



Modified QuEChERS technique and analysis methodology using UHPLC for residue analysis of thiacloprid and emamectin benzoate in chilli

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ABSTRACT: The presence of insecticide residues in chilli fruits limits the export and poses a health risk to consumers. Thus, it is necessary to develop quick and effective methods for conducting residue analysis of commonly used insecticides. The present research validates a modified QuEChERS technique for analysing thiacloprid and emamectin benzoate residues in chilli using UHPLC. The proposed methods recovered thiacloprid and emamectin benzoate by 80.33-88% and 79.33-96%, respectively. LOQ for both the insecticides was 0.01 mg/kg. Matrix effect values were within an acceptable range of -20 to 20%. The developed methodology can be used for periodical monitoring of the residue levels of the target insecticides in chilli.

Keywords: Chilli, emamectin benzoate, Quechers technique, thiacloprid, UHPLC.

INTRODUCTION

Chilli (*Capsicum annum* L.) is a major commercial spice crop cultivated in India. With a production of 4363.17 thousand metric tonnes, chilli is grown over 410.9 thousand hectares in the country (Anonymous, 2022a), making India one of the largest chilli producer, consumer and exporter in the world. Being a culinary ingredient of year-round availability, yield loss in chilli often goes unnoticed by the common man but is a matter of concern in the farmers' field. The incidence of insect pests is one of the major causes of reduced yield. Out of all the insect pests, sucking insects, especially thrips (*Scirtothrips dorsalis* Hood), attack alone can cause up to 50 to 90 per cent yield losses (Reddy and Reddy, 1999). In hopes of combatting pest attacks, chilli crop is repeatedly subjected to different insecticidal sprays. However, food contamination from pesticide residue has turned into an alarming case of human health risk. Not only the consumers, but the grower himself is in danger of the toxicants. Besides this, tonnes of Indian chilli exports are being rejected in the international market because our chilli samples still contain pesticide residue (Reddy *et al.* 2007). Therefore, it is necessary to develop methods to quickly and effectively estimate commonly used insecticides in chilli crop. With the need to use effective and safe insecticides two chemicals-thiacloprid and emamectin benzoate have been recently recommended by the Central Insecticides Board and Registration Committee (CIB&RC) to be used for the control of chilli thrips (Anonymous, 2022b).

Thiacloprid is a highly active, broad-spectrum novel insecticide belonging to the neonicotinoid group of systemic insecticides. It is an acute stomach and contact poison. It acts agonistically against the nicotinic

acetylcholine receptor (Elbert *et al.*, 2000). Emamectin benzoate, a highly selective novel macrocyclic lactone insecticide (Ishaaya *et al.*, 2002), is derived from avermectin B1. It is a non-systemic or semi-systemic chemical pesticide with an oral mode of entry and some contact action. It inhibits muscle contraction, which causes chlorine ions to flow continuously in the H-Glutamate and GABA receptor sites (Fanigliulo and Sacchetti, 2008). Residue analysis of thiacloprid in chilli has been previously done by Kumar *et al.* (2018) using methanol as a solvent. Similarly, Parmar *et al.* (2012) studied the persistence of thiacloprid in chilli and used a different approach for the extraction and cleanup of the sample. The dissipation kinetics of emamectin benzoate residue in chilli has been studied by Bhattacharyya *et al.* (2017) using a modified QuEChERS method with variation in the instrument method. The novelty of the current experiment lies in the methodology described, which is quick, suitable, gives satisfactory results and can be further used for residue studies of the two insecticides in chilli crop.

MATERIALS AND METHODS

Chemicals and reagents

Certified reference materials of thiacloprid (purity 99.9%) and emamectin benzoate (purity 99.3%) were purchased from Dr. Erhenstrofer, India. HPLC grade acetonitrile (ACN) and HPLC grade water were obtained from Merck Life Science Pvt. Ltd. Sodium chloride (NaCl), anhydrous sodium sulphate (Na₂SO₄), anhydrous magnesium sulphate (MgSO₄) and graphitic carbon black were all bought from HiMedia Laboratories Pvt. Ltd. Primary Secondary Amine (PSA) was obtained from Agilent Technologies.

