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# Development and validation of methodology for estimation of fenpropathrin and pyriproxyfen in chilli and soil through GC-ECD and NPD

**Chandrika Thanikanti, Sanjay Kumar Sahoo and Vijay Kumar**

**Abstract**
The objective of the study is to standardize methodology for estimation of fenpropathrin and pyriproxyfen in chilli and soil. QuEChERS technique is used for estimation of pyriproxyfen and fenpropathrin conducted through GLC equipped with NPD and ECD in chilli and soil. "Retention time" for pyriproxyfen and fenpropathrin was observed to be 4.10 and 4.73 min. LOQ and LOD of pyriproxyfen and fenpropathrin was quantified to be 0.01 mg $kg^{-1}$, 0.05 mg $kg^{-1}$ and 0.003 mg $kg^{-1}$, 0.02 mg $kg^{-1}$. Mean recoveries of pyriproxyfen in chilli and soil samples spiked of 0.01, 0.05 and 0.10 mg /kg, range about 80.97 to 88.33 per cent and found to be more than 80 per cent whereas for fenpropathrin in chilli and soil samples spiked with 0.05, 0.25 and 0.50 mg/ kg levels ranged from 80.03 to 89.51 per cent. $RSD_r$ for pyriproxyfen in chilli and soil at 0.01 - 0.50 mg/ kg ranged over 2.49 to 8.65, 2.73 to 10.13 per cent whereas for fenpropathrin in chilli and soil at 0.05-0.50 mg/ kg at 4.79 to 6.26, 2.90 to 6.95 per cent. The between-batch recoveries and reproducibility for pyriproxyfen and fenpropathrin in chilli and soil were examined at 0.01mg $kg^{-1}$and 0.05mg/ kg. The reproducibility of pyriproxyfen and fenpropathrin in distinctive substrates ranged from about 6.15 to 10.48 per cent and all measurements are within 15 per cent at all concentrations.

**Keywords:** QuEChERS, fenpropathrin, pyriproxyfen, GLC, LOQ, LOD, recoveries, standards

## Introduction
Development and validation of methodology for estimation of fenpropathrin and pyriproxyfen in chilli and soil through GC-ECD and NPD. Pyriproxyfen is a broad-spectrum insect growth regulator with insecticidal activity (WHO, 2008) [1]. "As a potent hormone agonist, pyriproxyfen is classified as an endocrine disruptor" (WHO, 2012) [2]. Fenpropathrin is a novel insecticide implemented by Sumitomo Co. Ltd. It is a pro synthetic pyrethroid compound. Fenpropathrin results in paralysis and death of pests by modulating the sodium channels in nerves (Agropages, 2020) [3]. It is the 4th generation of synthetic pyrethroid, resistant to sunlight, air and highly persistent. It is used as a broad spectrum insecticide which is useful to control mainly the pests of field crops, vegetables, fruit trees. Fenpropathrin functions as non systemic, contact and stomach poison at the same time act as sodium channel modulator. It is used as an acaricide and insecticide as well. It is effective against mites, whiteflies, leaf miners, armyworms, loopers, aphids, cutworms, stem borers. The rational recommendation for an insecticide must need effective control of target pest as well as residues which are left on the produce should be toxicologically unobjectionable. To estimate pesticide residues in any substrates, the methodology must be sound enough to give reliable results. The present studies, therefore, have been proposed to standardise and validate the methodology for estimation of residues of fenpropathrin and pyriproxyfen in chilli and soil.

## Materials and Methods
### Location of the experiment
The site for experimentation was done in the farm of "Dr. Rajendra Prasad Central Agricultural University (RPCAU), Pusa," which lies in the district Samastipur. The laboratory experiments were carried out in the Pesticide Residue Analysis Laboratory of Department of Entomology, Post Graduate College of Agriculture, RPCAU, Pusa.

