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Method Validation for Simultaneous Determination of Pyriproxyfen and Fenpropathrin in Okra Matrices

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Extraction and quantification of pesticide residue from the okra matrix at or below the established maximum residue limit (MRL) is a challenging task for both analytical chemists and regulatory institutions to take corrective actions for human health and safety. to develop a simple rapid and less expensive extraction and cleanup method for simultaneous analysis of pyriproxyfen and fenpropathrin residue in okra two methods: QuEChERS and Liquid-Liquid Extraction (LLE) were tested. Both methods produce reliable results, QuEChERS method was chosen for the present research due to its superior efficiency, low cost as well as reduced risk of exposure to solvents in comparison to Liquid-Liquid Partitioning. The residues of both pesticides were confirmed and quantified by hyphenated gas chromatography-tandem mass spectrometer (GC-MS/MS). The effect of spiking concentration, matrix effect (ME), measurement of inter- and intra-assay repeatability, reproducibility of recovery, and trueness of the results were investigated to validate the effectiveness of the method. Limit of determination (LOD) and limit of quantitation (LOQ) for both the analytes were 0.005 and 0.01 mg/kg. The % recovery of both pesticides ranged between 85.9 to 97.9 % with RSD ≤ 7.19 %. The method fulfilled all the SANTE guidelines (SANTE, 2021) and thus can be extended for routine analysis of multiclass pesticide residue in the okra matrix.

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Keywords: Okra; QuEChERS; dispersive solid-phase extraction; GC-MS/MS; pyriproxyfen; fenpropathrin.

1. INTRODUCTION

Pesticides have an important role in sustainable agriculture because they protect crops and commodities against pests and illnesses. Because of their intrinsic toxicity, they are not only destructive to the environment but also represent a risk to human health [1,2]. Several studies in the past have shown pesticide residues in a variety of food items [3]. Vegetables contribute significantly to food security as they're a great source of vitamins and minerals. After the pandemic, increased awareness of the need for good nutrition through high-quality food has caused a spike in vegetable demand. Okra (*Abelmoschus esculentus L. Moench*), often known as ladyfinger, belongs to the Malvaceae family and is a popular vegetable in India. It's most often grown for its immature fruits, which may be eaten raw, dried, fried, or cooked and used in dishes like salads, soups, and stews [4]. The new ready-mix formulation, Sumiprempt containing 5% EC pyriproxyfen (4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether) a pyridine-based juvenile hormone analogue insecticide, and 15% EC fenpropathrin [(RS)-a-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate] has good potential in the management of pests reported in okra [5]. However, the residues, which are left in different amounts in okra fruits after harvesting, are beyond the consumer's control and have a negative impact on human health after continuous long-term exposure. As a result, it becomes logical to monitor the residues of pyriproxyfen and fenpropathrin.

Due to the complexity of okra, its constituents (waxes) will always interfere with the extraction and quantification of pesticide residues. For eliminating interferences, okra samples are extracted and cleaned with materials such as silica, alumina, C-18, Florisil, graphite carbon black (GCB), and dehydrating agents such as sodium sulfate (Na_2SO_4), and magnesium sulfate ($MgSO_4$). In recent years, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique [6] has been increasingly used to extract pesticide residues from food in a fast, efficient, and economical manner with high recovery rates. There have been routine analyses of pesticide residue by gas chromatography (GC), Rahmiani et al., [7], gas chromatography-mass spectrometry (GC-MS) Hepsağ, [8], and liquid chromatography-tandem mass spectrometry (LC-MS/MS) Ngolo et al., [9].

Environmental quality legislation imposes detection limits that cannot be met without using sample preparation methods that enrich analytes while reducing matrix interference. In view of the challenging task of extracting residues from a waxy okra matrix, a method that achieved high sensitivity, accuracy, and repeatability was required. There are some conventional sample preparation methods that allow differential penetration of extraction solvents into the sample matrix, which enhances analyte recovery. In contrast, they require huge volumes of solvents for extraction. The comparison of conventional analytical methods with those that use fewer or no hazardous solvents can serve as a landmark in the field of green analytical chemistry. This study evaluated the efficiency of conventional Liquid-Liquid Extraction (LLE) sample preparation and compared it with QuEChERS (considered a modern approach to sample preparation in green analytical chemistry).These methods are validated through assessment of sensitivity, linearity, limit of determination (LOD), limit of quantitation (LOQ), and accuracy and precision of recoveries. The purpose of this study is to develop and validate a method for the simultaneous detection of pesticide residues in okra matrices that will be sensitive, reliable, and cost-effective. Therefore, we investigated the effect of different sample preparation methods on the extraction efficiency and percent recovery of pesticide residues from okra matrix in the present study.

