

Original Research Article

Method validation for simultaneous determination of pyriproxyfen and fenpropathrin in okra matrices

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 the regulatory institutions to take corrective actions for the human health and safety. to develop a simple rapid and less expensive extraction and cleanup method for simultaneous analysis of multiclass pyriproxyfen and fenpropathrin residue in okra matrixes two methods: QuEChERS and Liquid-Liquid Extraction (LLE) were tested. 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. The residues of both the pesticides were confirmed and quantified by hyphenated gas chromatography- tandem mass

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spectrometer (GC-MS/MS). 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 the pesticides ranged between 85.9 to 97.9 % with RSD \leq 7.19 %. The method fulfilled all the SANTE guidelines (Ref ?) and thus can be extended for routine analysis of multiclass pesticide residue in okra matrix.

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 agricultural 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 (Karami-Mohajeri and Abdollahi, 2011), (Zaganas et al., 2013). Several studies in the past have shown pesticide residues in a variety of food items (Gerage et al., 2017). 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 (Cycoń et al., 2014). 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 (Mankar et al., 2019). 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 (Na₂SO₄), and magnesium sulfate (MgSO₄). In recent years, the QuEChERS (Quick, Easy, Cheap, Effective,

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Rugged, and Safe) technique (Anastassiades et al., 2003) 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) (Elbashir et al., 2013), gas chromatography-mass spectrometry (GC-MS) (Elbashir et al., 2013), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Du et al., 2013). 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 multiclass pesticide residues in okra matrixes 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. The results of this study demonstrate that the developed method is able to effectively detect multiclass pesticide residues in okra matrixes. **The method is highly sensitive and can be used as a reliable alternative for pesticide residue analysis.**

2. Material methods

2.1. Chemical and reagents

Formulation under trade name Sumipremt (pyriproxyfen 5% EC + fenprothrin 15% EC) was purchased from a local retailer while the certified reference materials of Pyriproxyfen (CAS No.- 95737-68-1) and Fenprothrin (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

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).

Using LLE, a representative 15 g soil 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 ~~three~~ vigorous shaking for 1 min with dichloromethane (DCM) and hexane (100, 50, 50 mL) ~~thrice~~ to remove non-emulsifying contaminants. After passing through anhydrous Na₂SO₄, 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.

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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 (MgSO₄) 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.

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How the volume was recomposed to 3 ml, while 18 ml was taken for dSPE clean-up?

Comment [SA6]: Why n- hexane is used instead of MS grade hexane here?

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. 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 80 °C and remained at this temperature for 2 min, then start to increasing up to 180 °C at 20 °C/min ramp rate and attain the temperature of 300 °C, at 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 a 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 Fenprothrin (0.005- to 1 mg/L, n=3) was chromatographed by injecting 1µl under the conditions specified above, and the average peak areas were computed. The regression equations for both kinds of insecticides 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 R² for both of the insecticides. The analytical calibration curve was considered acceptable when R² was at least 0.99. Using the validated calibration curve, we determined the LOD and LOQ.

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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 (Cervera *et al.*, 2010).

2.4.3. Recovery experiments

Recovery tests were carried out by spiking the okra fruits with varied fortification levels of Pyriproxyfen and Fenprothrin to evaluate the validity of the analytical methods employed to analyze the pesticide traces in the product. The following equation (3) determines a sample's fortification level:

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

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.

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2.4.4. Accuracy

According to the guidelines of European Commission (2002), the criteria for evaluating the reliability of a procedure is values of % average recoveries must be in the range of 70-120% with the values of 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.

2.4.5.1. 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.

