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Premlata Meena

Anand Agricultural University,
Anand, Gujarat, India

Ajit Kumar Meena

Maharana Pratap University of
Agriculture & Technology,
Udaipur, Rajasthan, India

Mamta

ICAR-Indian Agricultural
Research Institute, New Delhi,
India

Rajendra Bairwa

Dr. Rajendra Prasad Central
Agricultural University,
Samastipur, Bihar, India

Method validation for the analysis of bifenthrin residue in different soils

Premlata Meena, Ajit Kumar Meena, Mamta and Rajendra Bairwa

Abstract

A modified QuEChERS method was optimized, validated and verified for the extraction of bifenthrin residues in agricultural soil samples. Bifenthrin was extracted using a single step, without clean-up, with matrix matched calibration, and by gas chromatography equipped with electron capture detector. The method was fully validated on a representative agricultural soil samples collected from Anand, Dantiwada, Junagadh and Khandha (sandy loam, sandy, medium black calcareous and heavy black soil, respectively). The recovery and matrix effect were critical parameters within each tested procedure. The optimal method without clean-up was validated. Accuracy (expressed as recovery), precision (expressed as RSD), linearity, LOQ, and uncertainty were determined. To avoid matrix effects, matrix-matched calibration curves ($R^2 \geq 0.99$) were used for Bifenthrin Analytes. The recoveries at the three spiking levels of sandy loam, sandy, medium black calcareous and heavy black soils were in the range of 86.75-119.03, 96.27-118.30, 82.00-96.67 and 81.71-98.32% and % RSD were in the range of 9.56-15.58, 5.28-6.30, 6.93-15.91 and 6.31-18.26%, respectively. The LOQ was $0.01 \mu\text{g g}^{-1}$. The QuEChERS protocol without the clean-up step is a promising option to make the method less expensive and faster.

Keywords: Bifenthrin, residue, soil, QuEChERS, gas chromatography

Introduction

Synthetic pyrethroids (SPs) are among the most powerful and effective insecticides for agricultural and indoor use (Singh and Singh, 2008) [19]. SPs are low in mammalian and avian toxicity, and they have a minimal propensity to contaminate ground water. With the phase-out of organophosphate pesticides, there has been a corresponding increase in the usage of SPs, resulting in widespread human exposure to this insecticide family (Superlock and Lee, 2008) [20]. However, most aquatic invertebrates and fish are highly susceptible to SPs, and they have been shown to cause lethality to fish with 96-h LC_{50} well below 1 g L^{-1} , 10-1000 times lower than corresponding values for mammals and birds (Köprücü *et al.*, 2006) [10]. Therefore, offsite movement of these compounds from treated areas to the adjacent water bodies is of great concern and casts doubt on their apparently low environmental impact (Zhao *et al.*, 2009) [23]. Bifenthrin (BF), a third-generation synthetic pyrethroid chemical, characterized by great photostability and insecticidal activity, is used as a miticide in orchards, nurseries and homes. The WHO classifies it as a moderately hazardous pesticide with toxicity class II.

Soil is an important agricultural resource that has the ability to retain agrochemicals. The presence of xenobiotic compounds in soil is caused by a variety of factors, ranging from industrial activities to inappropriate waste disposal to agricultural chemicals. Direct application, inadvertent spillage, runoff from plant surfaces, and assimilation of pesticide by plant components are all possible sources of pesticide chemicals in soils (Rashid *et al.* 2010) [18]. Because agricultural soil is a high-value component, it should be protected from irreversible degradation to ensure its fertility and current and future worth. Soil is a complex and heterogeneous matrix with a porous structure that contains both inorganic and organic components (Pinto *et al.* 2011) [16]. These compounds are characterized by the diverse chemical structure and physicochemical properties, which cause many analytical problems. Therefore, pesticide analysis at low concentration levels in these samples is a very difficult and challenging task.

Nowadays, in pesticide residue analysis, QuEChERS method (ang. *Quick, Easy, Cheap, Effective, Rugged and Safe*), developed by Anastassiades *et al.* (2003) [2], become a very popular technique for different matrix sample preparations such as: cereals (He *et al.* 2015) [8], fruit and vegetables (Lehotay *et al.* 2010) [11], honey (Bargańska *et al.* 2013) [3], tea (Lozano *et al.* 2012) [13] and tobacco (Łozowicka *et al.* 2015) [14], because of its simplicity, low cost,

Corresponding Author:

Ajit Kumar Meena

Maharana Pratap University of
Agriculture & Technology,
Udaipur, Rajasthan, India

amenability to high throughput, and high efficiency with a minimal number of steps. It involves two steps, extraction based on partitioning between an aqueous and an organic layer via salting-out and dispersive SPE for further cleanup using combinations of $MgSO_4$ and different sorbents, such as C_{18} , primary-secondary amine (PSA), or graphitized carbon (GCB) to remove interfering substances (Anastassiades *et al.* 2003) [3].