Preparation of standard solutions

Standard stock solutions of thiacloprid and emamectin benzoate of 400 µg/mL concentration were prepared from their CRMs concerning HPLC grade acetonitrile. The two stock solutions were subjected to serial dilution to prepare working standards of different concentrations. Both stock solutions and working solutions were stored in the refrigerator until further use.

Instrumentation

Residue estimation of thiacloprid and emamectin benzoate in chilli fruit sample extracts was done through Dionex Ultimate 3000 Ultra High Performance Liquid Chromatography (UHPLC) from Thermo Fisher Scientific Inc. A C18 column with 150 mm length and 3 mm diameter was used for stationary phase. The detection of insecticide was done by a Photodiode Array Detector (PDA). For estimation of thiacloprid, the instrument method was set with a mobile phase of ACN: HPLC water in 80: 20 ratio, flow rate of 3 mL/min, temperature 35°C and UV wavelength of 256 nm for a run time of 10 minutes. For estimation of emamectin benzoate, residues extract samples were run in a mobile phase of ACN: HPLC water in a 70: 30 ratio at a flow rate of 2 mL/min, with a temperature of 35°C and UV wavelength of 260 nm. The total run time for estimation was 15 minutes.

Sample collection

Chilli fruit samples were collected from the AICRP vegetable farm, Dr. Rajendra Prasad Central Agricultural University, Pusa. 200 grams of green chillies were plucked from the plots, which were free of any insecticide treatment. Samples were collected in polythene bags, properly labelled and brought to the Sample Processing Laboratory and Pesticide Residue Analysis Laboratory of the Department of Entomology, Post Graduate College of Agriculture, RPCAU, Pusa.

Sample preparation

Chilli samples were prepared for analysis by following a “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) technique with slight modifications. First step in QuEChERS technique is extraction of insecticide from sample. Chilli sample from each polythene bag was cut and macerated in a mixer grinder for homogenization of the sample. In an analytical balance, 15 g of this macerated chilli was weighed and transferred into clean and labelled 50 mL centrifugal tubes. Chilli samples in the tubes were fortified with the required amount of insecticide (thiacloprid or emamectin benzoate) and kept in the refrigerator for 30 minutes to one hour. Next, 30 mL HPLC grade acetonitrile and 10 g anhydrous sodium

chloride were added to the tubes. The tubes were first shaken vigorously by hand, then vortexed (Spinex) for 1 to 3 mins and then shaken on rotospin (Tarson®) at 50 rpm for 5 mins. After 5 mins the tubes were centrifuged at 3000 rpm for 5 mins. From the centrifuged tubes 16 mL of supernatant was carefully pipetted out into another clean and labelled 50 mL polypropylene centrifugal tube.

Clean-up of the extracted sample began with the addition of 10 g of anhydrous sodium sulphate. This was followed by cleanup of the sample through “dispersive solid phase extraction (DSPE)”. 0.15 ± 0.01 g primary secondary amine (PSA) sorbent, 0.90 ± 0.01 g anhydrous magnesium sulphate and 0.05 ± 0.01 g graphitic carbon black was weighed into a clean and labelled 15 mL DSPE tube. To this tube, 6 mL aliquot was pipetted from the 16 mL extract, shaken thoroughly by hand and vortexed. The tubes were centrifuged at 3000 rpm for 5 mins and finally 3 mL supernatant was pipetted into separate 15 mL glass tubes. This terminal 3 mL volume was labelled properly and stored in a refrigerator until its analysis. For final quantification, 1 mL from the terminal volume was pipetted into a small glass vial and placed in the sampler compartment of the UHPLC.

METHOD VALIDATION

The quantitative determination of thiacloprid and emamectin benzoate residues in chilli samples was validated as per the instructions of bio-analytical method recommendations mentioned in the SANCO guidelines.

Selectivity

Selectivity of the method was obtained by comparing the peaks of the chromatograms from running the blank ACN samples with the peaks obtained from the chromatograms of standard solutions. Selectivity is determined to distinguish the peaks of the analyte from those of the matrix.

