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A COMPREHENSIVE HPLC METHOD FOR MEASURING ABAMECTIN, CHLORANTRANILIPROLE, AND DELTAMETHRIN IN HUMAN PLASMA: DEVELOPMENT, VALIDATION, AND COMPARATIVE EVALUATION

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Abstract:

Purpose: Pesticides play a critical role in preventing vector-borne diseases, safeguarding crops, and food preservation. Still, the exposure poses significant risks to people and the environment. Thus, an accurate analytical method to detect and quantify this hazardous compound in biological matrices is required. This work is intended to provide an analytical method for the evaluation of Abamectin, Chlorantraniliprole, and Deltamethrin using High-Performance Liquid Chromatography and UV-visible spectrophotometry.
Method: Initially, the pesticides were identified based on their physical properties, melting point, ultraviolet spectroscopy, and the Fourier transform infrared. Further, the HPLC method was developed and validated as per FDA guidelines. The HPLC method involves preparing a standard stock solution of pesticides in acetonitrile: water, making serial dilutions of the stock solution for calibration over a range of linear curve.
Result: The physical identity of the compounds was established as to its purity. The linearity of the data is graphically represented, based on which a strong correlation was reported. Accuracy and precision were done with Quality control samples in different concentrations to get the standard deviation and percentage relative standard deviation. Recovery study results are 60.47-61.87% for abamectin, 54.74-66.32% for chlorantraniliprole, and 49.87-53.86% for deltamethrin. Limits of detection and Limit of quantification are abamectin 0.0126 & 0.0381 ppm; Chlorantraniliprole 0.019 & 0.057 ppm and Deltamethrin 0.023 & 0.07 ppm which shows a high sensitivity of the method.
Conclusion: The HPLC method developed must be precise, as precise as possible composite for the routine quantification of pesticides in various samples.
Novelty: This experiment intends to establish a novel High-Performance Liquid Chromatography method in FDA guidelines for determining Abamectin, Chlorantraniliprole, and Deltamethrin together. This HPLC validated drug usage method is quite sensitive and consistent. This method is acceptable as it includes Ultraviolet Fourier transform infrared and HPLC confirmation, with findings below parts per million. This work is a good activity for a routine study using an innovative mechanism and sophistication that aids in simple laboratory procedures.

Keywords: Pesticides, HPLC Method, Liquid Chromatography, Ultraviolet Fourier Transform Infrared.

1. INTRODUCTION

Pesticides are critical in modern agriculture as they control pests and enhance crop yield [1]. Abamectin, chlorantraniliprole, and deltamethrin are the common pesticides against various insects. Abamectin is a broad-spectrum macrocyclic lactone (Figure 1). Pesticide extracted from Streptomyces avermitilis. It is popularly used in agriculture and veterinary as it develops neurotoxic to invertebrates [2]. However, exposure to humans triggers neurotoxic, hepatotoxic, and other systemic reactions. Therefore, its monitoring in biological samples is necessary.

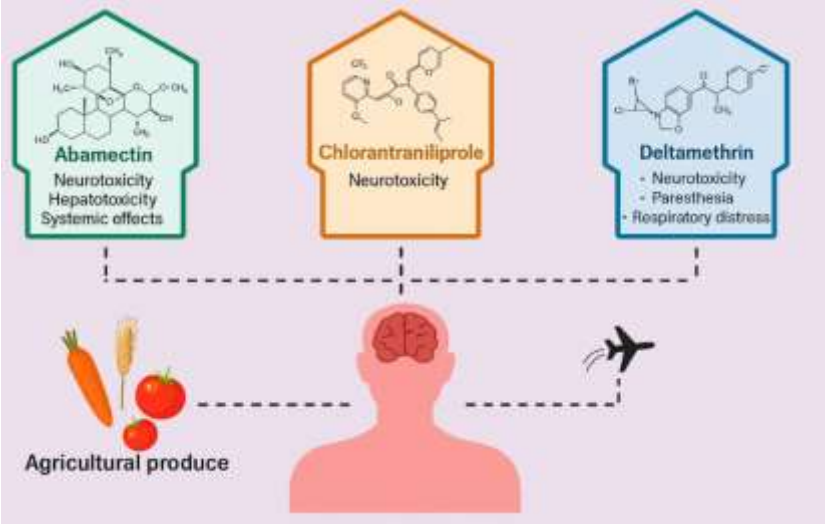


Figure 1. Integrated Pathways of Pesticide Exposure and Human Toxicity Mechanisms. (Generated by Microsoft copilot)