2. MATERIALS METHODS

2.1 Chemical and Reagents

Formulation under trade name Sumipremt (pyriproxyfen 5% EC + fenpropathrin 15% EC) was purchased from a local retailer while the certified reference materials of Pyriproxyfen (CAS No.- 95737-68-1) and Fenpropathrin (CAS No.- 39515-41-8) with a purity of 99.8% and 99.2% respectively, were acquired from Sigma Aldrich, Pvt, Limited. All the analytical organic solvents and reagents such as acetonitrile, acetone, sodium chloride, magnesium sulphate, and anhydrous sodium sulphate, were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA) was supplied by Agilent

Technologies Private Limited, Bangalore, India. Each of the chemicals used for the analysis was first subjected to glass distillation and then ran as reagent blank.

2.2 Sample Preparation

Using LLE, a representative 15 g okra sample was mixed with 100 mL of acetone in conical flasks and mechanically shaken for an hour. The extracted sample was filtered through a nylon filter of 0.22 mm in separate reagent bottles and concentrated to a volume of 10 mL using a rotary evaporator. The samples were cleaned by liquid-liquid partitioning with 600 mL of 10% NaCl brine solution, followed by vigorous shaking for 1 min with dichloromethane (DCM) and hexane (100, 50, 50 mL) thrice to remove non-emulsifying contaminants. After passing through anhydrous Na_2SO_4, the organic layer was collected to ensure that all moisture had been removed. The extracts were concentrated and reconstituted with 3 mL n-hexane for analysis.

In modern and green analytical chemistry, QuEChERS is considered to be a simple, flexible, and highly sensitive sample preparation method. Okra samples were processed using the QuEChERS method. A representative sample of 15 g macerated okra fruits was combined with 30 mL acetonitrile and homogenized using a low-volume homogenizer (Heidolph) for 3-4 minutes at 14,000 rpm. To separate the water (okra) and acetonitrile phases of the aforementioned representative sample, 3.3 g of sodium chloride (NaCl) is added to the extract and vortexed for 2 minutes. Following the 3 min centrifugation of the extract at 2500 rpm, the upper 18 mL acetonitrile layer was deposited over sodium sulphate to eliminate any remaining moisture traces. The dispersive solid phase extraction (d-SPE) technique was used for the cleanup of the extract with primary secondary amine (PSA) 0.4 g and 1.15 g magnesium sulphate ($MgSO_4$) as adsorbent. Then, the extract was recomposed to a volume of 3 mL in n-hexane and filtered through a 0.2-micron filter before GC–MS/MS analysis.

2.3 Instrumentation for Analysis

Pesticide analytes in samples were determined by GC-MS/MS (Shimadzu GC-MS TQ 8040) equipped with a capillary column (SH-Rxi-Sil MS column of 0.25 µm thick film having 30 m length and 0.25 mm internal diameter) using helium gas as the carrier gas at a constant flow rate of 1.5 mL min^{-1}. Samples were injected (1 µl) with an autosampler (20iAOC) in splitless injection mode. The temperature of the injection port was 250°C and programming of the oven temperature was done to optimize the working conditions. The oven temperature programming began from 80ºC and remained at this temperature for 2 min, then start to increase up to 180 °C at 20 °C/min ramp rate and attain the temperature of 300 °C, at the rate of 5 °C/min and remains for 10 min. Pesticide residues could be confirmed and quantified by using GC-MS/MS in Multiple Reaction Monitoring (MRM) with an ESI(+) source of ionization throughout a scanning mass range of 40-1000 m/z. Peaks in the total ion chromatogram of the sample recorded in MRM mode were detected based on their particular retention time (RT) and their characteristic ion peaks in the mass chromatogram. The analysis was carried out in a completely air-conditioned laboratory with a temperature of less than 22°C and a relative humidity of less than 60%.

2.4 Method Validation Parameters

2.4.1 Linearity test

To validate the linearity of the data, 8-point calibration curves for both insecticides were developed. For the construction of the calibration curve, each of the aforementioned dilutions of Pyriproxyfen and Fenpropathrin (0.005-1 mg/L, n=3) was chromatographed by injecting 1 µ l under the conditions specified above, and the average peak areas were computed. The regression equations for both kinds of insecticide were constructed by graphing the mean peak area of three observations of each concentration against the corresponding concentration. Through the usage of the regression equations, it was feasible to obtain the values of R^2 for both of the insecticides. The analytical calibration curve was considered acceptable when R^2 was at least 0.99. Using the validated calibration curve, we determined the LOD and LOQ.