Linearity

A linear relationship was generated from the calibration curves of different concentrations (0.05, 0.1, 0.5, 1 and 2 µg/mL) of thiacloprid and emamectin benzoate against their area.

LOD and LOQ

Limit of Detection (LOD) refers to the minimum quantity of pesticide residue that can be detected by the analytical method. Meanwhile, the Limit of Quantification (LOQ) is the minimum amount of pesticide residue that can be quantified or measured by the instrument accurately and precisely. To determine, LOD and LOQ values, chilli

samples were spiked with different concentrations of thiacloprid and emamectin benzoate. The baseline of the chromatogram from the unfortified blank was magnified to obtain the noise response of the instrument. The noise response was converted to a concentration estimate from the known concentration of spiked extract.

Matrix effect

A matrix-match study was conducted to eliminate the matrix effect produced by various compounds. Matrix-matched calibration curves were obtained by spiking the matrix extracts with working standards of the insecticide at five different concentrations- 2, 1, 0.5, 0.1 and 0.05 mg/L. The area obtained from this set was compared with the area of standards prepared in ACN. The matrix effect percentage was calculated by the formula-

$$ME\% = \frac{\text{Peak area of standard in matrix} - \text{Peak area of standard in solvent}}{\text{Peak area of standard in solvent}} \times 100$$

Accuracy and precision

Recovery studies were carried out to determine the accuracy and precision of the methodology. For precision calculation, chilli fruit samples were spiked with three concentrations of the standard *viz.*, at LOQ, 5 times of LOQ and 10 times of LOQ. Three replications were made for each spike level. RSD values from these replications were used to evaluate the repeatability and reproducibility of the methodology. Intraday precision or instrumental repeatability was obtained from RSD_r value from standard deviation of recovery studies resulting from running the three replicates on the same day, using the same methodology, same operational conditions, and same operator and in the same laboratory. Interday precision or reproducibility was obtained from RSD_R value by analysing different batches of chilli extract and reagents by different analysts on three consecutive days.

RESULTS AND DISCUSSION

Selectivity

Upon observation of peaks in the blank samples, no significant peak was detected at the insecticide retention time. Thus, the methodology was determined to be selective for thiacloprid and emamectin benzoate without interference.

Linearity

Linearity curves of both the insecticides resulted in R² value greater than 0.99 (Figure 1), which implied that the prepared standards and detector used were fit and further residue analysis of chilli samples for thiacloprid and emamectin benzoate could proceed with their use.

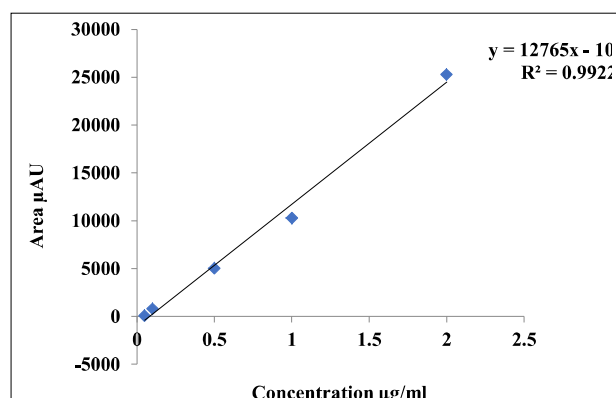


Fig. 1a

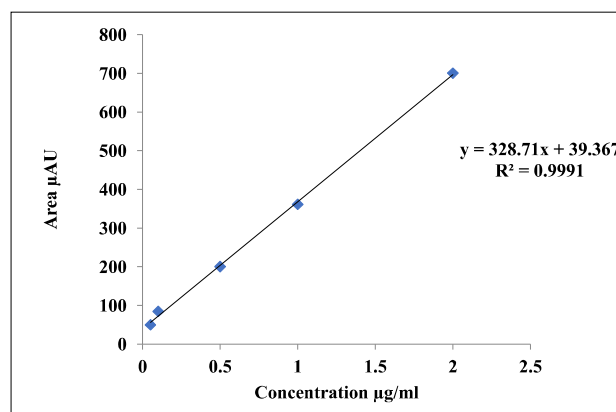


Fig. 1b

Fig 1. Linearity curve of (a) thiacloprid standards (b) emamectin benzoate standards