The diamide insecticide chlorantraniliprole is an ideal pesticide that interferes with calcium transport in insect muscle cells, paralyzing and killing insects [3]. The mammalian is less toxic though chronic ingestion may pose an issue, especially to human beings. Deltamethrin is another synthetic pyrethroid pesticide used in agriculture and public health for vector control. It targets the sodium ion passage in the nerve cells, which results in paralysis and death of parasites. Nevertheless, human exposure to the pesticide has several effects; sensory neuropathy and paresthesia, dizziness, respiratory problems and skin disruption may be of concern. Through food consumption, occupational exposure, and environmental poisoning, people may be exposed to these pesticides residues [7]. The World Health Organization (WHO) reports that 220,000 people die and around 3,000,000 cases of pesticide poisoning occur in underdeveloped nations annually [8].

Table 1. WHO Data on Pesticide Use and Toxic Effects.

Metric	Value / Statistic	Reference
Global annual pesticide usage	3.7 million tons (2022)	[34]
Main pesticide exporting region	Asia	[34]
Main pesticide consuming region	America	[34]
Unintentional pesticide poisonings per year	385 million cases	[34]
Annual pesticide poisoning deaths	11,000–20,000	[34]
Farmers affected by pesticide poisoning	44% out of 860 million	[34]
Highly hazardous pesticide health impact	Major contributor to death and toxicity	[35][36]
Preventable suicides by HHP bans	Up to 138,000 annually	[37]
ADI and MRL setting authority	JMPR (FAO/WHO)	[38]
Acute toxicity classifications (LD_{50} values)	Class Ia: <5, Ib: 5–50, II: 50–2000, III: >2000, U: >5000 mg/kg	[39]
Principal chronic health risks	Carcinogenicity, neurotoxicity, reproductive/endocrine disruption	[39]
Key WHO recommendations	Ban/phase-out HHPs; monitor residues; food safety	[40]

Therefore, it is highly essential to develop reliable analytical methods for the detection and quantification of abamectin, chlorantraniliprole, and deltamethrin in biological matrices, specifically human plasma. HPLC is a well-established method for the quantification of pesticide residues owing to its high sensitivity, selectivity, and reproducibility [9]. Accurate and sensitive quantification of abamectin, chlorantraniliprole, and deltamethrin in human plasma are critical for different exposure scenarios, pharmacokinetic studies, and risk analysis. Since biological matrices such as plasma are more chemically diverse and variable, effective sample cleanup and analyte pre-concentration are necessary to mitigate matrix effects and improve the sensitivity of the analytical methods [10]. Thus, we need robust and optimized extraction strategies such as ultrasonicator-aided cartridge-based extraction for successful isolation of the target analytes from endogenous components of plasma. Additionally, the approach ensures that co-eluting interferences do not affect subsequent chromatographic separation and detection positively, enhancing the reliability and accuracy of the bioanalytical method [12]. This research aims to develop and validate a new HPLC method for the simultaneous determination of abamectin, chlorantraniliprole, and deltamethrin in human plasma, bridging a research gap focusing on a single, accurate, and a matrix-effect-reduced analytical strategy for these specific pesticides. It will describe a complete method development workflow encompassing the chromatographic conditions and thorough validation to guarantee the method's specificity, linearity, accuracy, and precision for trace residue analysis in biological samples [12].