### Reagents and Chemicals
Analytical standard of pyriproxyfen (purity 99%) where as fenpropathrin (purity 98 %) was

obtained from Dr. Erhenstrofer, India. Standard stock solutions of pyriproxyfen along with fenpropathrin (1 mg $mL^{-1}$) were prepared in HPLC grade hexane and Supra Solv acetone, respectively. The standard solutions were further diluted to have different concentrations and injected into instrument to see the linearity by plotting a calibration curve. The storage temperature for all these standard solutions was kept around -4 $^0$C before use.

Different reagents and chemicals like Sodium chloride- E. Merck Ltd, Sodium sulfate anhydrous- SD Fine Chemicals, Primary Secondary Amine- Agilent Technologies, Magnesium sulphate anhydrous- E. Merck Ltd, Graphitic carbon black – Supelco, Solvents- HPLC grade Acetonitrile, Acetone –SupraSolv and Hexane- HPLC grade were purchased and its suitability was checked by running reagent blank.

**Residue analysis of chilli samples**

"Quick, Easy, Effective, Rugged and Safe (QuEChERS)" techniques with slight modification are used for processing of chilli samples for residue analysis. A macerated chilli sample (10g) was transferred to a 50 mL polypropylene centrifugal tube later kept overnight in refrigeration. Samples were taken from the refrigerator and 20 mL of acetonitrile (HPLC grade) was added to each tube. To each centrifuge tubes, sodium chloride (10 ± 0.1 g) was added and shaken for 10 min at 50 rpm on rotospin (Tarson®). Samples were centrifuged for 3 min at 2500 rpm. Moisture if any was removed from an aliquot of acetonitrile by anhydrous sodium sulfate followed by cleanup through"dispersive solid phase extraction (DSPE)". For this, a polypropylene tube constituting "0.15 ± 0.01 g PSA sorbent, 0.90 ± 0.01 g anhydrous $MgSO_4$ and 0.05 ± 0.01 g graphitic carbon black" was prepared for an aliquot of 6 mLwhich was thoroughly mixed by vortex spinix (Tarson®) . Once again centrifuged for 3 min at 2500 rpm and finally a 3 mL aliquot was taken and evaporated to near dryness. The terminal volume was marked up to 3 mL with acetone and hexane for fenpropathrin and pyriproxyfen residue analysis.

**Residue analysis of soil sample**

Soil sample (10 g) was measured into a polypropylene centrifuge tube and mixed with 20 mL acetonitrile in addition to 10 mL of distilled water.Then other steps described in the case of chilli samples were followed for soil samples also.

**Estimation**

The estimation of pyriproxyfen and fenpropathrin through gas liquid chromatography (GLC) developed with nitrogen-phosphorus detector and electron captured detector, respectively and a glass capillary column (length: 30 meters, 25mm i.d., film thickness: 0.25 mm). The conditions to operate for pyriproxyfen were as follows:

The conditions to operate for pyriproxyfen were as follows:

| **Temperature of** | | |
|---|---|---|
| Detector | : | 310 $^0$C |
| Oven | : | 270 $^0$C |
| Injector | : | 300 $^0$C |
| Flow rate of | | |
| $N_2$(Carrier gas) | : | 30 mL/ min |
| Zero Air | : | 145 mL/ min |
| Hydrogen | : | 3 mL/ min |

The conditions to operate for fenpropathrin were as follows:

| Temperature of | | |
|---|---|---|
| Detector | : | 280 $^0$C |
| Oven | : | 240 $^0$C |
| Injector | : | 260 $^0$C |
| $N_2$(Carrier gas) | : | 30 mL/ min |

The residues of pyriproxyfen and fenpropathrin in chilli and soil samples were matched with the "retention time" of respective standards, whereas, estimated by "peak heights". "Retention time"for pyriproxyfen and fenpropathrin was observed to be 4.10 and 4.73 min., correspondingly when injected under above mentioned conditions.