2.4.2 Determination of Limit of detection (LOD) and limit of quantification (LOQ)

Each insecticide's LOD and LOQ were adjusted to their lowest concentrations, which produced peaks in the chromatogram that were three and ten times more intense as compared to the noise in the chromatogram, respectively [10].

2.4.3 Recovery experiments

Recovery tests were carried out by spiking the okra fruits with varied fortification levels of

Pyriproxyfen and Fenpropathrin to evaluate the validity of the analytical methods employed to analyze the pesticide traces in the product. The following equation (1) determines a sample's fortification level:

$$\text{Fortification level} = \frac{\text{Std (ppm) x Amount to be added}}{\text{weight of the sample}} \quad (1)$$

Okra fruit samples were spiked at 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/L, respectively. Following spiking, these samples were processed to check recovery performance using QuEChERS and LLE.

2.4.4 Accuracy

According to the guidelines of the European Commission, 2021, the criteria for evaluating the reliability of a procedure is average recoveries must be in the range of 70-120% with relative standard deviation less than 20%. Consequently, data for the % average recovery and relative standard deviations were calculated and compared with the accuracy requirements of a method for both insecticides at a range of six different fortification levels: 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/kg (n=3).

2.4.5 Precision

The precision of the method was determined in two stages: intra-day assay (repeatability) and inter-day assay (within lab reproducibility). The degree of precision was measured in terms of the percent relative standard deviations from recovery trials conducted at six different fortification levels: 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/kg with three repetitions on the same day and three different days.

Repeatability (intra-day assay): To investigate the repeatability of the procedure, the analysis of the sample for recovery was carried out five times in a single day, and the values of the relative standard deviations (RSD_r) were computed for each replication of the single fortification level.

Reproducibility (inter-day assay): To evaluate procedure reproducibility, recovery trials were done on three separate days (n=3), and the relative standard deviation was determined for each replication. For the repeatability within laboratory, relative standard deviation (RSD_R) the standard deviations at each fortification level were pooled together using equation (2):

$$\text{Standard Deviation} = \sqrt{\frac{(V_1 x\, D_1 + V_2 x\, D_2 + V_3 x\, D_3)}{D_1 + D_2 + D_3}} \quad (2)$$

Where, V_1 is the variance obtained on day 1, V_2 on day 2, V_3 on day 3, and D_1, D_2, D_3 are the number of degrees of freedom on each measurement days:1, 2, 3, respectively.

2.4.6 Selectivity

The selectivity of the method was evaluated by determining the presence or absence of any interfering peaks at the retention time of each insecticide and by procuring two MS/MS transitions for each analyte through the appropriate selection of precursor and product ions. This made it possible to make an educated guess on the selectivity of the method.

2.4.7 Robustness

The robustness of a method was tested by making modest adjustments in mobile phase composition, detecting wavelength, and mobile phase flow rate.

From the above information on linearity, accuracy, precision, selectivity, and robustness, we were able to assess the effectiveness of each of the aforementioned procedures and pick the one that turned out to be the most promising for further experimental study.

3. RESULTS AND DISCUSSION

The method was validated by employing the performance parameters of % mean recovery in relation to linearity, selectivity, accuracy, and precision of intra- and inter-assay analysis in spiked okra and soil samples.

The method's quantification potential was evaluated using a linearity test, and the resultant coefficient of determination (R^2) demonstrated good linearity (0.999 and 0.999) between concentrations of Pyriproxyfen and Fenpropathrin and peak area over the calibration range of 0.005 to 1.00 mg/L (Fig. 1). The chromatographic behaviour of Pyriproxyfen and Fenpropathrin in the GC-MS/MS has been depicted in Fig. 2. The peaks for Pyriproxyfen and Fenpropathrin were detected at R_t (retention time) values of 21.8, and 20.3 minutes, respectively. Using an ESI+ source for ionisation,

