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Independent Laboratory Method Validation and Determination of Spiromesifen Residues in Tomatoes

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Abstract: A simple and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with PDA detection for determination of spiromesifen residues. The evaluated parameters include the extracts by ChemElut ®CE 1020 column using cyclohexane/ethyl acetate (85/15, v/v) mixture and acetonitrile solvents. The method was validated using tomato fruit samples spiked with spiromesifen at different fortification levels (0.05 and 0.5 µg/g). Average recoveries (using each concentration six replicates) ranged 84-94%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.05-10.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.02µg/g and 0.05µg/g respectively. Finally the tomato fruit residue samples were re analyzed by HPLC.
Key words : HPLC, ChemElut ®, spiromesifen and tomatoes.

Introduction

Spiromesifen is a non-systemic insecticide/acaricide belonging to the chemical class of cyclic ketoenoles[1,2]. Spiromesifen is an acetyl CoA carboxylase inhibitor[3]. The biological activity of cyclic ketoenoles correlates with inhibition of lipogenesis, resulting in decreased lipid contents, especially of triglycerides and free fatty acids[4]. In the present study, the determination of Spiromesifen residues in tomatoes followed by solid phase extraction and new validated HPLC method.

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[7,8]. However, none of the published researches to date have reported the residue analysis of spiromesifen in tomato fruit.

Experimental

Standards, Reagents and samples

The analytical standard of spiromesifen (99.5%) was obtained from Sigma Aldrich. HPLC grade acetonitrile and water was purchased from rankem, analytical grade solvents i.e., ethyl acetate, cyclohexane and Formic acid were supplied from Merck Limited and tomatoes were purchased from local market.

Standard stock solutions

The spiromesifen stock solutions was individually prepared in acetonitrile at a concentration level 1000 µg/g 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 50.0 gram portions of tomato fruit fortified with 0.1 mL of working standard stock solution. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction

The representative homogenized sample (50g –tomato peaces) was taken in a 500 ml extraction flask. Extracted it with Acetonitrile/water (9:1, v/v) 150 ml by keeping in an end-over-end shaker for 10 minutes. To this 3 g of celite was added and swirled it. Filtered it through a funnel with folded filter paper. The residual material was once again extracted with 100ml of same solvent and filtered. Collected the filtrate, concentrated upto 50 mL and added 5 ml of 5% formic acid and proceed for column clean up.

Column clean-up

Transferred the above solution on the top of the ChemElut ® CE 1020 column and eluted the column with 200 ml of cyclohexane/ethyl acetate (8:2, v/v). Collected the eluate in a 250 ml round bottomed flask and evaporated to dryness and then re-dissoved in 50 mL of acetonitrile. The sample was filtered through 0.45 µm filter and analysed by HPLC-PDA.

Instrumentation

HPLC-PDA separation parameters

The HPLC-PDA system used, consisted 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 10µL. Mobile Phases A and B was Acetonitrile and HPLC grade water (90:10 (v/v)). The flow- rate used was kept at 0.8 mL/min. A detector wavelength was 225 nm.

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[5,6]. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.05 and 0.5 µg/g. Linearity was determined by different known concentrations (0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD µg/g) 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/g) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

Results and Discussion

Specificity

Aliquots of spiromesifen, control sample solution, extracted solvents and mobile phase solvents were assayed to check the specificity. There were no matrix peaks in the chromatograms to interfere with the analysis of residues shown in (**Figure 1 and 2).** Furthermore, the retention time of spiromesifen was 5.5 min (Approximately).