Quantification of residues (mg/kg) was calculated as:

$$\text{Residues} = \frac{P_1 M_1 V_1}{P_2 M_2 V_2}$$

Where

$P_1$:"Peak area of the sample" $P_2$: "Peak area of the standard"

$M_1$:"Quantity (ηg) of standard injected" $M_2$: "Weight (g) of the sample"

$V_1$: "Final volume (ml) of the sample extract" $V_2$: "µl of the sample injected"

**Results and Discussions**

**Limit of detectability of pyriproxyfen and fenpropathrin residues**

The full scale deflection was obtained with 0.6 and 3 ng of the standard of pyriproxyfen and fenpropathrin respectively. Chromatograms for pyriproxyfen and spiked samples of chilli along with soil are given in Fig. 1 whereas chromatograms for fenpropathrin and spiked samples of chilli along with soil are given in Fig. 2. Samples of chilli were processed and terminal volume was composed to 3 mL and again concentrated to 0.5 mL from where 2 µL of the sample was injected to observed the maximum load of samples can be analysed without any interference peak in the area relating to the compound estimated. The limit of quantification (LOQ) of pyriproxyfen was quantified to be 0.01 mg $kg^{-1}$and the limit of detection (LOD) to be 0.003 mg $kg^{-1}$. The LOQ of fenpropathrin was observed to be 0.05 mg $kg^{-1}$and LOD was resulted to be 0.02 mg $kg^{-1}$.

**Recoveries of pyriproxyfen and fenpropathrin in chilli and soil**

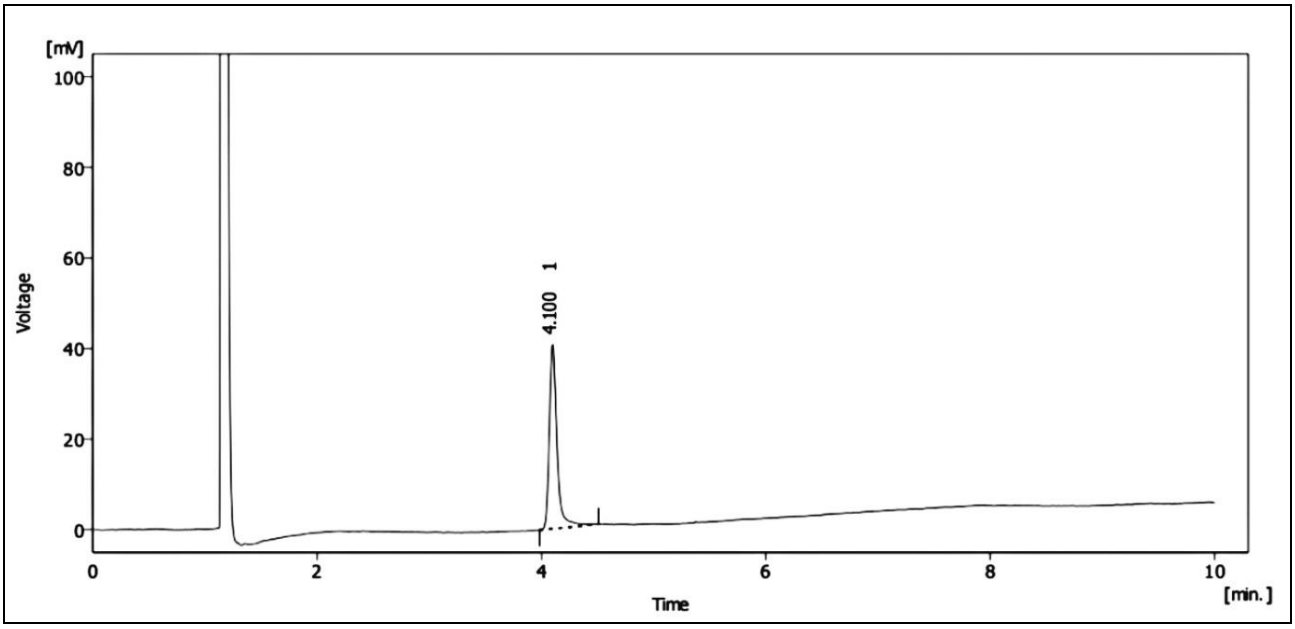
In supervised trials, a specified method sufficiently sensitive to detect low amounts of residues has to be developed to estimate a known insecticide in a crop. The efficacy of the analytical procedure needs to be assessed and determined for the samples being analysed by proportionate number of controls as well as recovery of the experiment (Leng, 1980) [4]. One set of recovery experiments must be carried out by spiking the desired pesticide below the tolerance limit proposed for that commodity (Kalra, 1996) [5].