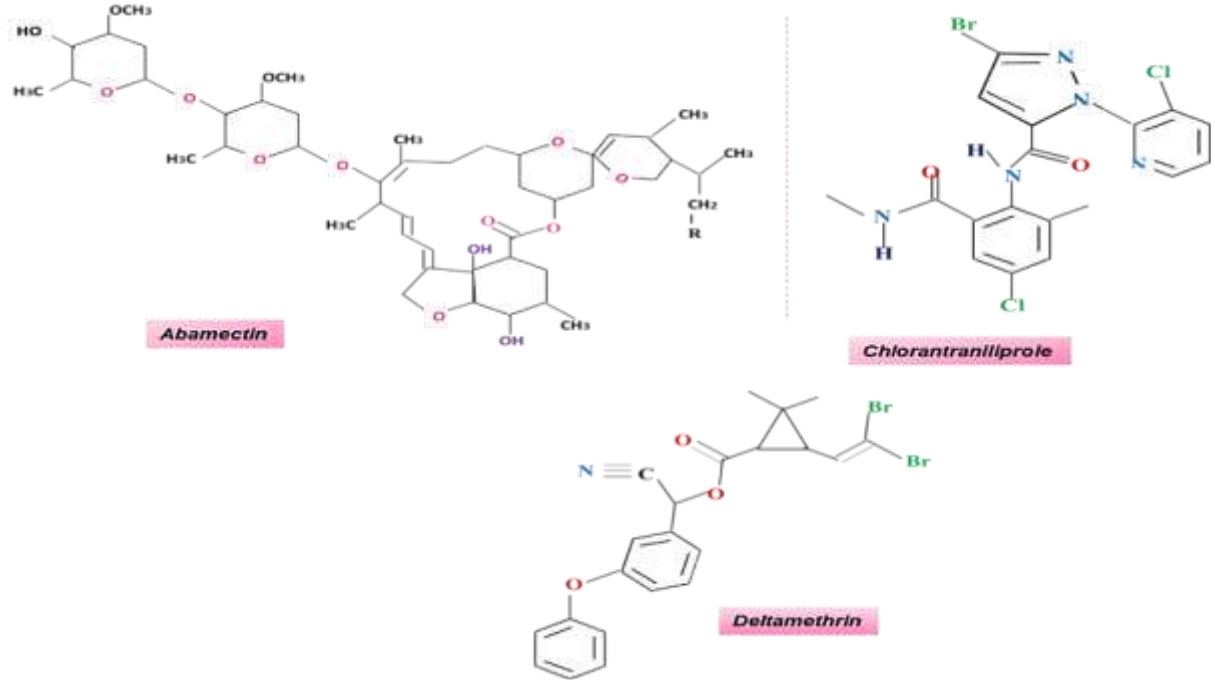


Figure 2. Structure of Abamectin, Chlorantraniliprole, and Deltamethrin (Own creation).

2. EXPERIMENTATION

The following section outlines the chemicals and procedures used in this research.

2.1 Chemicals

Abamectine (98%), Chlorantraniliprole (95%), and deltamethrin (95%) were obtained as gift samples from Vijay Agro Industries, Kasabe Sukene, Niphad. The plasma sample was procured from Adhar Blood Bank, Sangamner. Acetonitrile was obtained from HPLC grade Merck Company, Germany, and deionized water from HPLC grade.

2.2 Instrumentation

The setup of the laboratory consists of a Shimadzu Prominence HPLC Binary Gradient System from Shimadzu Analytical Pvt. Ltd., India, containing an SPD-20A detector and an LC-20AD UFLC pump with a maximum pressure of 40 MPa. Cosmosil C18 VP (250×4.6 mm ID, 5 µm particle size) was used as a column, and the analysis and control were done by LC solution software. Analytical Technologies Ltd. UV-Visible Spectrophotometer, Model 2012. This dual-beam instrument operates using UV-VIS Analyst software, and a wavelength variance of 0.1 nm from 190 to 1100 nm scanning range was used for UV-visible spectrophotometry.

Figure 3. Integrated Analytical System for Quantitative Determination of Pesticides Using HPLC and UV–Visible Spectrophotometry (ChatGPT).

2.3 Identification Study

2.3.1 Physical Appearance

The morphological properties of all three drugs were evaluated based on parameters such as color, odor, form, and state [12].

2.3.2 Assessment of Melting Point

The MP was determined using Mettler Toledo equipment (Greifensee, Switzerland) with the capillary fusion technique. Abamectin was filled into a glass capillary, with one end that was sealed with a flame. A suitable aperture was used to submerge the drug-containing capillary in liquid paraffin inside Thiele's tube. The silicon oil heater was used to heat the paraffin at a rate of 1°C/minute. The point at which the drug starts to melt is considered its melting point [13]. The same procedure was repeated for the other two pesticides.

2.3.3 Determination of λmax

The λmax of abamectin was determined using a UV-visible spectrophotometer (model 2012). Abamectin was dissolved in 100 mL of ACN: water in a volumetric flask to create a stock solution. A 10 µg/ml solution was obtained by further diluting the stock solution. The resulting mixture was scanned 200-400 nm, with the ACN: water solvent used as a blank for baseline correction [14]. The procedure was repeated for the other two pesticides.

