL separating funnel and partitioned with 100 mL methylene dichloride. The methylene dichloride layer was collected over anhydrous sodium sulphate and repeated partition with 100 mL dichloromethane and collected dichloromethane layer over anhydrous sodium sulphate. Combined dichloromethane was concentrated under vacuum using a buchi rotary vacuum evaporator.

2.5. Clean-up procedure

The concentrated material was transferred on a glass column pre-packed with silica gel in dichloromethane [3]. 50 mL dichloromethane was eluted through the glass column and discarded. Finally the column was eluted with 100 mL acetone and collected the elute into a round bottom flask. Concentrated to dryness and then re-dissolved in 20 mL of acetonitrile. The final extract solutions were analysed by HPLC and LC-MS/MS.

2.6. Instrumentation

2.6.1. HPLC-PDA separation parameters

The HPLC-PDA system used, is 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 250 mm x 4.6 mm and particle size 5 μm (Phenomenex Luna-C18) Column temperature was maintained at 30°C. The injected sample volume was 20μL. Mobile Phases A and B was Acetonitrile and 0.1% ortho phosphoric acid (80:20 (v/v)). The flow- rate used was kept at 0.8 mL min^{-1} and the detector wavelength was 235 nm.

2.6.2. LC-MS/MS separation parameters

A mass spectrum was recorded on an Agilent 6490 triple quadruple mass spectrometer equipped with an ESI source. System control and data acquisition were controlled by Agilent mass hunter software. Aliquots of 10 μL were injected to the LC-MS/MS system using Agilent SB C18 column (5μm particle size, 4.6 mm i.d. ,150 mm length) with flow rate of 0.5 mL per minute having acetonitrile as mobile phase A (80%) and 0.1% formic acid in HPLC grade water (20%) as mobile phase B were used. The nebuliser gas (nitrogen) flow was fixed to 10 L min^{-1}. MS/MS mode operation was done with helium as collision gas, with a pressure of 4 x 10^{-4} milli bar. A capillary voltage of 4.5 kV was used in positive ionization mode. The interface temperature was set at 360 °C. The scan range was 50 - 420 m/Z.

2.7. Method validation

Method validation ensures analysis credibility [1] [2]. 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 µg mL^{-1}. Linearity was determined by different known concentrations (0.03, 0.1, 0.5, 1.0, 2.0 and 10.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg mL^{-1}) 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^{-1}) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

3. Results and Discussion

3.1 Specificity

Aliquots of ethoxysulfuron, control sample solution, extracted solvents and mobile phase solvents were assayed to check the specificity [6], [7]. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (Fig. 1 and 2). Furthermore, the retention time of ethoxysulfuron was 5.4 min (Approximately) and the mass fragment selected for evaluation was m/z of base peak 199.14 and parent peak of m/z is 399.31. Shown in (Fig. 3).