Mean recoveries of pyriproxyfen in chilli and soil samples spiked of 0.01, 0.05 and 0.10 mg /kg,range about 80.97 to 88.33 per cent and found to be more than 80 per cent (Table 1). Whereas, mean per cent recoveries of fenpropathrin in chilli and soil samples spiked with 0.05, 0.25 and 0.50 mg/ kg levels ranged from 80.03 to 89.51 per cent (Table 2). In both the cases the amount recovered was greater than 80 per cent signifies the suitability of methodology,

**Method validation for pyriproxyfen and fenpropathrin residues**

The quantitative determination of pyriproxyfen and fenpropathrin in chilli and soil was validated as stated by bio analytical method recommendations described in the SANCO guidelines. The calibration curves of pyriproxyfen as well as fenpropathrin generate a linear relationship (Fig. 3 and 4).

Determination of Repeatability ($RSD_r$) by spiking fenpropathrin and pyriproxyfen through developed analysis method at different concentrations to different substrates. The repeatability ($RSD_r$) for pyriproxyfen in chilli and soil at 0.01 - 0.50 mg/ kg ranged over 2.49 to 8.65, 2.73 to 10.13 per cent respectively (Table 1). The repeatability ($RSD_r$) for fenpropathrin in chilli and soil at 0.05-0.50 mg/ kg ranged from 4.79 to 6.26, 2.90 to 6.95 per cent respectively (Table 2). The between-batch recoveries and reproducibility ($RSD_R$) examined at 0.01mg $kg^{-1}$ for pyriproxyfen in chilli and soil are given in Table 3. The between-batch recoveries and reproducibility ($RSD_R$) examined at 0.05mg/ kg for fenpropathrin in chilli and soil are expressed in Table 4. The reproducibility of pyriproxyfen and fenpropathrin in distinctive substrates ranged from about 6.15 to 10.48 per cent and all measurements are within 15 per cent at all concentrations.

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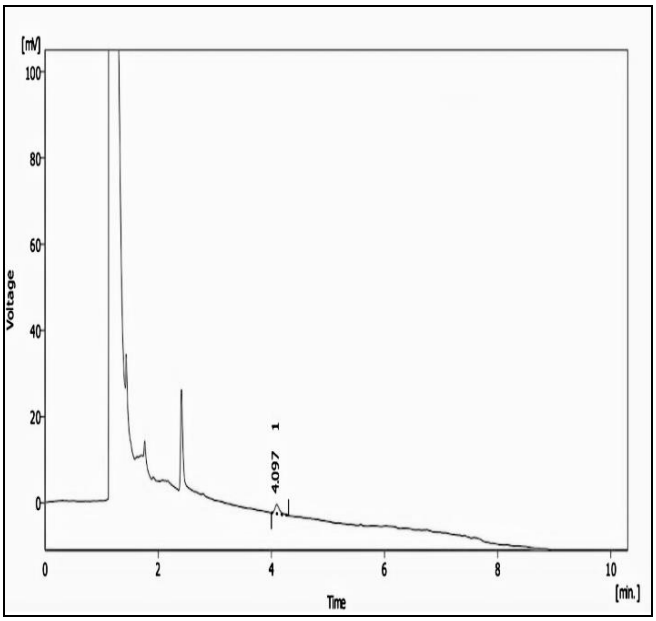