The QuEChERS methodology was the first time applied to the extraction of pesticides from soils in 2008 by Lesueur *et al.* (2008) [12]. The QuEChERS method has been described to a limited extent for the extraction of wide range of pesticides from soil. An existing knowledge needs to be filled (Vera *et al.* 2013; Bruzzoniti *et al.* 2014) [21, 51] by finding cheaper and faster method for the simultaneous analysis of pesticides

covering a wide range of polarities in complex matrix such as soil that has been carried out. In that study, the authors have applied the QuEChERS for the extraction of bifenthrin by using Gas chromatography (GC) with the electron capture detector (ECD) for residue analysis in different soils.

Material and Methods

A laboratory experiment was conducted at AINP on Pesticide Residues, ICAR Unit- 9, AAU, Anand to study the method validation for analysis of bifenthrin in different type of soils like sandy loam, sandy, medium black calcareous and heavy black soils collected from Anand, Dantiwada, Junagadh and Khandha, respectively, were used in this experiment. These soils were subjected to different physic-chemical properties which are listed in Table 1.

Table 1: The physicochemical properties of soils

Physico-chemical properties	Soil type				Standard analytical methods (Reference)
	Sandy loam	Sandy	Medium black calcareous	Heavy black	
Coarse sand (%)	2.60	51.32	1.20	1.24	International Pipette Method (Piper, 1966) [17]
Fine sand (%)	71.22	33.10	21.23	15.22	
Silt (%)	18.45	11.49	13.35	26.40	
Clay (%)	7.81	4.21	64.17	57.12	
Bulk Density ($g\ cm^{-3}$)	1.42	1.55	1.42	1.36	
WHC (%)	43.64	25.61	44.14	56.51	Measurement using Brass-cup with perforated base
pH(1:2.5soil to water ratio)	7.52	7.43	7.08	7.74	Potentiometer method (Jackson, 1979) [9]
EC at 25 °C ($dS\ m^{-1}$)	0.35	0.23	0.33	0.39	Conductivity meter method (Jackson, 1979) [9]
CEC (me/100 g soil)	23.67	10.81	38.66	40.25	Jackson (1979) [9]
Organic Carbon (%)	0.39	0.27	0.57	0.58	Walkley and Black method (Walkley & Black, 1934) [22]
$CaCO_3$ (%)	1.65	2.82	31.05	3.51	Rapid Titration Method (Jackson, 1979) [9]

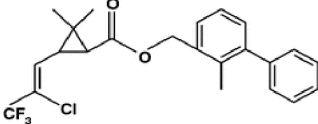
Chemicals and reagents

All the solvents like *n*- hexane, acetone, petroleum spirit, Magnesium sulphate ($MgSO_4$), PSA (Primary Secondary Amine) and toluene were used after distillation. Analytical grade acetonitrile (purity $\geq 99.9\%$) was used as such without distillation.

Certified reference material (CRM)

The Certified reference material (CRM) of bifenthrin (98.58% purity) was procured from Sigma Aldrich India Limited. Bifenthrin is a synthetic pyrethroid insecticide/ acaricide which is classified as a non-cynopyrethroid. It is waxy beige solid with a faint, slightly sweet smell. The elementary properties of bifenthrin are listed in Table 2.

Table 2: Physico- chemical properties of bifenthrin

Common name	Bifenthrin
IUPAC name	2-methyl-3-phenylbenzyl (<i>IRS</i>)-cis-3- [2-chloro-3,3,3-trifluoroprop-1- enyl]-2,2-dimethylcyclo- propanecarboxylate
Molecular weight	422.9 $g\ mol^{-1}$
Specific gravity (at 25 °C)	1.212 $g\ mL^{-1}$
CAS No	82657-04-3
Molecular formula	$C_{23}H_{22}ClF_3O_2$
Molecular structure	
Melting point	68-70 °C
Boiling point	Decomposition at 285 °C before boiling
Relative Density	1.316 $g\ cm^{-3}$ at 24 °C
Water solubility (25 °C)	<1 $\mu g\ L^{-1}$ (pH 4.05) <1 $\mu g\ L^{-1}$ (pH 7.04) 3.76 $\mu g\ L^{-1}$ (pH 9.22)
Octanol water coefficient (K_{ow})	1.0×10^6
Henry's constant (at pH 7, 25 °C)	$7.20 \times 10^{-3} atm. m^3 mol^{-1}$
Hydrolysis half-life (in natural water, at pH 6.7 & 25 °C)	Stable
Anaerobic half- life	97- 156 days
Solubility in other solvents	Bifenthrin is soluble in methylene chloride, acetone, chloroform, ether and toluene. It is slightly soluble in heptane & methanol.
Adsorption coefficient	K_{ow} (octanol/water) = 1,000,000
Vapour pressure ($mm\ Hg^{-1}$ at 25 °C)	1.8×10^{-7}
Vapour pressure	2.431×10^{-5} Pa at 25 °C
Common product name	Talstar®, Bifenthrin®, Brigade®, Capture®
Mode of action	ATPase inhibitor affects the nervous system and causes paralysis in insects.