LOQ and LOD

Full scale deflection in both thiacloprid and emamectin benzoate was achieved with 1 ng of their standards. Chilli samples underwent processing to result in the terminal volume of 3 ml. The terminal volume was concentrated up to 1.5 ml from where 10 µL of sample was injected into a vial in UHPLC observe maximum load of samples that could be analysed by the machine without the occurrence of any interference peak in the area of the compound getting estimated. The limit of quantification (LOQ) for both thiacloprid and emamectin benzoate was quantified at 0.01 mg/kg, and the limit of detection (LOD) was 0.003 mg/kg.

Matrix effect

The linearity curve of the matrix matched the standards of thiacloprid, giving the determination coefficient (R²) value 0.9877 and linearity equation $y = 1207.1x + 117.25$. Similarly, the linearity curve of matrix matched standards of emamectin benzoate gave the determination coefficient (R²) value 0.9972 and linearity equation $y = 328.71x + 39.367$.

Table 1. Matrix effect of thiacloprid and emamectin benzoate standards in chilli fruits

Spiking level	ME % Thiacloprid	ME % Emamectin benzoate
2	-17.25	-17.40
1	-16.40	-12.25
0.5	-11.37	-12.49
0.1	-18.01	-17.95
0.05	-17.05	-18.23

Table 2. Recovery (%) of thiacloprid and emamectin benzoate from spiked chilli samples with respect to matrix matched standard

Substrate	Spiked Level (mg/kg)	Thiacloprid		Emamectin benzoate	
		Recovered (%) *Mean \pm SD	RSD _r	Amount Recovered *Mean \pm SD	RSD _r
Chilli	0.25	98.67 \pm 6.79	6.89	109.33 \pm 16.44	15.03
	0.05	99.33 \pm 2.49	2.51	90 \pm 3.26	3.63
	0.01	95.33 \pm 3.39	3.56	116.67 \pm 9.42	8.08

Table 3. Recovery (%) of thiacloprid and emamectin benzoate from spiked chilli samples

Substrate	Spiked Level (mg/kg)	Thiacloprid		Emamectin benzoate	
		Amount Recovered *Mean \pm SD	RSD _r	Amount Recovered *Mean \pm SD	RSD _r
Chilli	0.25	88.00 \pm 5.35	6.08	89.33 \pm 8.22	9.20
	0.05	81.33 \pm 4.11	5.05	79.33 \pm 2.49	3.14
	0.01	80.33 \pm 4.03	5.01	96.00 \pm 2.83	2.95

*Mean of six replications

SD = "Standard Deviation"

RSD_r = "Relative Standard Deviation" (Repeatability)**Table 4. Reproducibility for thiacloprid and emamectin benzoate at 0.01 mg/ kg**

Substrate	Day	Thiacloprid			Emamectin benzoate		
		Amount recovered (%)	Standard deviation (%)	RSD _r (%)	Amount recovered (%)	Standard deviation (%)	RSD _r (%)
Chilli	1	113.33	12.47		92	13.95	
	2	97.33	0.94	5.99	93.67	12.71	10.47
	3	80.33	4.03		96	2.83	

RSD_r = "Relative Standard Deviation" (reproducibility)

