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A NEW METHOD FOR DETERMINATION OF TOLFENPYRAD RESIDUES IN MANGO FRUIT

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ABSTRACT

A simple and inexpensive method was developed using solid-phase extraction, together with high performance liquid chromatographic method with UV detection for determination of tolfenpyrad residues. The evaluated parameters include the extracts by fluorosil packed column using ethyl acetate: cyclohexane (9:1) and acetonitrile: HPLC grade water (9:1) mixture of solvents. The method was validated using mango fruit samples spiked with tolfenpyrad at different fortification levels (0.03 and 0.3 µg/g). Average recoveries (using each concentration six replicates) ranged 84-93%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01-10.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01µg/g and 0.03µg/g respectively. Finally the mango fruit residue samples were re analyzed by HPLC.

Key words: HPLC, fluorosil, tolfenpyrad and mango fruit.

INTRODUCTION

Tolfenpyrad is a pesticide developed by Mitsubishi chemical Co[1]. That wasfirst approved in 2002 in japan under the trade name of Hachi-hachi. It is used against a broad range of pests such as hemiptera, coleopteran, Diptera, Lepidoptera, tysanoptera and acarina. It is especially effective against pests that are resistant to existing insecticides such as organophosphates and carbametes, because it supposedly possesses a new mode of action: inhibition of complex in the respiratory electron-transfer chain of mitochondria[2].

Various methods have been described for the determination of these residues, using solid-phase micro extraction (SPME)[3] Supercritical fluid extraction (SFE)[4] and liquid – liquid extraction. However, none of the published researches to date have reported the residue analysis of tolfenpyrad in mango fruit.

EXPERIMENTAL

Standards, Reagents and samples

The analytical standard of tolfenpyrad (99.0%) was obtained from Sigma Aldrich. HPLC grade acetonitrile and water was purchased from rankem, analytical grade solvents i.e., ethyl acetate, cyclohexane and fluorosil sorbet were supplied from Merck Limited and mango fruit was purchased from local market.

Standard stock solutions

The tolfenpyrad 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.

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Sample preparation

Representative 50.0 gram portions of mango 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 and clean up

The representative homogenized mango fruit 50 g was taken into a 500 mLerlenmeyer flask and added 5.0 mL water followed by 95 mL ethyl acetate : cyclohexane (90:10) and kept in end-over-end mechanical shaker for about 15 minutes. The sample was centrifuged for 15 minutes at 6000 rpm and then 12 mL of supernatant was passed through glass column packed with fluorosil[6] material and eluted the residue with ACN:H2O (90:10) and elute was collected into a flask and Concentrated to dryness and then re-dissoved in 20 mL of acetonitrile. The sample was filtered through 0.45 µm filter and analysed by HPLC-DAD.

Instrumentation

HPLC-UV separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software[5], 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 40°C. The injected sample volume was 20µL. 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 230 nm.

Method validation

Method validation ensures analysis credibility[7,8]. 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/g. 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/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 tolfenpyrad, 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 tolfenpyrad was 6.2 min (Approximately).

Linearity

30.30 mg of tolfenpyrad 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. 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) 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[9]. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation. This was Y=10407.80X + 30.44 with correlation coefficient of 1.0000 respectively. A calibration curve showed in (**Figure 3).**

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Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/g fortification levels for tolfenpyrad in mango fruit. 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 (tolfenpyrad) 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 0.03 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 0.03 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 tolfenpyrad before storing and at the end of storage period.The percentage dissipation observed for the above storage period was only less than 3% for tolfenpyrad showing no significant loss of residues on storage. The results are presented in T**able 3 and 4.**

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

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This paper describes a fast, simple sensitive analytical method based on HPLC-UV to determine the tolfenpyrad residues in mango fruit. The SPE extraction procedure is very simple and inexpensive method for determination of tolfenpyrad residues in mango 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 mango fruit is very short (around 15 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 tolfenpyrad residues in different commodities (fruit, juice, seed, oil, fruit, and water and soil samples).