2.3.4 Fourier Transform Infrared Spectroscopy

The FTIR study was conducted using the Agilent Cary 630 spectrometer, which helped identify functional groups related to the drug parts and assisted in testing its purity [15]. According to this procedure, an accurately weighed abamectin powder drug was mixed with a weight of 50 mg IR-grade potassium bromide and directed into compression using hydraulic pressure. Keeping in view the established range, an open pathway to scan the process was maintained during 4000–400 cm^{-1}, corresponding peaks were recorded [16]. The above process was continued for both the abamectin and subsequently chlorantraniliprole and deltamethrin samples.

2.4 Preparation of Stock and Working Solutions

Ten milligrams of carcinogenic pure pesticide were weighed and weighed in ACN solvent, NSF was diluted to make a 1000 ppm standard stock solution. An aliquot (0.1 mL) of the STD stock solution was transferred to a 10 mL volumetric flask, and the volume was made up to the mark with the same solvent to prepare a 10 ppm working solution. Hence after that, the stock and working solution was used for the further analysis described later below.

2.5 System Suitability Parameters/tests (SST)

Further discussed are the SST that are essential study was validated with Pallidness testing to evaluate system performance. SSTs are intended to assess the instrument's analytical performance and are a critical component of the valid test system related to the analytical measurement system, such as chromatographic. These SSTs are established for utilization to ensure that the testing system's capabilities are appropriate for the attitude of the measurements of the intended analytical requirement, making weaknesses more apparent. SSTs assist in establishing a strategy to conduct critical experiments to ensure that instrumental measurements are consistent and that the experimental values agree with referenced patterns. Thus should be applied to below-discussed parameters [19].

2.5.1 Resolution

The parameter that describes the chromatographic system's overall separation power concerning the specific mixture components is called resolution. According to convention, resolution (R) is the ratio of the mean peak width at the base line to the distance between two peak maxima. It must be more than 1.75. This factor is particularly relevant when analyzing a mixture of two compounds. In the case of a single sample, the resolution will show a value of zero (0), as no separation between peaks is expected [19, 20]. The R is measured using equ. (1)

$$R = \frac{2(t2 - t1)}{w1 + w2} \quad (1)$$

Where,

- t1 and t2 are the retention times of two peaks,
- w1 and w2 are their respective peak widths.

2.5.2 Theoretical Plates

Depending on the design of the column, the number of theoretical plates is proportionate to the length of the column. To determine the height equivalent of a theoretical plate, or simply plate height H, the column length L is divided by the number of plates, N (equ.2):

$$H = \frac{L}{N} \quad (2)$$

Using retention time and peak width (baseline method)

$$N = 16\,(\frac{tR}{w})^2 \quad (3)$$

The number of HETP should exceed 2000. This parameter indicates the efficiency of the chromatographic column, with a higher number of theoretical plates reflecting better column performance [21].

2.5.3 Tailing/ Asymmetry Factor

The asymmetry factor is used to measure the symmetry of a chromatographic peak. In an ideal scenario, peaks ought to exhibit symmetry resembling a Gaussian shape. However, various factors such as column overloading, inadequate packing, or secondary interactions can lead to peaks that are either tailing or fronting. The asymmetry factor must remain below 2. A value approaching 1 signifies optimal peak symmetry. This is determined using equ.4:

$$Asymmetry\ factor = \frac{B}{A} \quad (4)$$

Where,

- A = Distance from the peak center to the leading edge at 10% peak height.
- B = Distance from the peak center to the tailing edge at 10% peak height [22].

The tailing factor (T) is another way to measure peak asymmetry, measured as:

$$T = \frac{B}{2A} \quad (5)$$

Where A and B are measured at 5% peak height instead of 10% [19].

2.6 Sample Preparation

A sample of blood plasma was acquired from the Adhar Blood Bank, Sangamner. Before carry out the analysis, the whole plasma was stripped (in this procedure, plasma was first filtered through cotton to remove macro solid particles and any other contaminants so that a clean sample was taken for further examination) and then kept at −20°C until use [23].

2.6.1 Blank Plasma Extraction

1 mL of plasma sample was added to a centrifuge tube and then a blank (1 mL ACN: water). The added mixture was vortexed for 4-5 min and then centrifuged for 5 minutes at 3000 rpm. This separation was carried to separate the heavy material from the plasma and give a clear supernatant. Note that 1 mL of the supernatant was withdrawn and made to 10 mL using the solvent. After that, a 100 µL volume of the prepared sample was cleaned with a syringe filter and injected into HPLC system while maintaining a 1.0 mL min^{-1} flow rate [24].