scans were performed in a positive ion mode, yielding a fragmentation pattern for Pyriproxyfen with m/z 226, 136, 96, 78 and Fenpropathrin with m/z 265, 210, 172, 89 (Table 1). The LOQ and LOD were found to be 0.01 mg/kg and 0.005 mg/kg, respectively, which were in agreement with the values intended by Ahlawat et al. (2017) and fulfilled the requirement of European Union, EU protocols [11]. Similar operational conditions were found for Pyriproxyfen by Schenck et al., [12], who employed GC-MS/MS in MRM modes with ESI+ ionisation source to generate a fragmentation pattern for the analyte with m/z 226.109 and 136 for confirmation of Pyriproxyfen. Cervera et al., [10] also used GC-MS/MS to validate Pyriproxyfen by finding ions with m/z values of 136 and 226. Nasiri et al., [13] found very comparable conditions for confirmation and quantification of Fenpropathrin, with m/z 210 and 172. Considering the well-defined peaks (responses) of Pyriproxyfen and Fenpropathrin, GC-MS/MS was considered to be suitable for use in the present research.

Table 1. Multiple reaction monitoring (MRM) table showing retention time and m/z

Insecticide	m/z	Retention time (R_t)
Pyriproxyfen	226 > 136 > 96 > 78	21.8
Fenpropathrin	265 > 210 > 172 > 89	20.3

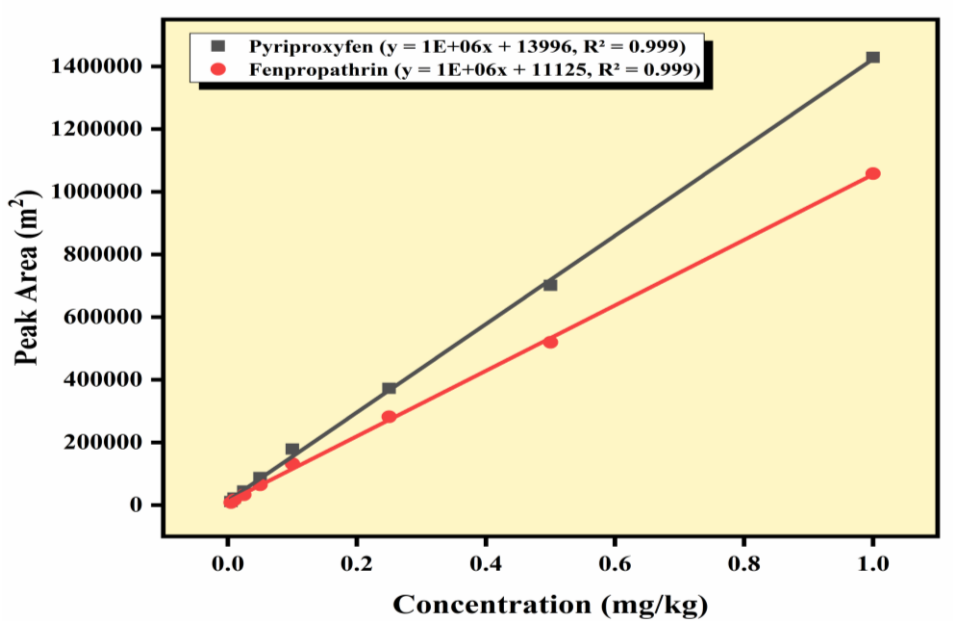

Fig. 1. Standard curve of pyriproxyfen and fenpropathrin on GC-MS/MS

Table 2. Pyriproxyfen and fenpropapthrin recovered from spiked okra samples processed on the same day by the QuEChERS

Fortification level (mg/kg)	Pyriproxyfen		Fenpropathrin	
	Average Recoveries* ±SD (%)	RSD_r (%)	Average Recoveries* ±SD (%)	RSD_r (%)
0.50	87.7±4.46	5.08	85.9± 5.19	6.04
0.25	90.2±3.59	3.98	87.2±3.31	3.80
0.10	89.4±3.07	3.43	86.9±4.94	5.68
0.05	91.3±3.93	4.30	90.3±2.98	3.30
0.025	92.2±4.05	4.39	88.8±4.96	5.59
0.01	88.5±3.12	3.52	92.1±4.41	4.79