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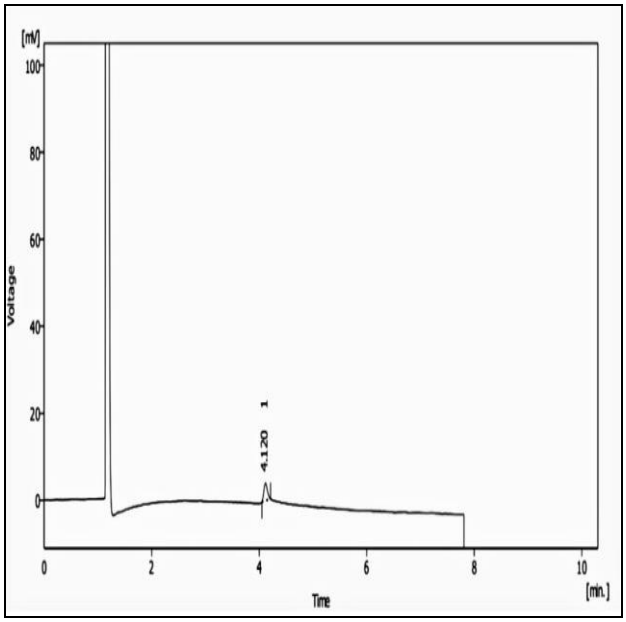
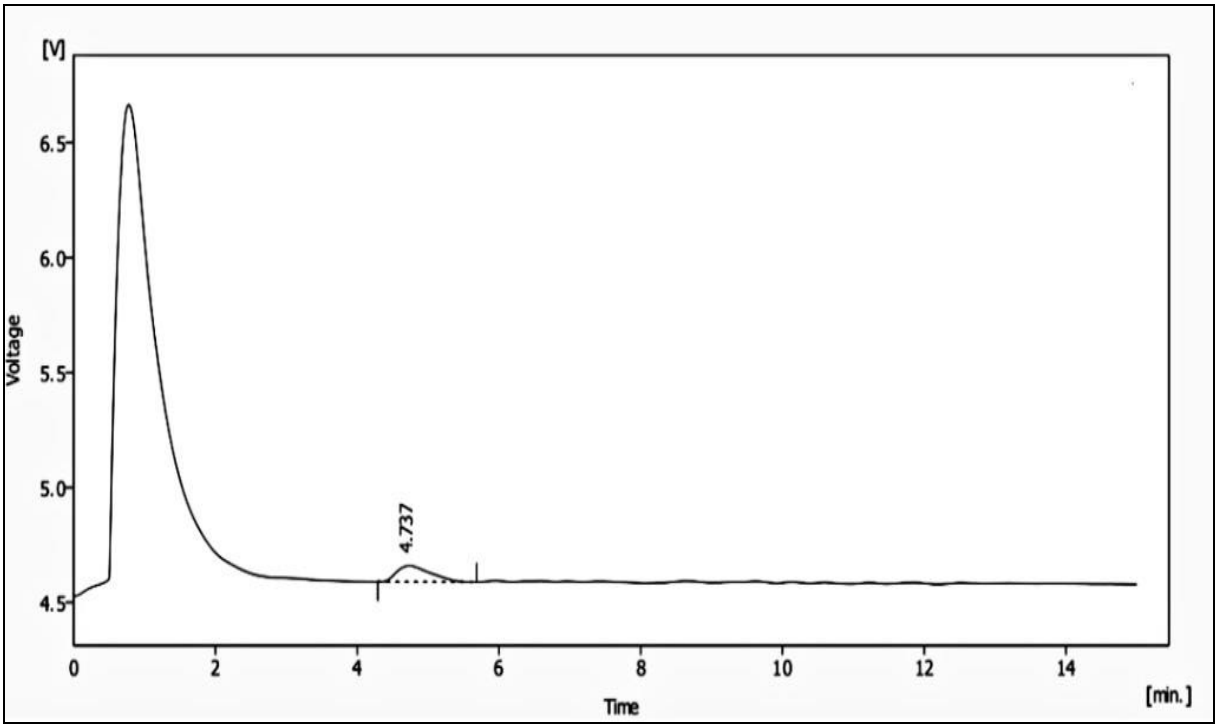



C.