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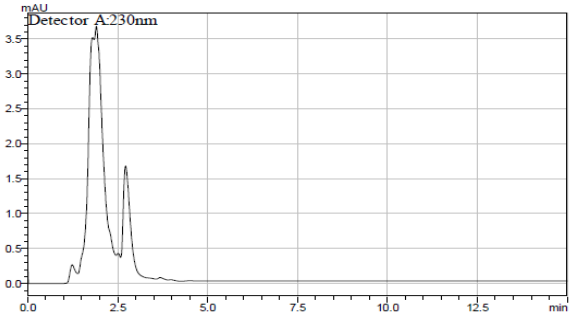

Figure.1. Representative Chromatogram at mango fruit control

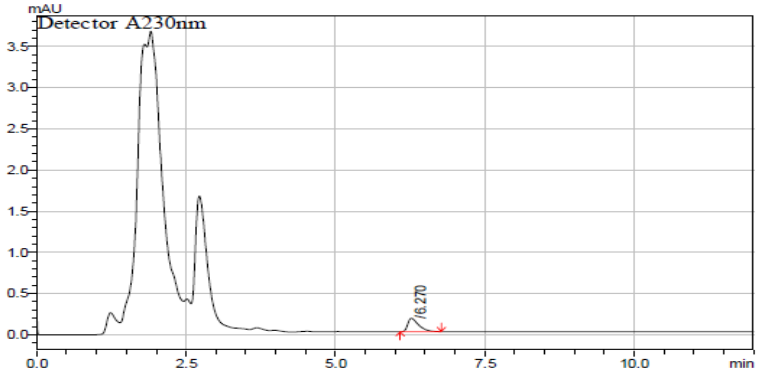

Figure.2. Representative Chromatogram at fortification level of 0.03 µg/g

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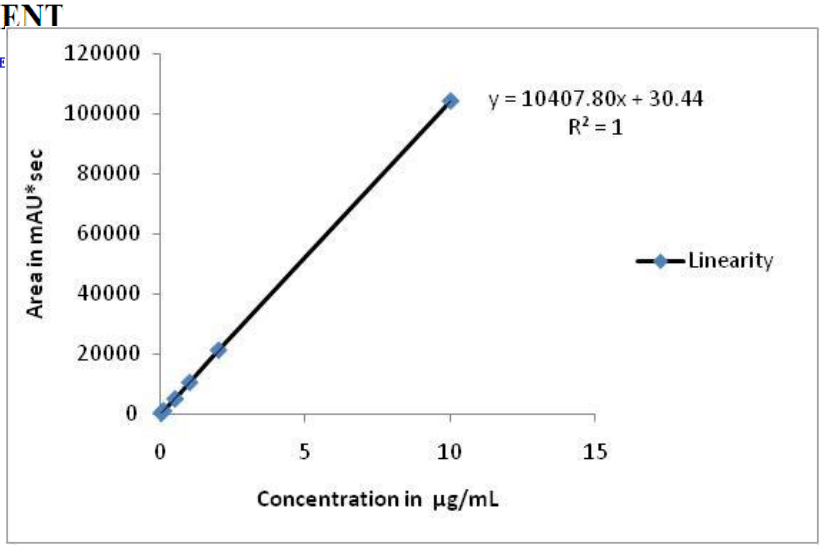

Figure.3. Representative Calibration curve of tolfenpyrad

Stock solution concentration (µg/mL)	Volume taken from stock solution (mL)	Final make up volume (mL)	Obtained concentration (µg/mL)
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

Table 1. Serial dilutions of linearity standard solutions

Fortification	Replication	Recovery (%)

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Concentration in µg/g		
0.03	R1	84
	R2	84
	R3	86
	R4	83
	R5	84
	R6	85
	Mean	**84.33**
	STDEV	**1.03**
	RSD in %	**1.22**
0.3	R1	92
	R2	94
	R3	93
	R4	95
	R5	96
	R6	94
	Mean	**94.00**
	STDEV	**1.41**
	RSD in %	**1.50**

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

Fortification Concentration in µg/g	Storage Period in Days	Recovery in %
0.1	0	95
		92
		94
		93
		92
		93
	Average	**93.2**
	STDEV	**1.17**
	RSD in %	**1.25**
	30	90
		89
		90
		91
		90
		91
	Average	**90.2**
	STDEV	**0.75**
	RSD in %	**0.83**

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

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Fortification Concentration in µg/g	Storage Period in Days	Recovery in %
0.1	0	94
		92
		91
		93
		93
		92
	Average	92.5
	STDEV	1.05
	RSD in %	1.13
	30	89
		90
		89
		90
		91
		89
	Average	89.7
	STDEV	0.82
	RSD in %	0.91

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

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