Primary standard solution

The primary standard solution (100 ppm) was prepared in petroleum spirit. Technical grade pesticide standards (10 mg) were accurately weighed on sartorius basic plus balance (maximum capacity 210 g and sensitivity 0.01 mg). It was then transferred to 100 ml of volumetric flask (A grade). The standard was initially dissolved with 10 to 15 ml of petroleum spirit and final volume made up with petroleum spirit. Details of primary standards are listed in Table 3.

Table 3: Details of primary/stock solution of bifenthrin

Compound	Weight (mg/50 mL)	Purity (%)	Final concentration (mg/l)
Bifenthrin	30.24	100	604.8
		98.58	596.21

Intermediate and final working standard solution

From the primary standard, 5 mL aliquot was diluted to 50 mL with petroleum spirit in volumetric flask. This gave a concentration of 10 µg/mL intermediate standard solution. Suitable aliquots were diluted from intermediate standard to

required final volume using petroleum spirit, to obtain final concentration of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 µg/mL.

Extraction and clean up (QuEChERS method)

Extraction and clean-up was carried out as per method of Caldas *et al.*, (2011) [6] with minor modifications. A representative 10 g soil was taken into a 50 mL centrifuge tube and added 20 mL acetonitrile. After it the mixture of 4 g MgSO₄ + 1 g NaCl was added and shaken vigorously by hand (1 min.) before centrifugation. Thereafter tubes were centrifuged at 3500 rpm for 3 minutes. A 10 mL aliquot was taken into 15 mL centrifuge tubes that contained 1.5 g MgSO₄ and 0.25 g PSA (Primary Secondary Amine), by auto pipette. Following it, centrifugation was done at 2500 rpm for 2 minutes. A 4 mL aliquot was transferred into glass test tube that was completely evaporated on Turbo Vap® LV. Final volume was made to 2 mL in petroleum spirit: acetone (1:1 v/v) and the residues were quantified on Gas Chromatography (GC) equipped with electron capture detector (ECD). Extraction process is showed in Figure 1.