**Fig 1:** GLC chromatograms of a. standard of pyriproxyfen b. chilli fortified with pyriproxyfen @ 0.01 ppm c. Soil fortified with pyriproxyfen @ 0.01ppm

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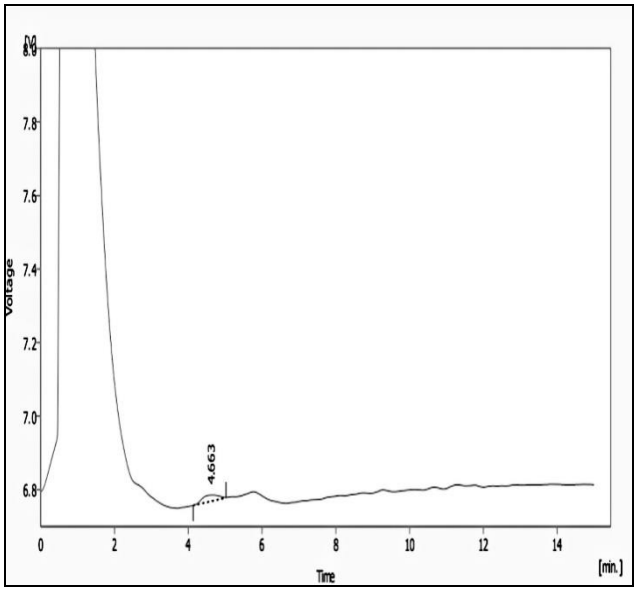


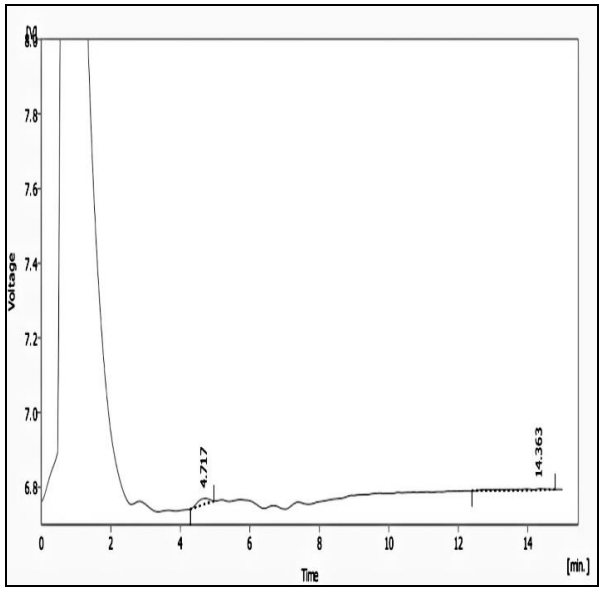


B. C.

**Fig 2:** GLC chromatograms of a. standard of fenpropathrin b. chilli fortified with fenpropathrin @ 0.05 ppm c. Soil fortified with fenpropathrin @ 0.05 ppm

**Table 1:** Amount of pyriproxyfen recovered from spiked chilli samples and soil

| Substrate | Spiked Level (mg/kg) | Amount Recovered* Mean ± SD | $RSD_r$ |
|---|---|---|---|
| Chilli | 0.10 | 88.33 ± 7.64 | 8.65 |
| | 0.05 | 84.56 ± 2.11 | 2.49 |
| | 0.01 | 82.50 ± 4.50 | 5.45 |
| Soil | 0.10 | 86.51 ± 3.14 | 3.63 |
| | 0.05 | 84.73 ± 8.58 | 10.13 |
| | 0.01 | 80.97 ± 2.21 | 2.73 |

*Mean of six replications
SD = "Standard Deviation"
RSDr = "Relative Standard Deviation" (Repeatability)

**Table 2:** Amount of fenpropathrin recovered from spiked chilli samples and soil

| Substrate | Spiked Level (mg/ kg) | Amount Recovered* Mean ± SD | $RSD_r$ |
|---|---|---|---|
| Chilli | 0.50 | 89.51 ± 5.60 | 6.26 |
| | 0.25 | 87.52 ± 4.80 | 5.48 |
| | 0.05 | 82.07 ± 3.93 | 4.79 |
| Soil | 0.50 | 86.03 ± 2.50 | 2.90 |
| | 0.25 | 80.03 ± 3.39 | 4.23 |
| | 0.05 | 84.94 ± 5.90 | 6.95 |