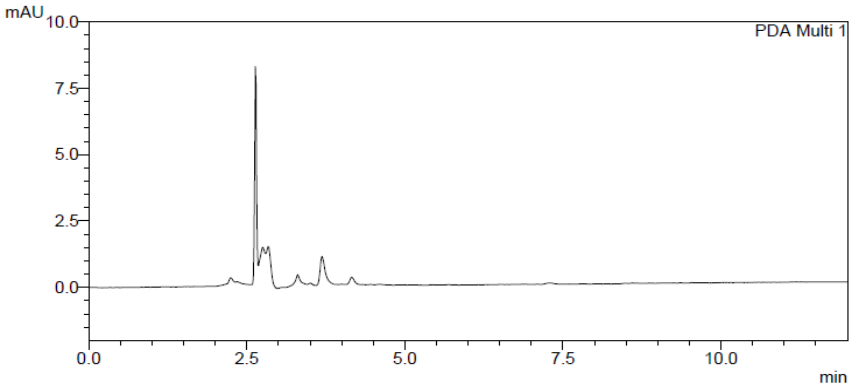

Fig. 1. Representative Chromatogram at sugarcane juice control

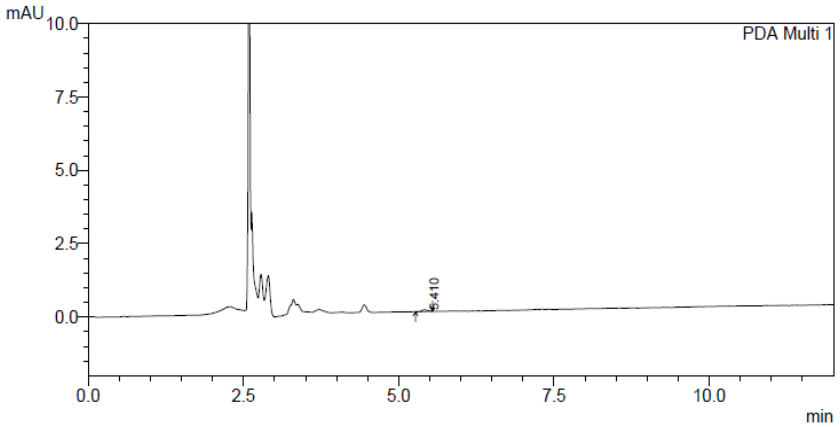

Fig. 2. Representative Chromatogram at fortification level of 0.03 µg mL^{-1}

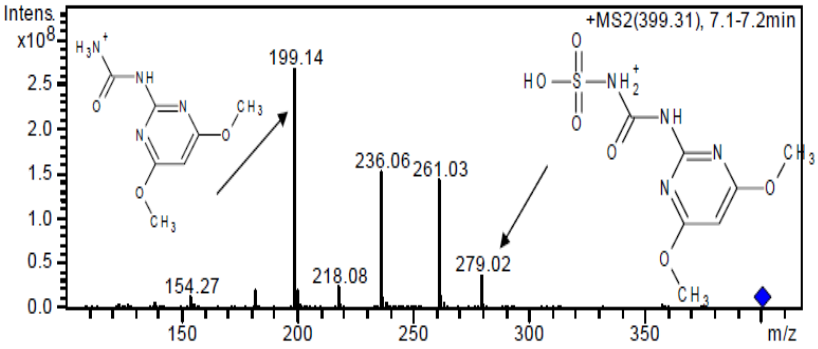

Fig. 3. LC-ESI-MS/MS Spectra of Ethoxysulfuron

3.2. Linearity

30.03 mg of ethoxysulfuron reference standard was taken into 10 mL volumetric flask and dissolved in acetonitrile, sonicated and made upto the mark with the same solvent. The concentration of the stock solution was 3000 µg mL^{-1}. From this stock solution prepared by different known concentrations of standard solutions (0.03, 0.1, 0.5, 1.0, 2.0 and 10.0 µg mL^{-1}) were prepared into a different 10 mL volumetric flasks and made upto the mark with acetonitrile. The serial dilution details were presented in Table 1. These standard solutions were directly injected into a HPLC. 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 standards were used to calculate linear regression equations. These were Y=10666.52X + 12.56 with correlation coefficient of 0.9999 ethoxysulfuron respectively. A calibration curve showed in**. 4).**

Table 1. Serial dilutions of linearity standard solutions

Stock solution concentration (µg mL^{-1})	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg mL^{-1})
3000	0.333	10	100
100	1.000	10	10
100	0.200	10	2
100	0.100	10	1
10	0.5	10	0.5
10	0.1	10	0.1
1	0.3	10	0.03

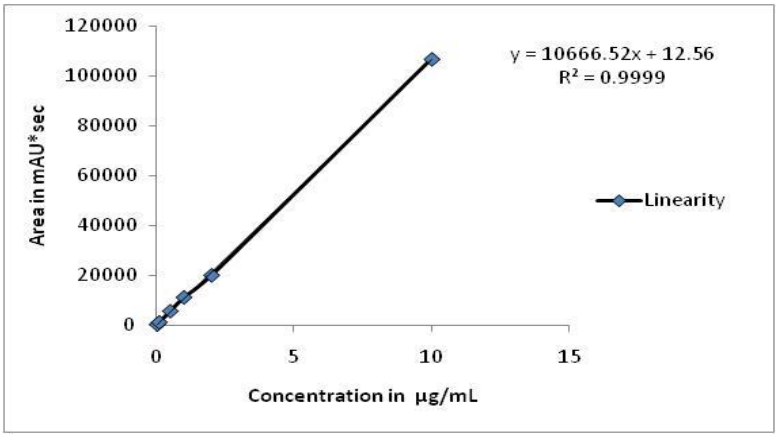

Fig. 4. Representative calibration curve of ethoxysulfuron

3.3. Accuracy and Precision

Recovery studies[8], [9] were carried out at 0.03 and 0.3 μg mL^{-1} fortification levels for ethoxysulfuron in juice. The recovery data and relative standard deviation values obtained by this method are summarized in Table 2.