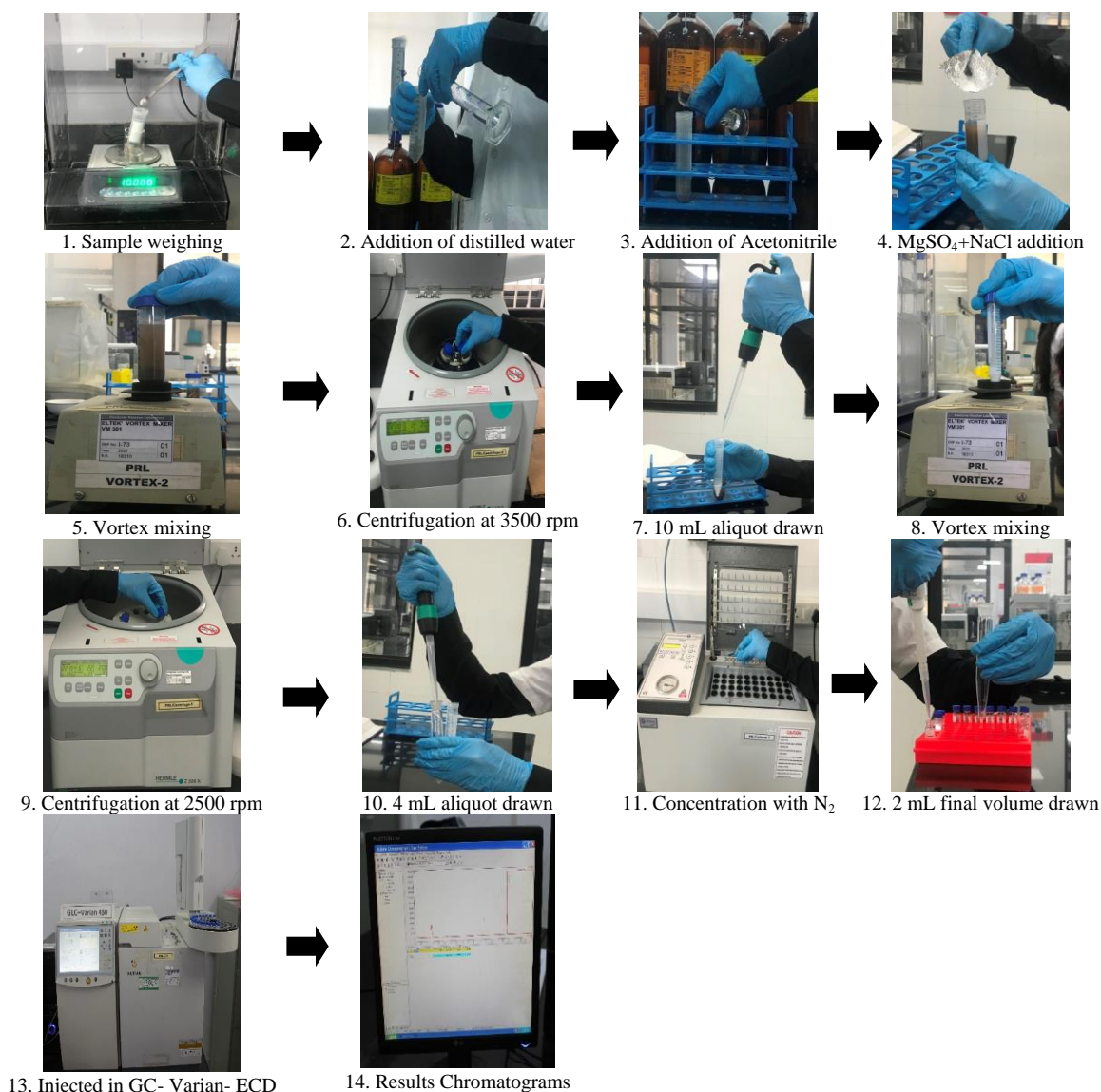


Fig 1: Extraction of bifenthrin from soil GC-ECD

Bifenthrin residues were detected and quantified in the gas chromatography (model: Varian 450 GC) equipped with an

electron capture detector (ECD ⁶³Ni). The DB-5 capillary column (30 m × 0.25 mm × 0.25 µm) was used to resolve the

target compound. It was programmed to maintain the column temperature as follows: It was increased from 160 to 250 °C with a ramp rate of 15 °C/min. Furthermore, it was increased to 280 °C with the ramp rate 10 °C/min, and the column was held at 280 °C for 2 min. The AOC-20s auto sampler and AOC-20i auto injector were used to inject 1 µL of sample in the split mode with a split ratio of 1:10. The temperature of both injector and detector was maintained at 280 °C. The detector temperature was increased to 300 °C during the post-run analysis. Ultra-pure grade N₂ (99.9995%) was used as carrier gas, and the column flow rate of which was maintained at 1 mL/min. The total flow of N₂ was 30 mL/min. The retention time of bifenthrin at the above mentioned operating conditions was 11.3 min. The chromatograms of this study were analyzed in the GC solution software.

Method validation studies of bifenthrin

Validation method of pesticide residues analysis is the process of verifying that the method is fit for the intended purpose. Method validation is an essential requirement of accreditation bodies which is required to be supported by ongoing method performance verification during day-to-day analysis. The method may then be adopted or modified to match the requirements and capabilities of laboratory and/or the purpose for which the method was being used.

Validation of method was performed to analyze the residue of bifenthrin with different in terms of linearity, recovery studies, accuracy, precision, LOQ.

1. Linearity Study

Linearity of an analytical procedure is defined as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Linearity is determined by a series of injections of five or more standards whose concentration span varies from 80- 120 percent of the expected concentration range. The response should be directly proportion to the concentration of the analyte or proportional by means of a well- defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

A linearity study was performed to determine the performance of ECD detector. To work out the linearity, detector response (height/area) vs concentration graph was plotted. To establish the linearity of gas liquid chromatography, seven different concentration of the standards viz., 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm were injected and their response (nA) were recorded. The volume of the standard used for the injection was 1.0 µL. A correlation coefficient and equation was determined by using best fit model of linear relationship.