*Mean of six replications
SD = "Standard Deviation"
RSDr = "Relative Standard Deviation" (Repeatability)

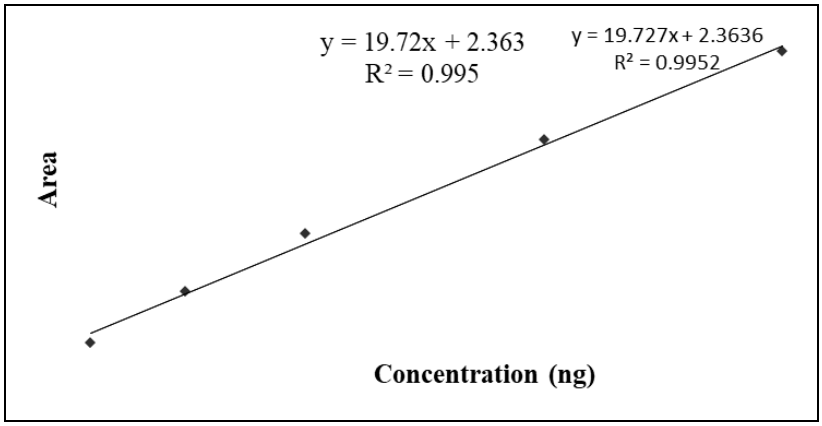


**Fig 3:** Linearity curve of pyriproxyfen standards

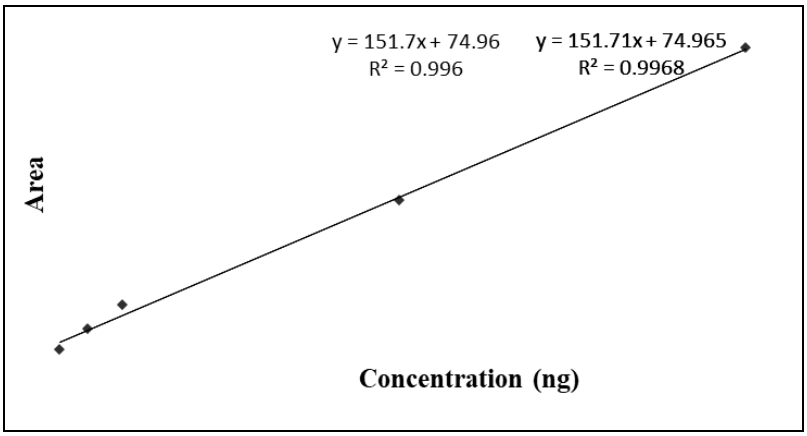


**Fig 4:** Linearity curve of fenpropathrin standards

**Table 3:** Reproducibility for pyriproxyfen at 0.01 mg/kg

| Substrate | Day | Amount recovered (%) | Standard deviation (%) | $RSD_R$ (%) |
|---|---|---|---|---|
| Chilli | 1 | 82.50 | 4.50 | 5.13 |
| | 2 | 83.67 | 1.53 | |
| | 3 | 81.33 | 6.66 | |
| Soil | 1 | 82.04 | 9.50 | 6.15 |
| | 2 | 80.97 | 2.21 | |
| | 3 | 79.80 | 3.52 | |

$RSD_R$= "Relative Standard Deviation" (reproducibility)

**Table 4:** Reproducibility for fenpropathrin at 0.05 mg/ kg

| Substrate | Day | Amount recovered (%) | Standard deviation (%) | $RSD_R$ (%) |
|---|---|---|---|---|
| **Chilli** | 1 | 82.07 | 3.93 | 10.48 |
| | 2 | 81.00 | 6.93 | |
| | 3 | 86.16 | 15.28 | |
| **Soil** | 1 | 84.94 | 5.90 | 6.50 |
| | 2 | 87.89 | 7.19 | |
| | 3 | 82.00 | 3.46 | |

$RSD_R$= "Relative Standard Deviation" (reproducibility)

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