International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.4, pp 1473-1477, Oct-Dec 2012

Determination Of Paclobutrazol Residue In Mango Fruit Using A Matrix Solid-Phase Dispersion Method Coupled To High-Performance Liquid Chromatography With Ultraviolet Detection

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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 paclobutrazol in mango fruite. The evaluated parameters included the type and amount of sorbent (silica gel and Alumina) and the nature of eluent (Tetrahydrofuron, Acetonitrile and MilliQ water).The best results were obtained using 1.0 g of mango fruit sample, 1.0 g of silica gel as sorbet and 20ml of Tetrahydrofuron - Acetonitrile - MilliQ water (1:1:1), (v/v)).The method was validated using mango fruit samples spiked with paclobutrazol at different concentration levels (0.03 and 0.3 µg/mL). Average recoveries (using each concentration six replicates) ranged 89-93%, 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, paclobutrazol, method validation and HPLC-UV.

Introduction

Paclobutrazol (PBZ), also known as “cultar“, is an important agrochemicals in the culture of mango, being a regulator of growth inhibitor of gibberellins synthesis, making it possible to control the growth of the trees, reducing the need for pruning and handling of the crop, with achieving a high degree of response in growth and flowering, factors essential for the development of orchards. This product is applied directly to soil and brazil there are few diagnostic environmental impact, becoming, therefore, require a detailed study on the behavior of paclobutrazol.

Various methods have been described for the determination of paclobutrazol, using solid-phase extraction (SPE), solid-phase micro extraction (SPME)[3] and supercritical fluid extraction (SFE), However, none of the published researches to date have reported the paclobutrazol in mango fruit followed by matrix solid-phase dispersion (MSPD) technique.

The matrix solid-phase dispersion (MSPD) technique was developed by Barker in 1989[1]. 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 fungicide recovery depends on the solubility of the pesticide 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 pesticides belonging to different chemical classes from plants, this paper presents an MSPD method for determination of residue of paclobutrazol[2,4] in mango fruit. So, the present research considered paclobutrazol which analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV).

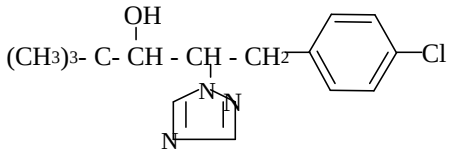

Fig.1. Structure of the Paclobutrazol

Experimental

Standards, Reagents and samples

Certificated analytical standards of Paclobutrazol (99.2%), was obtained from international institute of biotechnology and toxicology (IIBAT). Common names and structures of the paclobutrazol evaluated here are shown in **Fig. 1**. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, tetrahydrofuron, was supplied from Merck Limited, Mumbai, silica gel (50 µm) from phenomenex (Torrance, CA, USA), alumina (30-50 mesh) from Merck Limited, Mumbai, AR grade sodium sulphate from Merck Limited, Mumbai and mango fruit 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 fungicide standard stock solutions were individually prepared in acetonitrile at a concentration level 100 µg/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 mango fruit fortified with 100 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 mango fruit sample was weighed out and homogenized with 1.0 g of silica gel for 5 min. The homogenized sample was transferred to an MSPD column consisting of a 20mL capacity polyethylene syringe containing 1.0 g alumina and 1.0 g of anhydrous sodium sulfate. The elution was performed under vacuum with 20 mL of Tetrahydrofuron - Acetonitrile - MilliQ water (1:1:1).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.

Chromatographic 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, equipped with a reversed

Phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (Lichrospher) Column temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B were Acetonitrile and MilliQ water (50:50(v/v)). The flow- rate used was Kept at 1.5 ml/min. A detector wavelength was 225nm. The external standard method of Calibration was used for this analysis.

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 paclobutrazol giving a response of 10 times the baseline noise.