2.4.5.2. 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 (6):

$$\text{Standard Deviation} = \sqrt{\frac{(V_1 \times D_1 + V_2 \times D_2 + V_3 \times D_3)}{D_1 + D_2 + D_3}} \quad (6)$$

Where, V₁ is the variance obtained on day 1, V₂ on day 2, V₃ on day 3, and D₁, D₂, D₃ 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 Fenprothrin and peak area over the calibration range of 0.005 to 1.00 mg/L (Fig. 1). The chromatographic behaviour of Pyriproxyfen and Fenprothrin in the GC-MS/MS has been depicted in Fig. 2. The peaks for Pyriproxyfen and Fenprothrin were detected at R_t (retention time) values of 21.8, and 20.3 minutes, respectively. in the chromatogram obtained from the GC-MS/MS in MRM modes with a mass range of 40-1000 m/z. 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 Fenprothrin 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 (European Commission, 2002). Similar operational conditions were found for Pyriproxyfen by Schenck *et al.*, (2008), who employed GC-MS/MS in MRM modes with ESI+ ionisation source to generate a fragmentation pattern for the analyte with ions of m/z 226.109 and 136 of the ions for confirmation of Pyriproxyfen. Cervera *et al.*(2010) also used GC-MS/MS to validate Pyriproxyfen by finding ions with m/z values of 136 and 226. Nasiri *et al.* (2016) found very comparable conditions for confirmation and quantification of Fenprothrin, with m/z 210 and 172. Considering the well-defined peaks (responses) of Pyriproxyfen and Fenprothrin, GC-MS/MS was considered to be suitable for use in the present research.

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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 |
| Fenprothrin | 265 > 210 > 172 > 89 | 20.3 |