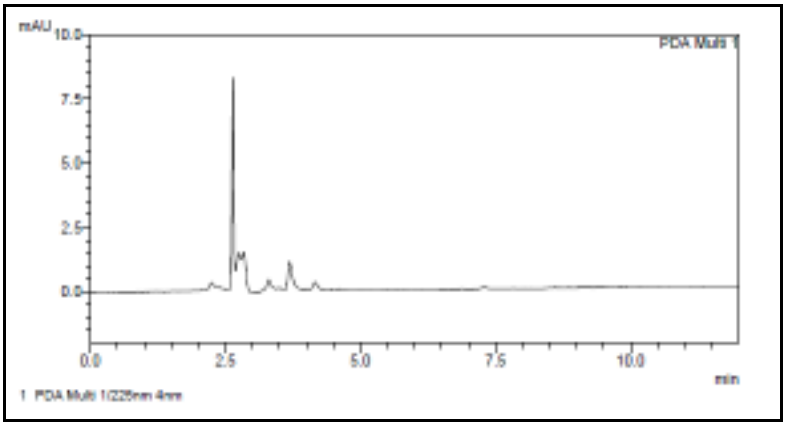

Figure.1. Representative Chromatogram at tomato fruit control

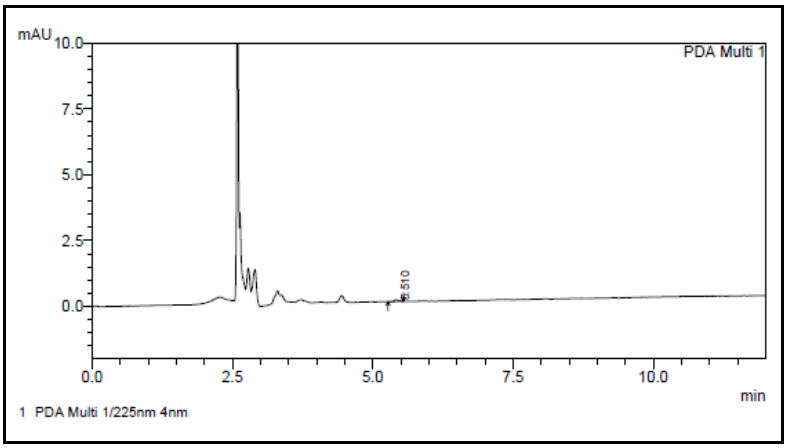

Figure.2. Representative Chromatogram at fortification level of 0.05 µg/g

Linearity

40.20 mg of spiromesifen reference standard was taken into 20 mL volumetric flask and dissolved in acetonitrile, sonicated and made upto the mark with the same solvent. The concentration of the stock solution was 2000 µg/mL. From this stock solution prepared by different known concentrations of standard solutions (0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/mL) 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 equation. This was Y=7302.07X + 29.75 with correlation coefficient of 1.0000 respectively. A calibration curve showed in (**Figure 3).**

Table 1. Serial dilutions of linearity standard solutions

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
2000	0.500	10	100
100	1.000	10	10
100	0.500	10	5
100	0.100	10	1
10	0.5	10	0.5
10	0.1	10	0.1
1	0.5	10	0.05

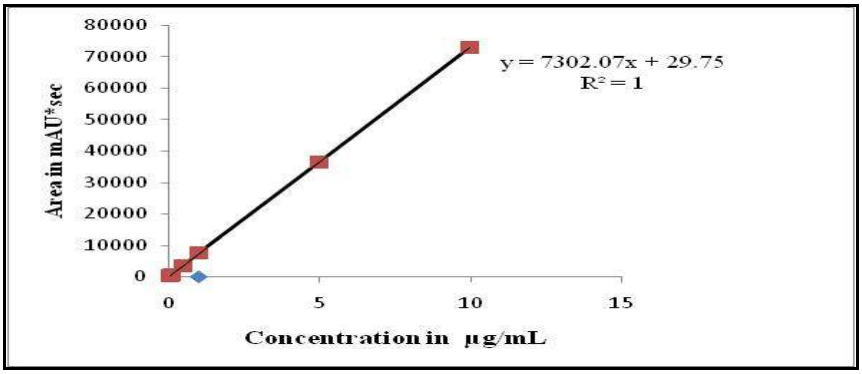

Figure.3. Representative Calibration curve of spiromesifen

Accuracy and Precision

Recovery studies were carried out at 0.05 and 0.5 µg/g fortification levels for spiromesifen in tomato fruit. The recovery data and relative standard deviation values obtained by this method are summarized in **Table 2.**

Table 2. Recoveries of the spiromesifen from fortified tomato fruit control sample (n=6)

Fortification Concentration in µg/g	Replication	Recovery (%)
0.05	R1	83
	R2	82
	R3	85
	R4	84
	R5	86
	R6	83
	Mean	**83.83**
	STDEV	**1.47**
	RSD in %	**1.76**
0.5	R1	93
	R2	93
	R3	96
	R4	95
	R5	94
	R6	95
	Mean	**94.33**
	STDEV	**1.21**
	RSD in %	**1.28**

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

Detection and Quantification Limits

The limit of quantification was determined to be 0.05 µg/g. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (84-94%, 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 0.05 µg/g 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 refrigerator condition (5 ± 3°C) and Ambient temperature

(25 ± 5°C) of 0.1 µg/g level fortified fruit samples were stored for a period of 30 days at this temperature. Analysed for the content of spiromesifen before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 3% for spiromesifen showing no significant loss of residues on storage. The results are presented in T**able 3 and 4.**

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

Fortification Concentration in µg/g	Storage Period in Days	Recovery in %
0.1	0	96
		93
		92
		94
		93
		91
	Average	**93.17**
	STDEV	**1.72**
	RSD in %	**1.85**
	30	91
		91
		90
		89
		90
		90
	Average	**90.17**
	STDEV	**0.75**
	RSD in %	**0.83**

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

Fortification Concentration in µg/g	Storage Period in Days	Recovery in %
0.1	0	92
		91
		95
		92
		93
		94
	Average	92.83
	STDEV	1.47
	RSD in %	1.59
	30	89
		90
		91
		91
		90
		89
	Average	90.00
	STDEV	0.89
	RSD in %	0.99

Calculations

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

$Y = mx + c$
Where,
Y = peak area of standard (mAU*sec)
m = the slope of the line from the calibration curve
x = concentration of injected sample (mg/L)
c = 'y' intercept of the calibration curve
The recovered concentration or Dose concentration was calculated by using the formula:

$$\text{Recovered concentration or Dose concentration} = \frac{(x-c)\ X\ D\ X\ 100}{m\ X\ P}$$

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

$$\% \text{ Recovery} = \frac{\text{Recovered Concentration}}{\text{Fortified Concentration}} \times 100$$

Conclusions

This paper describes a fast, simple sensitive analytical method based on HPLC-PDA to determine the spiromesifen residues in tomato fruit. The SPE extraction[9] procedure is very simple and inexpensive method for determination of spiromesifen residues in tomato fruit. The mobile phase Acetonitrile and HPLC grade water showed good separation and resolution and the analysis time required for the chromatographic determination of the tomato fruit 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 spiromesifen residues in different commodities

(fruit, juice, seed, oil, and water and soil samples).

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