Residue analysis of thiacloprid and emamectin benzoate in chilli

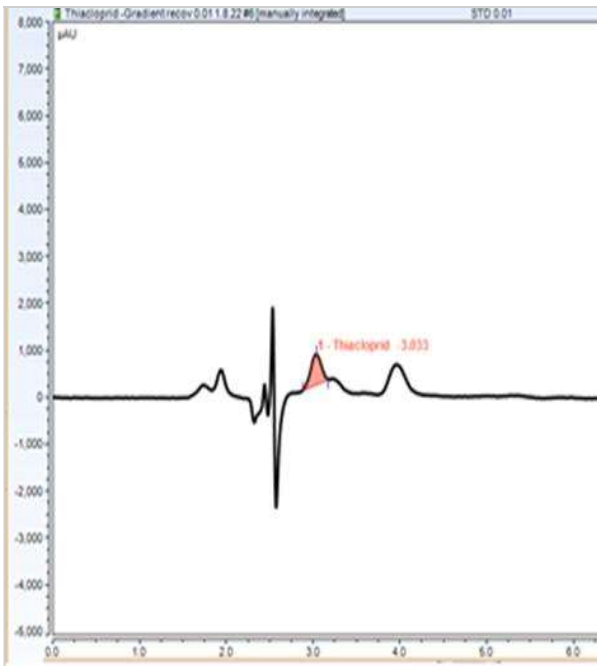


Fig. 3a

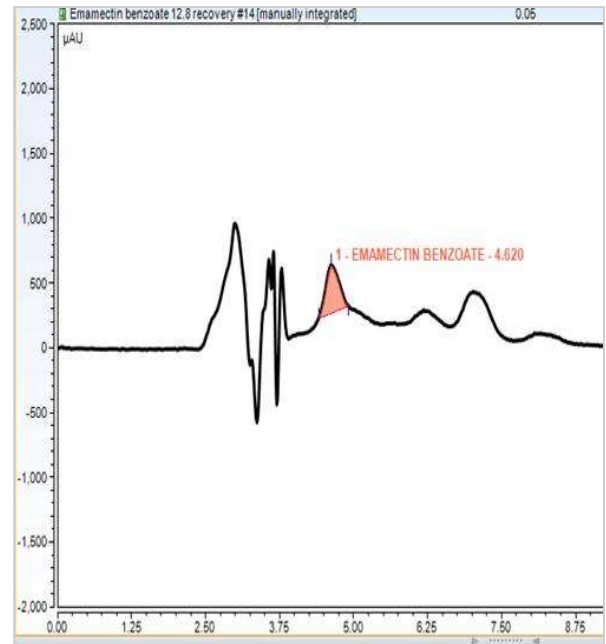


Fig. 3b

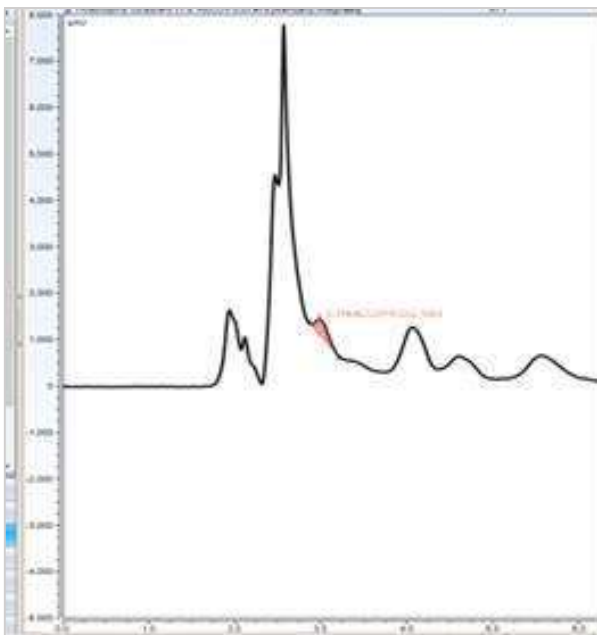


Fig. 3c

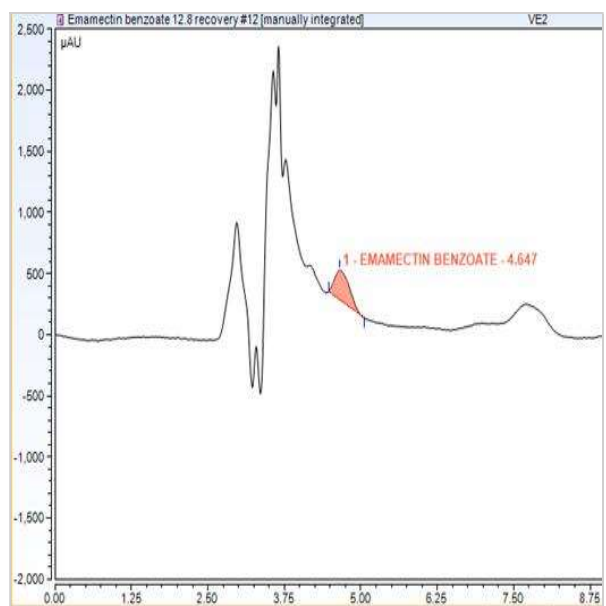


Fig. 3d

Fig. 3. UHPLC Chromatograms of (a) thiacloprid standard (0.01 $\mu\text{g/ml}$) (b) chilli spiked with thiacloprid (0.01 mg/kg) (c) emamectin benzoate standard (0.01 $\mu\text{g/ml}$) (d) chilli spiked with emamectin benzoate (0.01 mg/kg)

= $284.19x + 19.411$ (Fig. 2). For the matrix matched standards of thiacloprid and emamectin benzoate in chilli fruits ME% at all concentrations was found within the acceptable range of -20% to 20% (Table 1). The recovery percentage of thiacloprid and emamectin benzoate from the spiked chilli sample with respect to matrix matched standard is given in Table 2.

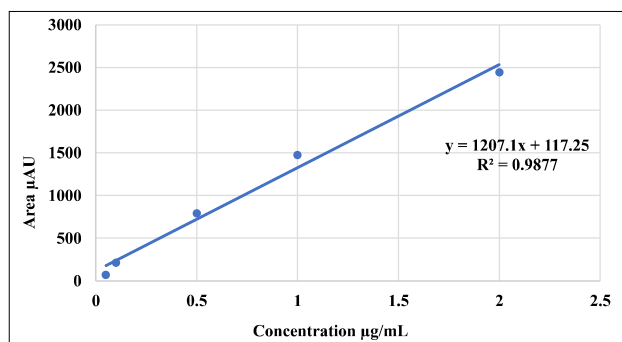


Fig. 2a

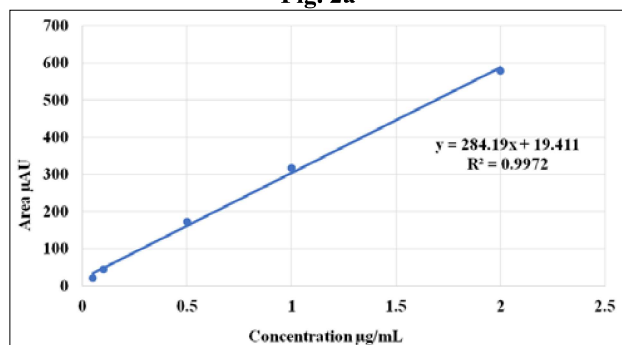


Fig. 2b

Fig 2. Linearity curve of matrix-matched standards of (a) thiacloprid (b) emamectin benzoate

Accuracy and precision

Chromatograms of thiacloprid and emamectin benzoate standards at 0.01 mg/kg and chilli samples spiked with 0.01 mg/kg of the insecticides are presented in Fig. 3. The instrumental repeatability (RSD_p) or intraday precision for thiacloprid in chilli at 0.25, 0.05 and 0.01 µg/ml corresponded to 6.08, 5.05 and 5.01%, respectively. The intraday precision (RSD_p) for emamectin benzoate was obtained at 9.2, 3.14 and 2.95% for the concentrations 0.25, 0.05 and 0.01 µg/ml concentrations, respectively (Table 3). Interday precision (between-batch recoveries) and reproducibility (RSD_R) were examined for thiacloprid and emamectin benzoate residue in chilli at 0.01 mg/kg. The reproducibility of thiacloprid and emamectin benzoate from the separate batches of chilli samples was obtained as 5.99 % and 10.47 %, correspondingly (Table 4). For both the insecticides, the values were obtained within 15 per cent at a concentration of 0.01 mg/kg.

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