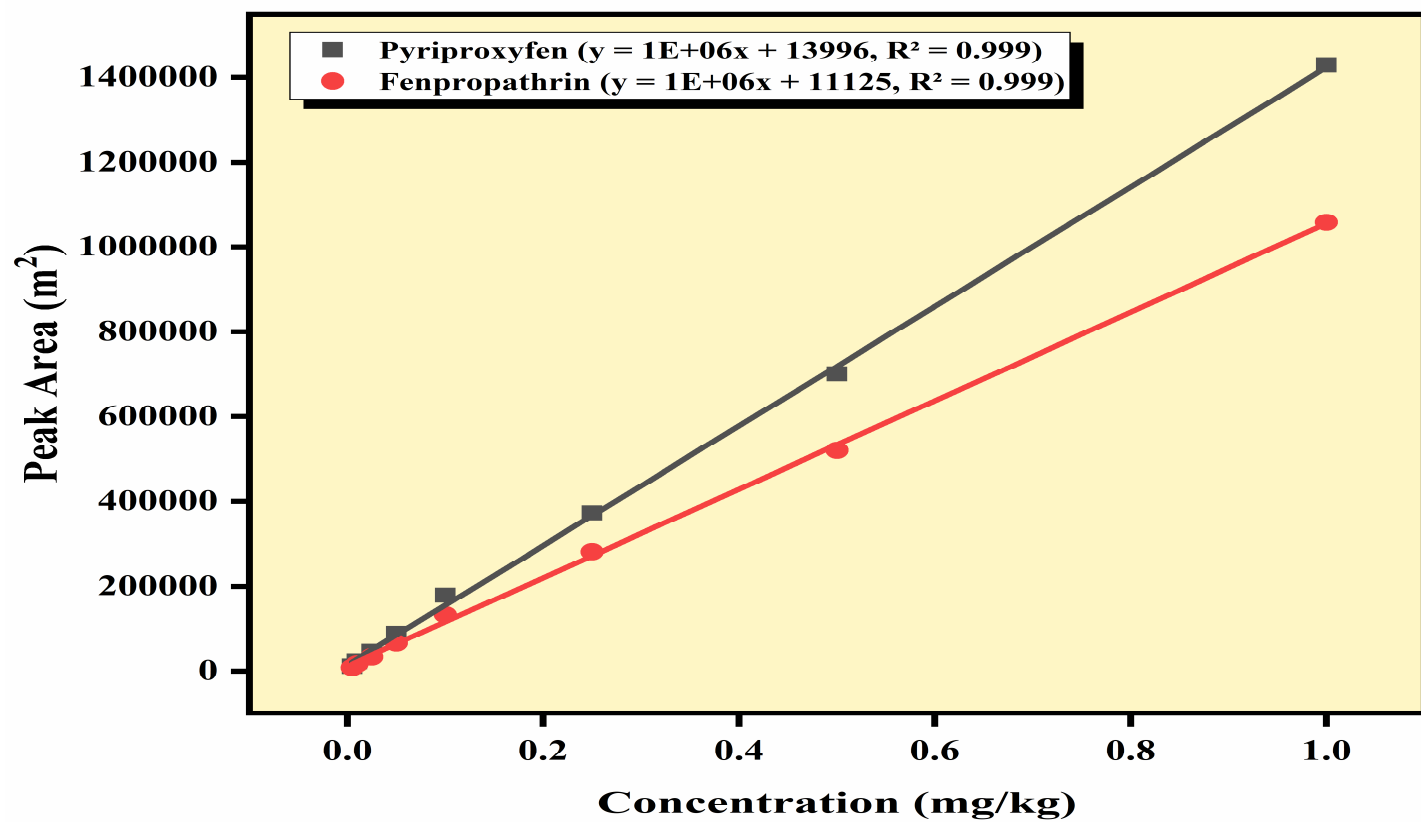


Fig. 1: Standard curve of Pyriproxyfen and Fenpropathrin on GC-MS/MS

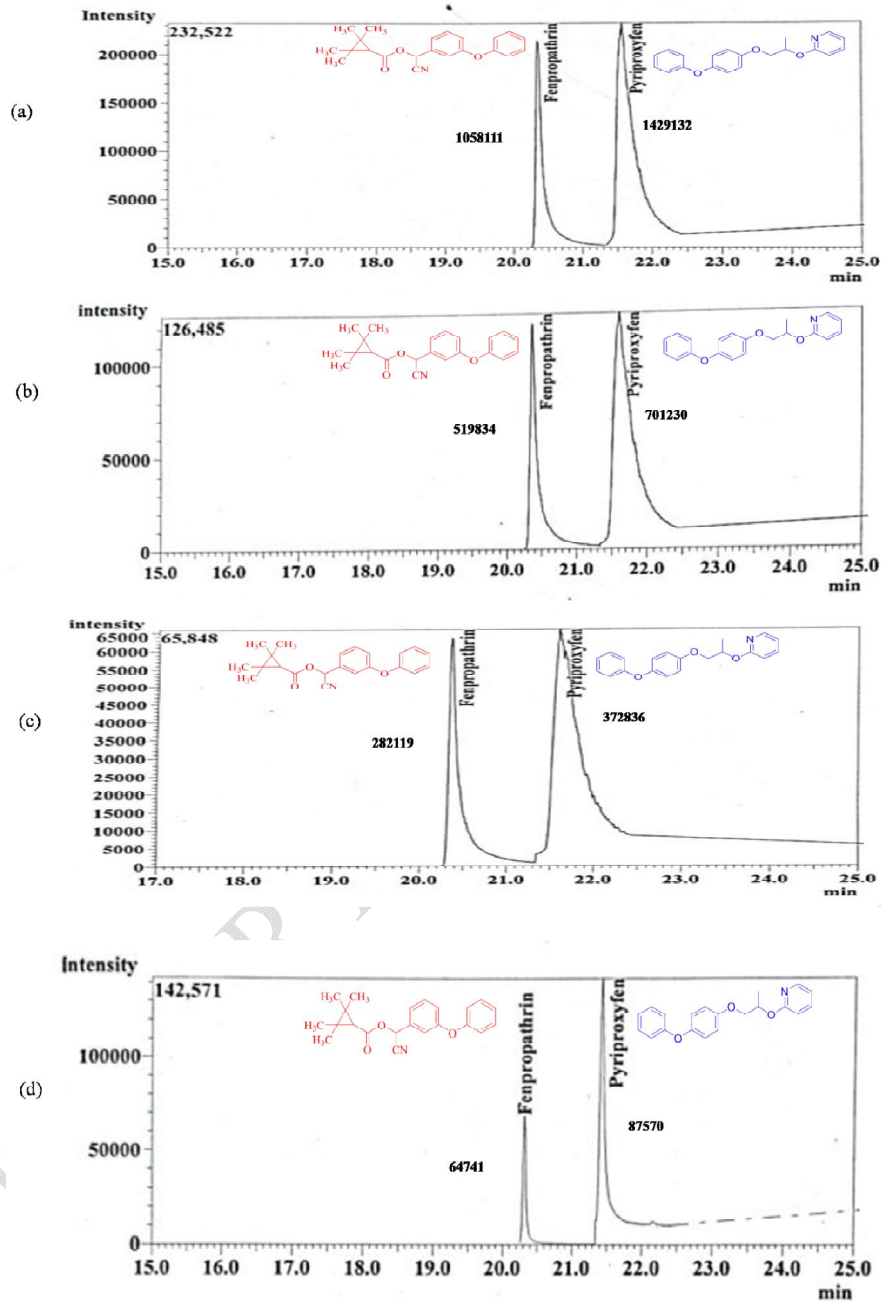


Fig. 2: Chromatogram of standard of Pyriproxyfen and Fenpropathrin on GC-MS/MS at (a) 1 mg/L (b) 0.5 mg/L (c) 0.25 mg/L (d) 0.05 mg/L

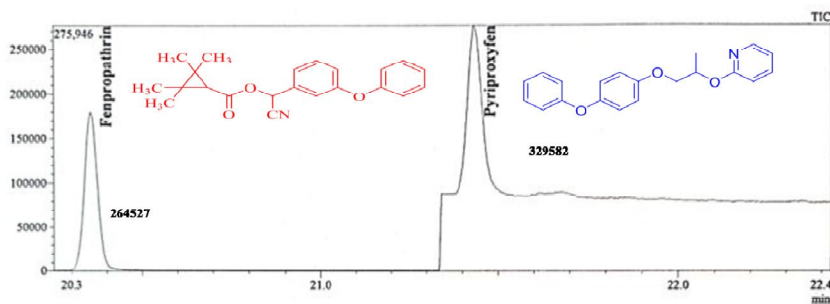


Fig. 3: Chromatograms of fortified okra samples at 0.25 mg/L processed by the QuEChERS method

Validation of the quantitative determination of Pyriproxyfen and Fenpropathrin in okra fruits and soils was carried out in accordance with the recommendations for bioanalytical methods outlined in the SANTE guidelines (SANTE, 2017). 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 the course of 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 analysed for pesticide residue analysis. The recovery chromatograms of Pyriproxyfen and Fenpropathrin are represented in Fig. 3. While both methods produce reliable results with significant difference in 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. The values of % Mean Recoveries for both insecticides at spiking levels of 0.5- to 0.01 mg/kg ($n=3$) for the QuEChERS method in okra samples ranging from 85.9 % with RSD \leq 7.19 % demonstrate the accuracy of the method by meeting the European Commission (2002) guidelines for evaluating the accuracy of a procedure (% Mean recoveries must be in the range of 70-120% with the values of RSD \leq 20 %). Comparable results were obtained by Cervera *et al.*, (2010) 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 (2019) also validated the QuEChERS method using GC-MS/MS for

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Pyriproxyfen and Fenproprathrin 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 Fenproprathrin recoveries varies 85.4 to 92.8% in cucumber, 84.9 to 89.4% at two different fortification levels with RSD below 20%.