** Average of three replicates*
SD= Standard deviation
RSD_r = Relative Standard Deviation for Repeatability

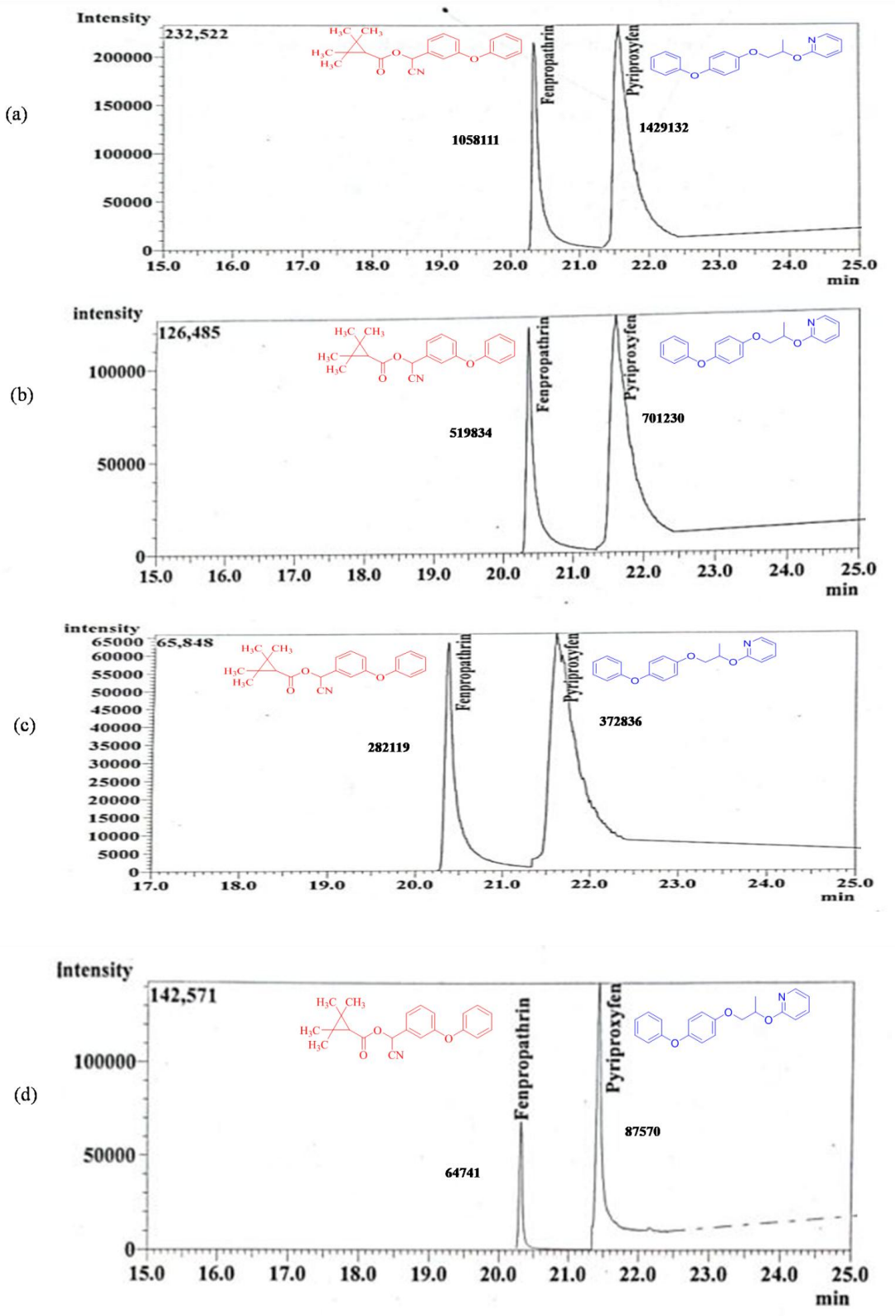

Fig. 2. Chromatogram of standard of Pyriproxyfen and Fenpropathrin on GC-MS/MS at (a) 1 mg/L (b) 0.5 mg/L (c) 0.25 mg/L (d) 0.05 mg/L

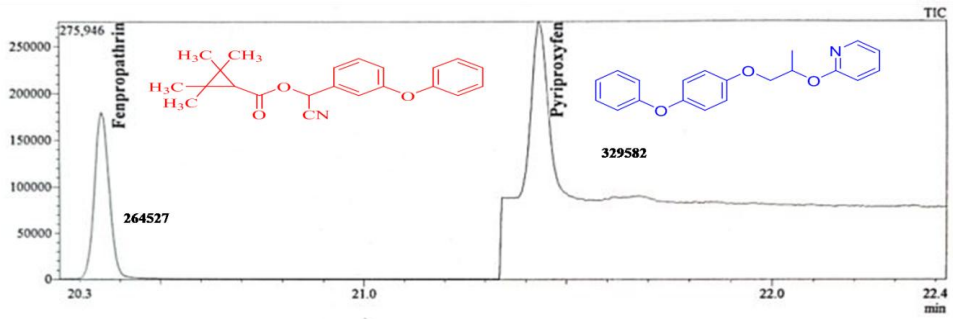

Fig. 3. Chromatograms of fortified okra samples at 0.25 mg/L processed by the QuEChERS method