2. Accuracy and Precision

As per SANTE guidelines (2017), % recovery and % Relative Standards Deviation (RSD) is the indicator of trueness and precision of any analytical method employed for the quantitative estimation of insecticide. The recovery study was carried out from soil before taking up analysis of test sample for each treatment 0.05, 0.25 and 0.5 µg/g levels.

$$\text{Recovery (\%)} = \frac{\text{Recovery Value}}{\text{Fortified Level}} \times 10$$

$$\text{RSD (\%)} = \frac{\text{SD in response}}{\text{Mean Response}} \times 10$$

3. Limit of Quantification (LOQ)

LOQ were determined by using the following formula proposed by Adou *et al.*, (2001) [1].

$$\text{LOD (mg/kg)} = \frac{\text{Cal}_{\min} \times v}{W \times R} \times 1$$

Where,

V is the extract final volume in mL,

W is the sample weight in g,

R is the average recovery with an RSD of no more than 15,

Cal_{min} is the minimum standard concentration detectable by the analytical instrument as a peak having S:N ratio 3.

Prior to quantification of bifenthrin in different soils, the LOQ were worked out. This was carried out by injecting matrix-match bifenthrin in gas liquid chromatography to get signal to noise ratio 1:10 for LOQ.

Results and Discussion

1. Linearity study

To establish the linearity of bifenthrin on Gas Chromatography equipped with electron capture detector, equal volume of seven different concentrations of bifenthrin viz., 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm were injected and their corresponding response were recorded. The volume of the standard used for the injection was 1.0 µL. Linearity of the detector for bifenthrin was calculated (graphically depicted in Figure 2.) by plotting response (area) vs concentration. As per the data obtained in linearity study (Table 4.), it was found linear in the range 0.01 to 1.0 ppm and the R² values obtained from the correlation equation were calculated by adopting positive linear correlation model (Y = a + bX) which was >0.99. The gas chromatogram of standards of bifenthrin at 0.05 ppm is depicted in Figure 3.

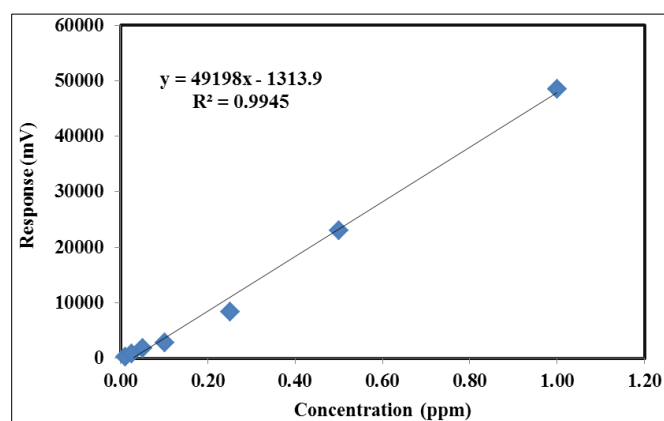


Fig 2: Linearity study of bifenthrin

Table 4: Response of bifenthrin on ECD at different concentrations

Concentration (ppm)	Detector Response (Area) mV
0.01	333.5
0.025	831.1
0.05	1859.7
0.10	2878.5
0.25	8383.7
0.50	23069.6
1.00	47773.8

