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Determination of valifenalate fungicide residues in tomato fruit

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ABSTRACT

A simple, sensitive and inexpensive method was developed using Liquid liquid extraction, together with high performance liquid chromatographic method with UV detection for determination of valifenalate residues. The evaluated parameters include the extraction procedure using different solvents and buffers (acetonitrile, n-hexane, water and 0.02 M triethylamine). The method was validated using tomato fruit samples spiked with valifenalate at different concentration levels (0.01 and 0.1 µg/mL). Average recoveries (using each concentration six replicates) ranged 88-90%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01-5.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.003 µg/mL and 0.01 µg/mL respectively.

Key words: Liquid liquid extraction, valifenalate, LOQ, LOD.

INTRODUCTION

Valifenalate is an acylamino acid fungicide. It acts systemically. Valifenalate once absorbed and trans located throughout the plant, gives a long term protective and curative effect. It acts on cell wall synthesis and thus affects all growth stages of the pathogen, reducing spore germination and mycelia growth. The fungicide is used to control late blights in tomato's and potato's, downy mildews in vines ad in tobacco. Thus, the fungicide controls many pathogens belonging to the class Oomycetes, particularly viral diseases may be difficult to identify. The symptoms can vary from one plant to the next and also with age and growing conditions [1].

Various methods have been described for the determination of these residues, using solid-phase micro extraction (SPME) [2], Supercritical fluid extraction (SFE) [5,7] and liquid – liquid extraction[3,4,6]. However, none of the published researches to date have reported the determination of valifenalate residues in tomato fruit.

MATERIALS AND METHODS

Standards, Reagents and samples

The reference standard of Valifenalate (99.9%), were obtained from Carbosynth. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents and reagents, n-hexane, triethylamine and Sodium Chloride were supplied from Merck Limited and tomato fruit was purchased from local market.

Standard stock solutions
The Valifenalate standard stock solutions were individually prepared in methanol at a concentration level 200 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 methanol, immediately prior to sample preparation.

Sample preparation
Representative 50.0 g portions of tomato fruit fortified with 100 µL 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.

Extraction procedure for Tomato fruit
Accurately weighed 50 g of representative tomato fruit. The sample was homogenized with 100 mL extraction solvent (80 mL of 80: 20 (v/v) acetonitrile: triethylamine (0.02 M)) using an homogenizer for 15 min at about 3000 rpm.

After decanting, the liquid was filtered under vacuum through a Buchner funnel using Whatman filter paper. The extraction was repeated with solid residue using 80 mL aliquot of extraction solvent and eventually the solvent was collected through filtration.

Purification
The 250 mL pooled liquid extract was transferred in to a 1.0L separatory funnel. After adding 25 g of sodium chloride and 200 mL of n-hexane saturated with acetonitrile, the solution was shaken vigorously for 1 min at least. The separatory fennel was left to stand (at least 1 hour) until the three phases (water, acetonitrile and n-hexane, in ascending order) were separated. The lower aqueous layer (containing un-dissolved NaCl at the bottom) was discarded and the intermediate acetonitrile phase was transferred quantitatively in a round bottomed flask. The upper organic phase (n=hexane) was discarded. Acetonitrile was reduced to small volume by Buchi rotavapour at 30°C maximum temperature and filtered through 0.45 micron in order to get rid of possible sodium chloride. The filtered acetonitrile was evaporated to dryness firstly by Buchi Rotavapour as above and at last by gentle nitrogen stream and analysed by HPLC.

Chromatographic 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 RP-18 Phenomenox (250 mm x 4.6 m i.d x 5µparticle size), Column temperature was maintained at 30°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.1% trifluro acetic acid (85:15 (v/v)). The flow- rate used was kept at 0.7 mL/min. A detector wavelength was 220 nm. The external standard method [8, 9] 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 0.01 and 0.1 mg/kg. Linearity was determined by different known concentrations (0.01, 0.1, 0.5, 1.0, 2.0 and 5.0 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 fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity
Specificity was confirmed by injecting the fruit control. There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in (**Figure 1 and 2).** Furthermore, the retention time of valifenalate was constant at 5.4 ± 0.2 min.