Validation of the quantitative determination of Pyriproxyfen and Fenpropathrin in okra fruits was carried out following the recommendations for bioanalytical methods outlined in the SANTE guidelines [14]. To ensure the accuracy (reliability), repeatability (intra-day precision), and reproducibility (inter-day precision) of the procedures to be used in the examination of the various test samples, recovery experiments were conducted. The matrices of okra fruit were fortified with Pyriproxyfen and Fenpropathrin insecticides at six different fortification or spiking levels: 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/kg, and then processed on the same day so that the repeatability (intra-day assay) could be determined. For the determination of methods' reproducibility, recovery experiments were conducted over three days (inter-day assay), with fortified okra fruit matrices and soil at six different fortification or spiking levels (0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 mg/kg). The okra fruit matrices that were fortified were processed using two separate procedures, Liquid-Liquid Partitioning, and QuEChERS, followed by cleanup to evaluate the residues and effectiveness of the methods utilized, and the resulting extract was analyzed for pesticide residue analysis. The recovery chromatograms of Pyriproxyfen and Fenpropathrin are represented in Fig. 3. While both methods produce reliable results with significant differences between the recoveries ($p = 0.05$), the QuEChERS method was chosen for present research due to its superior efficiency, low cost as well as reduced risk of exposure to solvents in comparison to Liquid-Liquid Partitioning. Mean Recoveries for both insecticides at spiking levels of 0.5-0.01 mg/kg (n=3) for the QuEChERS method in okra samples ranging from 85.9 to 97.9 % with RSD ≤ 7.19% demonstrate the accuracy of the method by meeting the European Commission, 2002 guidelines for evaluating the accuracy of a procedure. Comparable results were obtained by Cervera et al., [10] who performed the recovery experiments using orange, spinach, and nectarine spiked with Pyriproxyfen @ 0.01 mg/kg and 0.05 mg/kg. The observed recoveries for Pyriproxyfen were in the range of 111-115%, 91-108%, and 90-104% with RSD below 20% for orange, spinach, and nectarine, respectively. Hepsağ, [8] also validated the QuEChERS method using GC-MS/MS for Pyriproxyfen and Fenpropathrin by fortifying cucumber and grapefruits @ 0.002 mg/kg and 0.001 mg/kg. The average recoveries for Pyriproxyfen range from 93.2 to 96 % in cucumber, 82.9 to 87.8% in grapes whereas for Fenpropathrin recoveries varies 85.4 to 92.8% in cucumber, 84.9 to 89.4% at two different fortification levels with RSD below 20%.

The precision of the method was determined in two stages: Intra-day assay (repeatability) and inter-day assay (within lab reproducibility) as provided in Tables 2, 3. The recovery for intra-day assays of okra matrices processed by the QuEChERS method ranged from 87.7-92.2%, 85.9-92.1% with $\%RSD_r$ in the range of 3.43-5.08%, 3.30-6.04% for Pyriproxyfen and Fenpropathrin, respectively (Table 2). The recovery for inter-day assays of okra matrices for Pyriproxyfen and Fenpropathrin ranged from 86.3-91.4%, 86.9-93.3% with $\%RSD_R$ in the range of 4.74-6.20%, 4.68-7.19%, respectively (Table 3). Similar findings were represented by Farouk et al., [15] who performed recovery experiments for Pyriproxyfen residues in Egyptian tomatoes. The recoveries ranged from 86.03 to 94.55 % for Pyriproxyfen with $\%RSD_s$ below 20% at fortification levels of 1, 3, and 5.6 mg/kg. Rahmiani et al. [7] also validated a method for the determination of the pesticides Fenpropathrin in potatoes and tomatoes with average recoveries ranging from 93to 102% for

analytes in the two samples with a relative standard deviation below 7% at 5, 20, 30 ppb spiking levels. Our findings are also supported by Payá et al., [16] who conducted recovery experiments to analyse the residues of Pyriproxyfen in fresh and canned peaches using HPLC-DAD. The average recoveries of Pyriproxyfen from the fortified samples ranged from 83.52-89.62% at two different spiking levels of 0.05 and 1.0 mg/kg [17,18].