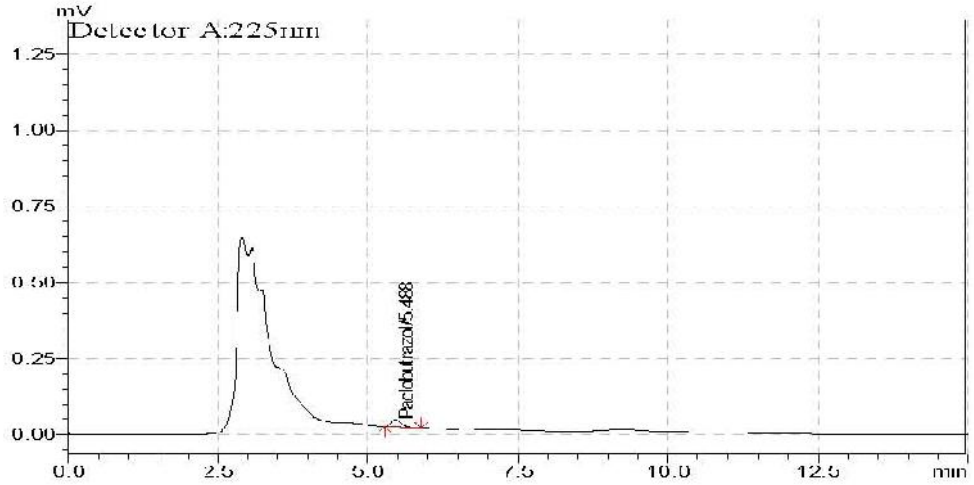

Fig.2.Representative Chromatogram at fortification level of 0.03µg/mL

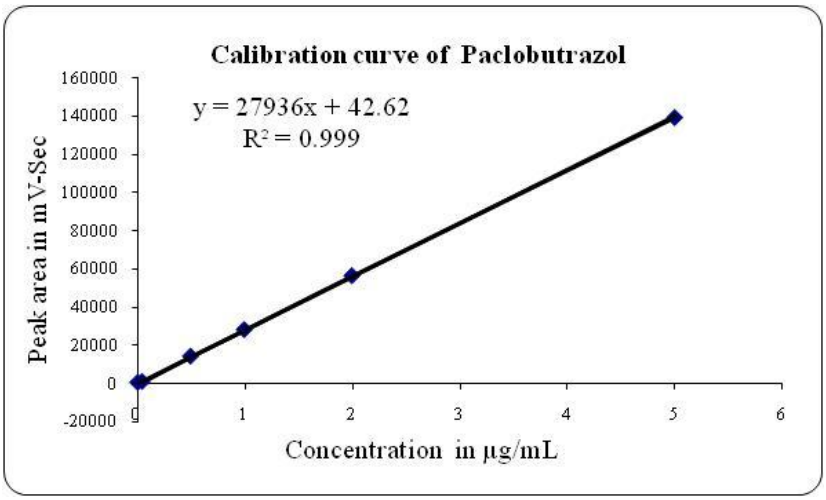

Fig.3. Representative Calibration curve of Paclobutrazol

Results and discussion

Specificity

Specificity was confirmed by injecting the mango fruit control. There were no matrix peaks in the chromatograms to interfere with the analysis of paclobutrazol residue shown in **Fig.2.** Furthermore, the retention time of paclobutrazol was constant at 5.5 ± 0.2 min.

Linearity

Different known concentrations of paclobutrazol (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 paclobutrazol were used to calculate linear regression equation. These were Y=27936X+42.62, with correlation coefficient of 0.999 for paclobutrazol respectively. A calibration curve showed in **Fig. 3.**

Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for paclobutrazol in mango fruit. The recovery data and relative standard deviation values obtained by this method are summarized in **Table 1.**

These numbers were calculated from (6) replicate analyses of given sample (paclobutrazol) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<3 %).

Table1. Recoveries of the Paclobutrazol from fortified mango fruit control sample (n=6)

Fortification Concentration in µg/mL	Replication	Recovery in %
0.03	R1	89
	R2	87
	R3	91
	R4	89
	R5	89
	R6	90
	Mean	**89**
	RSD	**1.49**
0.3	R1	94
	R2	93
	R3	93
	R4	94
	R5	93
	R6	92
	Mean	**93**
	RSD	**0.81**

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 (89-93%, 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 background 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 mango fruit samples spiked with 0.1 µg/mL of paclobutrazol was stored for a period of 30 days at this temperature. Analysed for the content of paclobutrazol before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 2% for paclobutrazol showing no significant loss of residues on storage. The results are presented in **table 2.**

Table2. Storage stability Details (n=6)

Fortified concentration in µg/mL	Storage Period in Days	Replication	Recovery in %
			Paclobutrazol
0.1	0	R1	93
		R2	91
		R3	93
		R4	92
		R5	93
		R6	91
		Mean	92
		RSD	1.07
	30	R1	90
		R2	92
		R3	90
		R4	89
		R5	91
		R6	90
		Mean	90
		RSD	1.14

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 determination of paclobutrazol residue in mango fruit.

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 mango fruit 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 milliQ water yields good separation and resolution and the analysis time required for the chromatographic determination of the paclobutrazol is 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[7].

Acknowledgement

The authors are thankful to the Management, and Dr. P. Balakrishnamurthy, Director, IIBAT, for providing necessary facility to conduct the experiment.

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