Figure. 1. Representative Chromatogram at fruit control

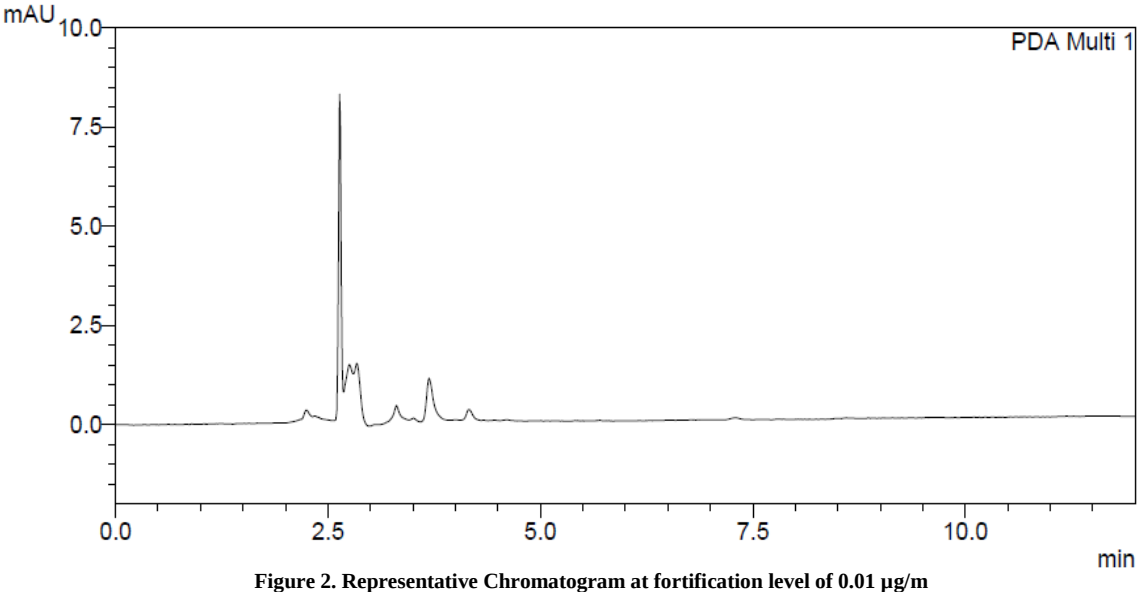


Figure 2. Representative Chromatogram at fortification level of 0.01 µg/m

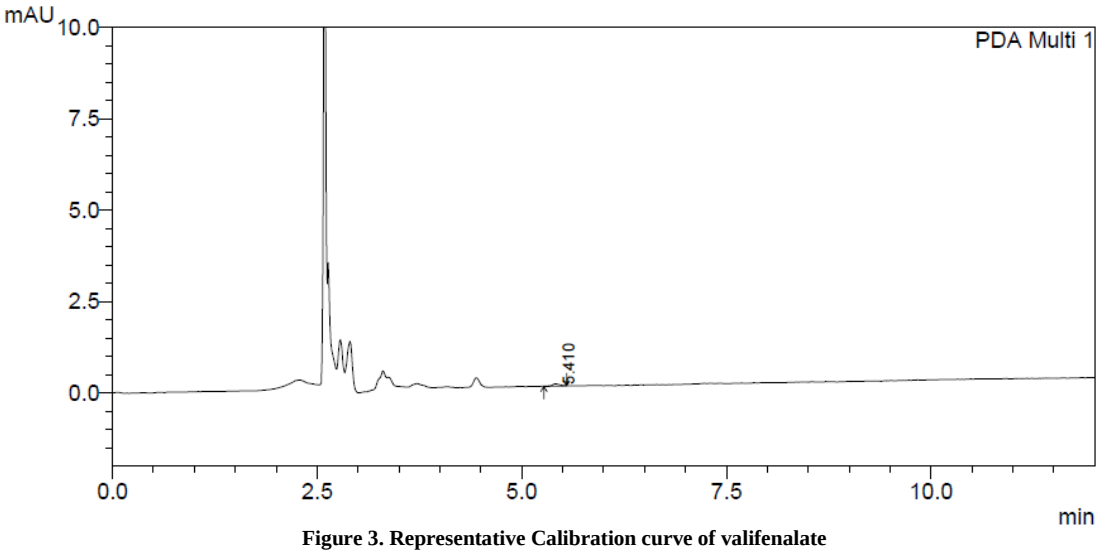
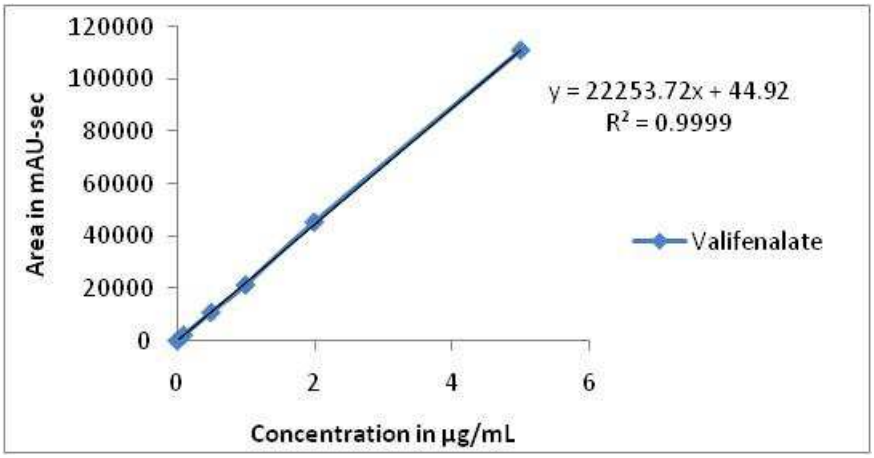


Figure 3. Representative Calibration curve of valifenalate



Linearity

Different known concentrations of standards (0.01, 0.1, 0.5, 1.0, 2.0 and 5.0 µg/mL) were prepared in methanol 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 standards were used to calculate linear regression equations. These were Y=22253.72X + 44.92 and 0.9999 for valifenalate. A calibration curve showed in (**Figure 3).**

Accuracy and Precision

Recovery studies were carried out at 0.01 and 0.1 µg/mL fortification levels for valifenalate in tomato fruit. The recovery data and relative standard deviation values obtained by this method are summarized in **Table 1.**

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

Table1. Recoveries of the valifenalate from fortified tomato fruit Control sample (n=6)

Fortification Concentration in µg/mL	Replication	Recovery (%)
0.01	R1	88
	R2	87
	R3	90
	R4	89
	R5	88
	R6	88
	Mean	88
	RSD	1.17
0.1	R1	90
	R2	89
	R3	90
	R4	89
	R5	90
	R6	90
	Mean	90
	RSD	0.58

Detection and Quantification Limits

The limit of quantification was determined to be 0.01 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (88-90%, 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 0.01 µg/mL at a level of approximately two 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 tomato fruit samples spiked with 0.1 µg/mL of valifenalate Samples were stored for a period of 30 days at this temperature. Analysed for the content of valifenalate before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 2% for valifenalate showing no significant loss of residues on storage. The results are presented in **Table 2.**

Table2. Storage stability Details (n=6)

Fortification Concentration in µg/mL	Storage Period in Days	Recovery in %
0.1	0	94
		93
		94
		92
		94
		95
	Mean	**94**
	RSD	**1.10**
	30	92
		90
		91
		93
		90
		91
	Mean	**91**
	RSD	**1.28**

CONCLUSION

This paper describes a fast, simple sensitive analytical method based on SPE-HPLC-UV simultaneous determination of valifenalate residues in tomato fruit. The SPE extraction and derivatization procedure is very simple and inexpensive method for determination of valifenalate residues in tomato fruit. The mobile phase composition was showed good separation and resolution and the analysis time required for the chromatographic determination of the valifenalate is very short (around 12 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[10]. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of valifenalate residues on a large number of juice, fruit, and water and soil samples.

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