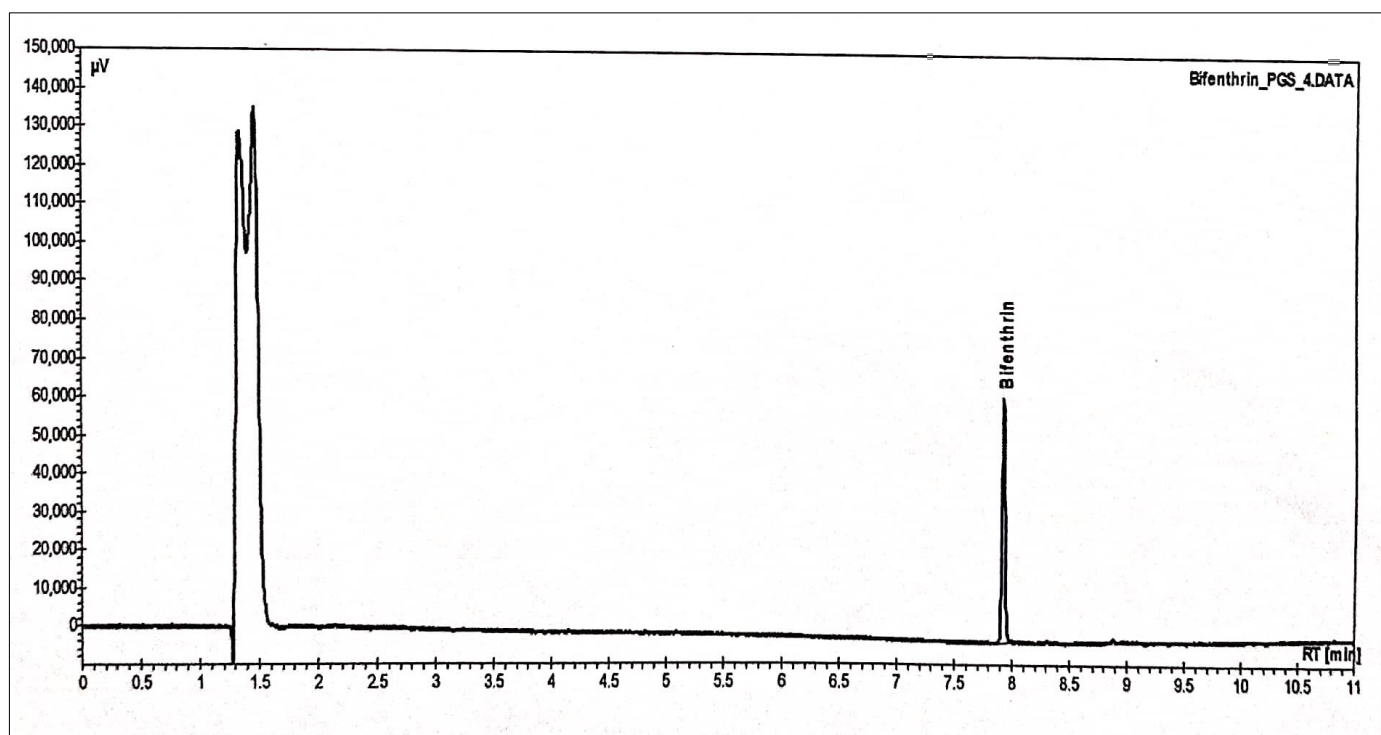


Fig 3: The chromatogram of the bifenthrin standard at 0.05 ppm

2. Precision study

Precision represents random errors of a set of replicate measurements. This is calculated as a (relative) standard deviation of replicate measurement. Less precision is reflected by a large standard deviation. Precision depends on the conditions and therefore can be expressed as repeatability and reproducibility. This has nothing to do with true or reference value. Repeatability is a multiple measurements of a standard by the same analyst over a short period of time. The RSD (%) was worked out as 0.35%, as measure of instrument precision.

Table 5: Response of bifenthrin on ECD at 1.00 ppm

Replicate	R-I	R-II	R-III	R-IV	R-V	Average	SD	% RSD
Area (µV)	6839.5	6880.8	6895.9	6843.1	6861.6	6880.8	24.19	0.35

3. Limit of Quantification (LOQ)

The method quantification limits (LOQ) of the bifenthrin were worked out by injecting the seven different concentrations *viz.*, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm of the standard prepared in control matrix of soil in GLC (ECD) so as to quantify and detect minimum response and to get repeatable and constant peak area. In this case 0.01 ppm gave the peak area more than 10 times the noise in all the matrices which were considered for LOQ.

LOQ for instrument is 0.01 ppm and final soil sample volume being 2 g/2 mL, thus the sample injected in GLC was 1 mg and therefore, LOQ for method worked out to be 0.01 µg g⁻¹. The highest concentration of this range is usually selected as

an acceptable LOQ for pesticide residue analyses in soil (EC, 2019b).

4. Accuracy study

The mean recovery of bifenthrin from sandy loam, sandy, medium black calcareous and heavy black soils were recorded at three spiking levels, *i.e.* 0.05, 0.25 and 0.5 ppm and the mean recovery was in the range of 86.75- 119.03, 96.27-118.30, 82.00- 96.67 and 81.71-98.32%, respectively. The percent relative standard deviation within the replicates (RSD_{WR}) obtained for respective soils at different spiking levels were in the range of 9.56- 15.58, 5.28- 6.30, 6.93-15.91 and 6.31- 18.26%. The gas chromatogram of bifenthrin recovery of sandy loam, sandy, medium black calcareous and heavy black soils at 0.05 ppm are depicted in Figure 4, 5, 6 and 7, respectively. Similar results reported by Łozowicka *et al.*, (2017) [15] that the recoveries for almost all pesticides were satisfactory and ranged from 71 to 120% (RSD 1–17%). In a study which Quechers method was applied for the analysis of pesticide residues (atrazine, fipronil, endosulfan alpha, and endosulfan beta) in water and sediment were detected in the samples with 63% and 116% recoveries (Brondi *et al.* 2011) [4]. The matrix effect (ME) was evaluated comparing the response obtained for each analyte in the soil extract with that given in the solvent at the same concentration in both equipment, extracting the blank to the soil matrix signal. No significant matrix effects were considered when ME was between 80 and 120% (EC, 2019b) [7].