Table 2: Amount of Pyriproxyfen and Fenproprathrin recovered from spiked okra samples processed on the same day by the QuEChERS

| Fortification level (mg/kg) | Pyriproxyfen | | Fenproprathrin | |
|-----------------------------|-----------------------------|----------------------|-----------------------------|----------------------|
| | 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

Table 3: Amount of Pyriproxyfen and Fenproprathrin recovered from spiked okra samples processed on three different days by the QuEChERS

| Fortification level (mg/kg) | Day | Pyriproxyfen | | Fenproprathrin | |
|-----------------------------|-----|-----------------------------|----------------------|-----------------------------|----------------------|
| | | 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 | 5.41 | 90.7±7.84 | 7.19 |
| | 2 | 89.5±4.44 | | 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 | |

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|------|---|-----------|------|------------|------|
| 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: Amount of Pyriproxyfen and Fenproprathrin recovered from spiked okra samples processed on the same day by the Liquid-Liquid Extraction (LLE)

| Fortification level (mg/kg) | Pyriproxyfen | | Fenproprathrin | |
|-----------------------------|-----------------------------|----------------------|-----------------------------|----------------------|
| | 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: Amount of Pyriproxyfen and Fenproprathrin recovered from spiked okra samples processed on three different days by the Liquid-Liquid Extraction (LLE)

| Fortification level (mg/kg) | Day | Pyriproxyfen | | Fenproprathrin | |
|-----------------------------|-----|-----------------------------|----------------------|-----------------------------|----------------------|
| | | 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 | |