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Table 3. Pyriproxyfen and fenpropapthrin recovered from spiked okra samples processed on three different days by the QuEChERS

Fortification level (mg/kg)	Day	Pyriproxyfen		Fenpropathrin	
		Average Recoveries* ±SD (%)	RSD_R (%)	Average Recoveries* ±SD (%)	RSD_R (%)
0.50	1	88.8±4.99	4.74	87.4± 5.29	4.68
	2	88.3±5.13		86.7±4.05	
	3	87.4±4.02		88.1±4.63	
0.25	1	89.9±6.10	5.57	92.1±5.56	5.99
	2	90.5±4.72		91.5±6.63	
	3	89.1±5.79		90.8±5.74	
0.10	1	90.9±5.56		90.7±7.84	7.19
	2	89.5±4.44	5.41	91.4±6.54	
	3	90.7±6.09		90.1±7.12	
0.05	1	91.4±3.50	5.17	90.7± 3.99	5.58
	2	89.5±6.47		93.3±5.15	
	3	90.7±5.12		91.5±7.13	
0.025	1	88.9±7.56	6.20	92.6±3.84	5.28
	2	87.5±3.08		93.3±7.54	
	3	91.4±6.99		89.4±3.45	
0.01	1	86.3±6.33	5.22	88.7± 7.10	5.56
	2	90.5±4.78		91.7±5.45	
	3	90.2±4.34		92.2±3.56	

** Average of three replicates*
SD= Standard deviation
RSD_R = Relative Standard Deviation for Reproducibility

Table 4. Pyriproxyfen and fenpropapthrin recovered from spiked okra samples processed on the same day by the Liquid-Liquid Extraction (LLE)

Fortification level (mg/kg)	Pyriproxyfen		Fenpropathrin	
	Average Recoveries* ±SD (%)	RSD_r (%)	Average Recoveries* ±SD (%)	RSD_r (%)
0.50	82.1±4.01	4.88	81.5± 5.09	6.25
0.25	85.7±3.19	3.72	83.7±4.31	5.15
0.10	83.4±3.70	4.44	80.9±4.04	4.99
0.05	81.2±3.39	4.17	84.3±1.98	2.35
0.025	84.2±4.52	5.37	83.6±3.06	3.66
0.01	86.5±3.22	3.72	82.9±5.41	6.53

** Average of three replicates*
SD= Standard deviation
RSD_r = Relative Standard Deviation for Repeatability

Table 5. Pyriproxyfen and fenpropapthrin recovered from spiked okra samples processed on three different days by the Liquid-Liquid Extraction (LLE)

Fortification level (mg/kg)	Day	Pyriproxyfen		Fenpropathrin	
		Average Recoveries* ±SD (%)	RSD_R (%)	Average Recoveries* ±SD (%)	RSD_R (%)
0.50	1	81.4±5.23	4.05	82.4± 5.67	4.00
	2	83.9±2.89		81.7±3.15	
	3	82.1±3.67		85.1±2.45	
0.25	1	80.9±7.45	6.04	82.3±2.38	5.29
	2	78.5±6.21		83.7±5.29	
	3	83.4±3.90		81.9±7.10	
0.10	1	83.5±4.09	4.33	82.1±8.45	6.99
	2	84.3±5.46		85.4±7.67	
	3	80.1±3.11		83.3±4.02	
0.05	1	82.7±6.50	5.15	81.6± 4.55	4.72
	2	80.6±5.77		84.2±6.34	
	3	79.0±2.02		83.1±2.45	
0.025	1	81.2±3.67	5.31	83.3±3.35	6.48
	2	83.7±7.09		84.9±7.48	
	3	82.8±4.56		81.9±7.67	
0.01	1	81.3±6.11	4.67	81.7± 7.10	5.56
	2	80.5±4.08		82.7±5.45	
	3	82.2±4.12		80.2±3.56	