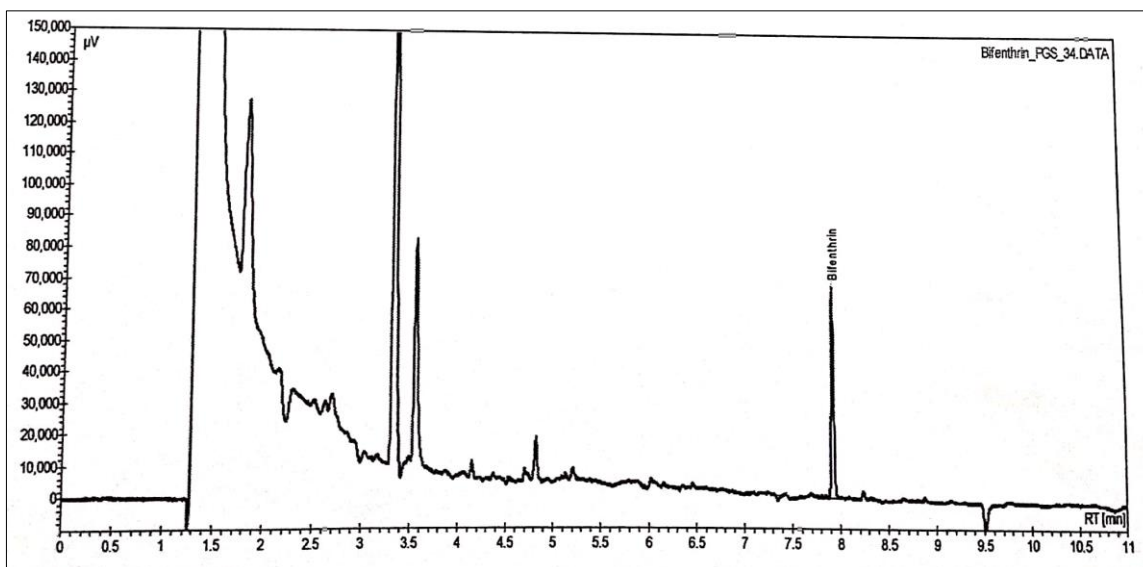


Fig 4: The chromatogram of the bifenthrin recovery in sandy loam soil at 0.05 ppm