^a Average of three replicates

SD= Standard deviation

RSD_R = Relative Standard Deviation for Reproducibility

The precision of the method was determined in two stages: intra-day assay (repeatability) and inter-day assay (within lab reproducibility) as provided in Table 2-3. ~~The repeatability was represented by the %RSD_r of the data from three replicates tested on the same day using the same instrument. The intermediate precision (reproducibility) was represented by the %RSD_R of the findings of the analysis on three distinct days using the same instrument.~~ The values of percent 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 values of percent 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.*, (2014) 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.*, (2018) also validated a method for the determination of the pesticides Fenpropathrin in potatoes and tomatoes with % average recoveries ranging from 93% to 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.*, (2007) 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.

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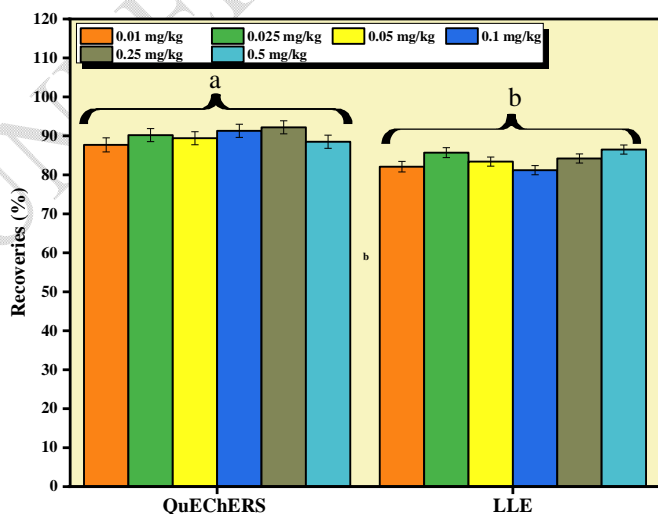


Figure 4: Percent recoveries of pyriproxyfen from okra matrices using QuEChERS, and LLE at six fortification levels.

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

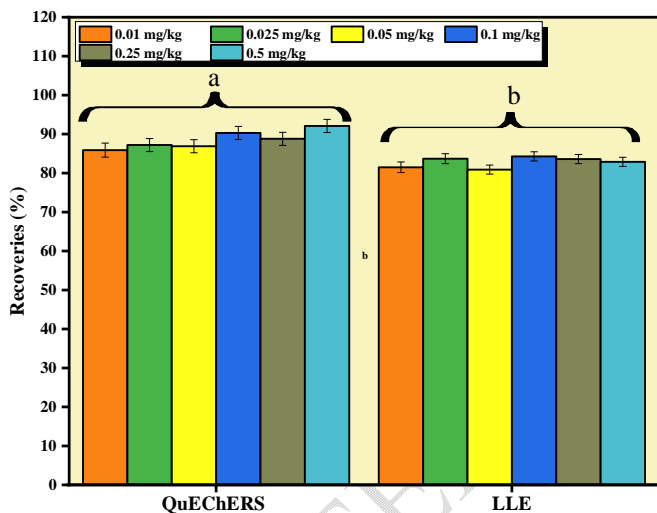


Figure 5: Percent recoveries of Fenprothrin from okra matrices using QuEChERS, and LLE at six fortification levels.

(Alphabets a, and b represent significant relationship between recoveries obtained in different methods; Error bars represent \pm 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, 2002) indicating the robustness of the method. The results of linearity, accuracy, precision, selectivity, and robustness of our experiments complied with SANTE (SANTE, 2017), and European Commission (European Commission, 2002) recommendations with the values of % mean recoveries falling in the

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range of 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-ECD and GC-MS analysis for simultaneous detection and quantification of 41 multiclass pesticide residues in milk 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. ~~The method is sensitive and can be applied for routine analysis of pesticide contaminants in milk at or below the MRL.~~

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