These numbers were calculated from four (6) replicate analyses of given sample (ethoxysulfuron) made by a single analyst on the day. The repeatability of the method as satisfactory (RSDs<2 %).

Table 2. Recoveries of the ethoxysulfuron from fortified sugarcane juice control sample (n=6)

Fortification Concentration in μg mL^{-1}	Replication	Recovery (%)
0.03	R1	84
	R2	85
	R3	85
	R4	86
	R5	88
	R6	86
	Mean	85.67
	STDEV	1.37
	RSD in %	1.59
0.3	R1	92
	R2	91
	R3	92
	R4	94
	R5	93
	R6	92
	Mean	92.33
	STDEV	1.03
	RSD in %	1.12

3.4. Detection and Quantification Limits

The limit of quantification was determined to be 0.03 μg mL^{-1}. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (86-92%, RSD<2%) 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 0.03 μg mL^{-1} at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

3.5. Storage Stability

A storage stability study was conducted at refrigerator condition (5 ± 3°C) and Ambient temperature (25 ± 5°C) of 0.1 μg mL^{-1} level fortified juice samples Samples were stored for a period of 30 days at this temperature. Analysed for the content of ethoxysulfuron before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 4% for ethoxysulfuron showing no significant loss of residues on storage. The results are presented in Table 3 and 4.

Table3. Storage stability Details at refrigerator condition (5 ± 3°C)

Fortification Concentration in μg mL^{-1}	Storage Period in Days	Recovery in %
0.1	0	95 95 94 95 94 96
	Average	94.8
	STDEV	0.75
	RSD in %	0.79
0.1	30	91 90 92 90 91 92
	Average	91.0
	STDEV	0.89
	RSD in %	0.98

Table 4. Storage stability Details at ambient Temperature (25 ± 2°C)

Fortification Concentration in μg mL^{-1}	Storage Period in Days	Recovery in %
0.1	0	93 92 94 93 92 93
	Average	92.8
	STDEV	0.75
	RSD in %	0.81
0.1	30	90 89 90 91 90 91
	Average	90.2
	STDEV	0.75
	RSD in %	0.83

3.6. Calculations

The concentration of ethoxysulfuron in the samples analyzed by HPLC was determined directly from the standard curve.

$$Y = mx + c$$

Where,

Y = peak area of standard (μV*sec)
m = the slope of the line from the calibration curve
x = concentration of injected sample (mg L^{-1})
c = 'y' intercept of the calibration curve

The recovered concentration or Dose concentration was calculated by using the formula:

$$Recovered\ concentration\ or\ Dose\ concentration = \frac{(x - c)xDx100}{mxP}$$

Where,

m = the slope of the line from the calibration curve
x = sample area of injected sample (μV*sec)
c = 'y' intercept of the calibration curve
D = Dilution Factor
P = Purity of Test item

$$Recovery, \% = \frac{Recovered\ concentration}{Fortified\ concentration} x100\%$$

4. Conclusion

This paper describes a fast, simple sensitive analytical method based on HPLC-PDA and LC-MS/MS to determine the ethoxysulfuron residues in sugarcane juice. The SPE extraction procedure is very simple and inexpensive method for determination of ethoxysulfuron residues in sugarcane juice. The mobile phase Acetonitrile and 0.1% ortho phosphoric acid and 0.1% formic acid showed good separation and resolution and the analysis time required for the chromatographic determination of the sugarcane juice is very short (around 12 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and LOQ were established by following South African National Civic Organization (SANCO) guidelines [10]. Therefore, the proposed analytical procedure could be useful for regular monitoring, residue labs and research scholars to determine the ethoxysulfuron residues in different commodities (juice, seed, oil, fruit, and water and soil samples.

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