** Average of three replicates*
SD= Standard deviation
RSD_R = Relative Standard Deviation for Reproducibility

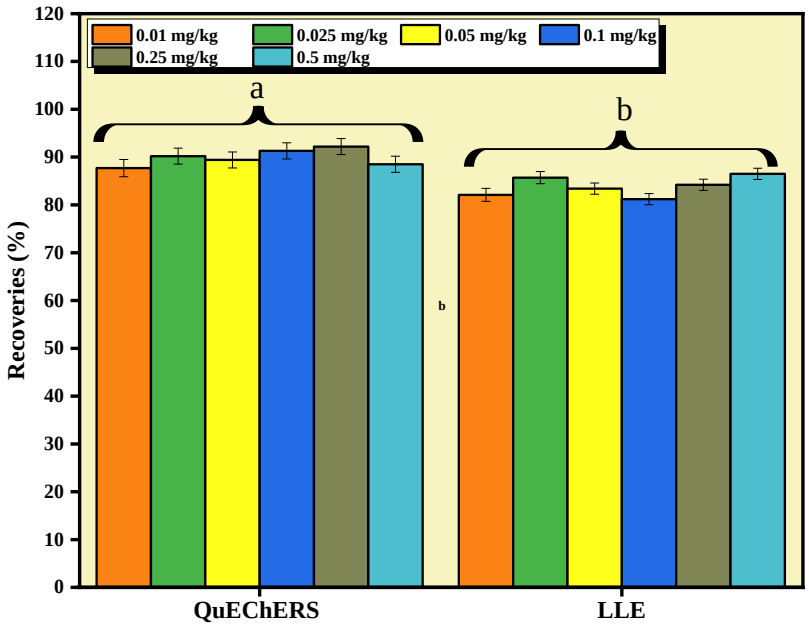

Fig. 4. Percent recoveries of pyriproxyfen from okra matrices using QuEChERS, and LLE at six fortification levels

(Alphabets a, and b represent significant relationship between recoveries obtained in different methods; Error bars represent ± standard deviation)

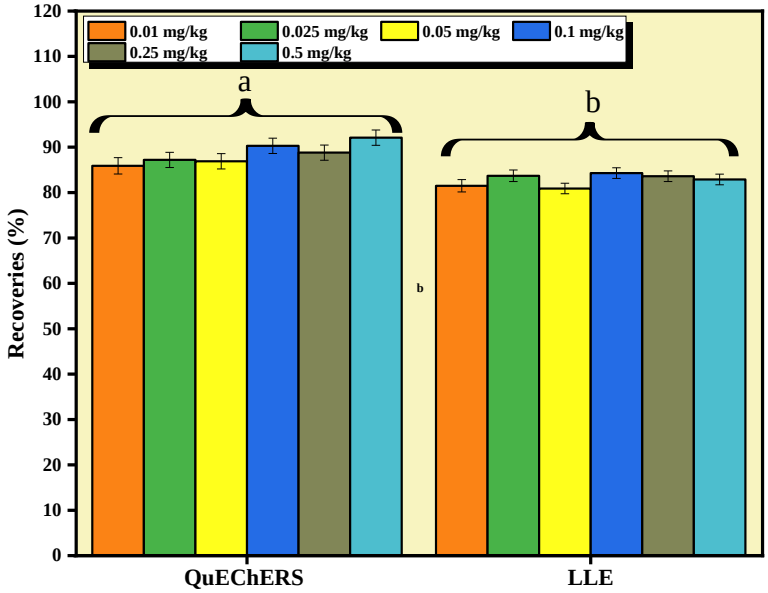

Fig. 5. Percent recoveries of Fenpropathrin from okra matrices using QuEChERS, and LLE at six fortification levels

(Alphabets a, and b represent the significant relationship between recoveries obtained in different methods; Error bars represent ± standard deviation)

The selectivity was assessed by comparing the blank okra or soil sample with the working mix standard for peak interference. There were no interfering peaks at the retention time of each insecticide in the chromatogram of the fortified okra and soil matrices (Fig. 3). This indicated that the optimised method was selective. The robustness of the method was also studied by performing the same analysis with a small change in chromatographic conditions i.e. temperature of column and injector, the flow rate of mobile phase, relative humidity, etc. Due to these changes, the variations in the GC – MS/MS analysis was ≤1.67 (less than 5%, according to the European Commission, 2021) indicating the robustness of the method. The results of linearity, accuracy, precision, selectivity, and robustness of our experiments complied with SANTE (SANTE, 2021), and European Commission [11] recommendations with mean recoveries falling in the 70–120% with less than 20% RSDs. Thus, the QuEChERS method was used to process the test samples, and residue analysis of these processed samples was done using the optimized GC-MS/MS conditions.

4. CONCLUSION

In this study, QuEChERS-based extraction cleanup followed by GC-MS/MS analysis for simultaneous detection and quantification of pyriproxyfen and fenpropathrin pesticide residues in okra samples has been developed. The method demonstrated acceptable inter- and intra-assay recovery at LOQ, good repeatability and within-lab reproducibility, and met all the SANTE guidelines of method validation. Besides, it is simple, less expensive, takes less time for analysis, and used minimal solvents, chemicals, and lab-wares.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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