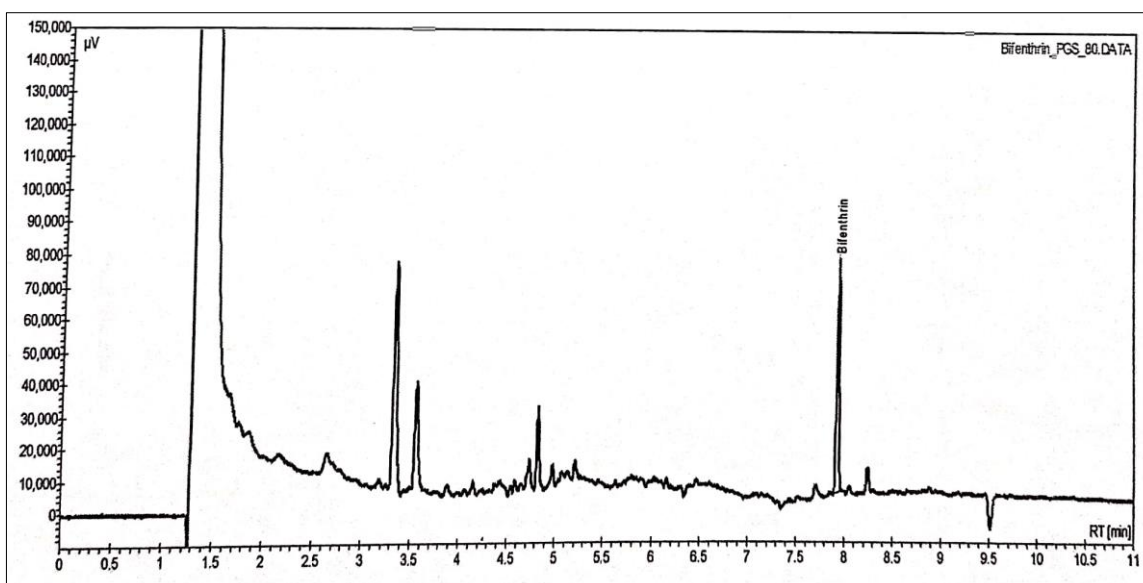


Fig 5: The chromatogram of the bifenthrin recovery in sandy soil at 0.05 ppm

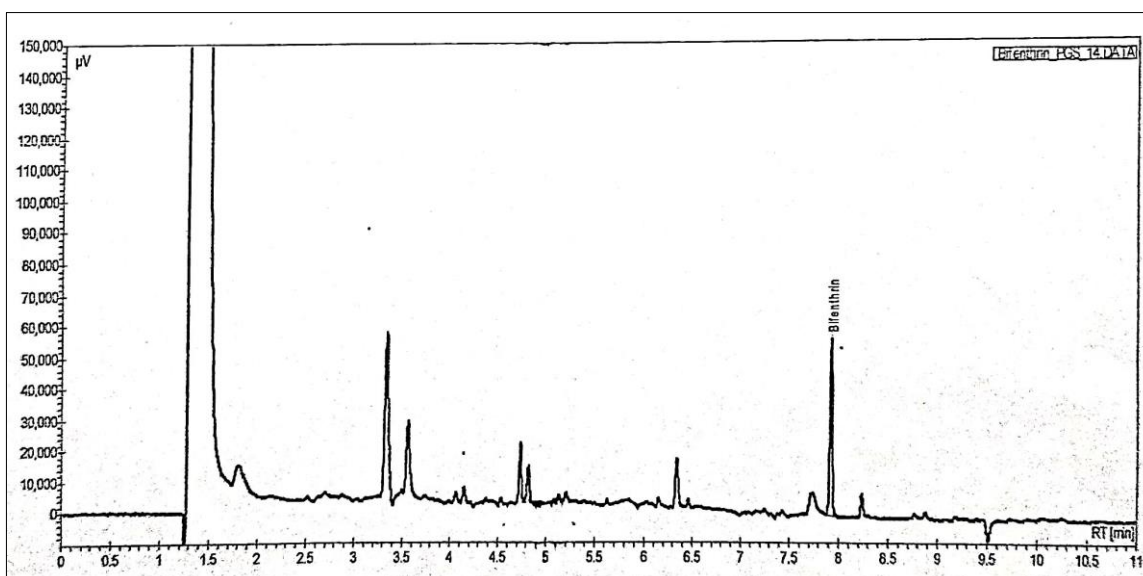


Fig 6: The chromatogram of the bifenthrin recovery in medium black calcareous soil at 0.05 ppm

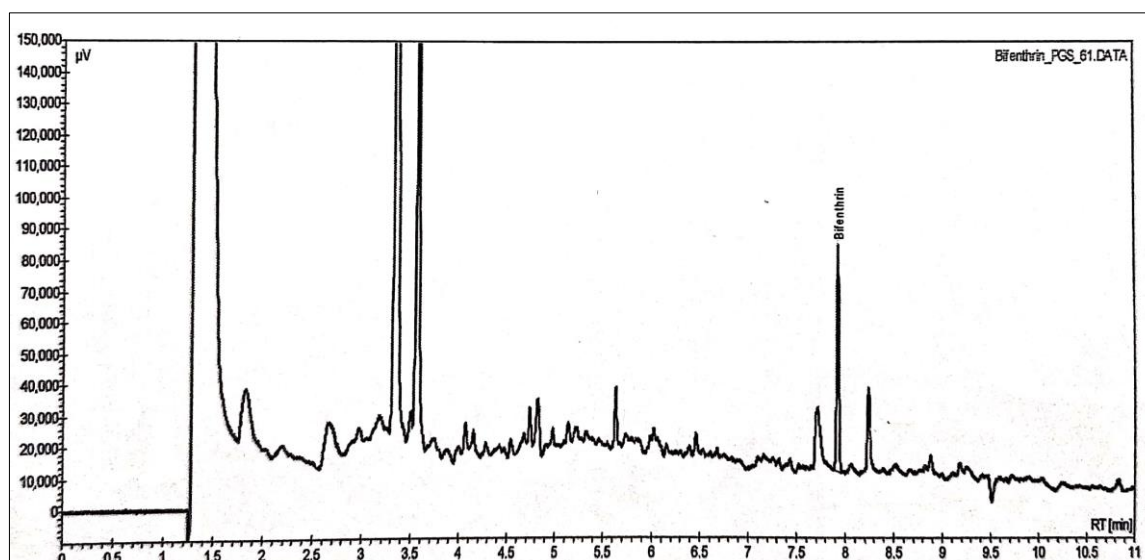


Fig 7: The chromatogram of the bifenthrin recovery in heavy black soil at 0.05 ppm

Conclusions

A one-step QuEChERS-based method has been developed and validated for the extraction of bifenthrin in agricultural soil samples and their analysis by GC–ECD. This makes the method a simpler, faster and cheaper process as the need to use more solvents and purification reagents were avoided. A successful linearity, precision, accuracy was obtained for the selected compounds and the LOQ was well below the typically fixed in soil. The method was tested on various farms soil samples and demonstrated to be well suited for monitoring pesticide residues in this matrix.

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