2.6.2 Spiked Plasma Extraction

1 mL of plasma was added to a centrifuge tube and vortexed for 4–5 min. Then, 0.01 mL of a 1000 ppm abamectin stock solution was added, and it was vortexed for 4-5 min. The mobile phase ACN: water 95:05 was added to obtain 0.9 mL of it, and then vortexed for 4-5 minutes. Finally, the added mixture was centrifuged at 3000 rpm for 5 minutes. The upended plasma settled down to give a clear supernatant. In that, 1 mL of the supernatant was withdrawn and filled to 10 mL using the same solvent used in the supernatant acetonitrile: water, 95:05, to obtain a 1 ppm concentration (1µg mL^{-1}). It was vortexed, passed through a syringe filter and injected into the high-performance liquid chromatography system while maintaining a flow rate of 1.0 mL min^{-1} [25], the same procedure was performed for chlorantraniliprole, and deltamethrin was done using acetonitrile: water 60:40 and acetonitrile: water 90:10, respectively.

2.7 Bioanalytical Method Validation

Validation tests are performed to reveal that the technique is reliable and appropriate for the intended application. The analytical developed method was validated as per FDA standards including precision, linearity, accuracy, LOD, LOQ, and recovery of analytical solutions [26].

2.7.1 Linearity

The initial concentration of 10 ppm of standard stock solution of individual drugs was made as discussed in the above section. Furthermore, serial dilutions of the samples were made by adjusting the volume to make 6 different dilutions of 0.015 to 0.3 ppm with the same mobile phase (acetonitrile: water), and then 20 µL were injected to the HPLC instrument. The peak area to concentration was calculated based on the calibration curve (linearity graph) along with R^2, slope, and regression equation [27]. The same procedure was repeated for chlorantraniliprole (0.5- 10ppm) and deltamethrin (0.05- 1.0 ppm).

2.7.2 Accuracy

The absolute ratio of the QC samples' computed mean values to their corresponding nominal values, represented as a percentage (%), was used to determine the assay's accuracy. At least 5 measurements of abamectin per 3 concentrations were used to gauge accuracy, i.e., (LQC=0.0225 ppm, MQC=0.09 ppm, HQC=0.30 ppm). The 20-µl aliquots of supernatant of each concentration were introduced to the HPLC, and finally, the SD was calculated. The precision was established by defining the RSD (equ.6) [28].

$$RSD = 100X\ \frac{SD}{Average} \qquad (6)$$

The same procedure was repeated for chlorantraniliprole and deltamethrin.

2.7.3 Percentage Recovery

By comparing the responses of the extracted plasma QC samples to unextracted QC samples at HQC and LQC levels, the percentage mean recoveries were calculated. For every sample, recovery from human plasma samples was assessed three times. Each concentration's supernatant was added to the HPLC apparatus in aliquots of around 20 µL, and the analytical results of these extracted plasma samples were compared with corresponding standard solutions, following FDA recommendations [26, 29].

2.7.4 Limit of Detection and Limit of Quantification

The LOD is the lowest concentration at which it may be identified but not quantified. A conc. at a specific signal-to-noise ratio of 3:1 is used to express it. The lowest conc. of an analyte can be identified with reasonable accuracy and precision under the method's specified operating conditions is called as the LOQ. The SD and the slope were determined the LOD and LOQ. Additionally, the formulas (7) and (8), respectively, were employed to measure the LOD and LOQ [30, 31].

$$LOD = 3.3\ X\ \left(\frac{SD}{Slope}\right) \qquad (7)$$

$$LOQ = 10\ X\ \left(\frac{SD}{Slope}\right) \qquad (8)$$

3 RESULTS AND DISCUSSION

3.1 Identification Study

3.1.1 Physical Appearance

The physical appearance of individual pesticides was studied using color, odor, form, and state. The findings obtained from this study are described in the following Table 2.

Table 2. Physical Appearance of Pesticides.

Drug Name	Properties			
	Color	**Odor**	**Form**	**State**
Abamectin	White to yellowish	Odorless	Crystalline powder	Solid
Chlorantraniliprole	Off-white	Odorless	Crystalline powder	Solid
Deltamethrin	White to beige	Characteristic Odor	Crystalline powder	Solid

3.1.2 Assessment of Melting Point

Determining the melting point is the initial phase in pesticide analysis, offering essential insights into purity, identity, stability, and formulation appropriateness. A precise melting point suggests high purity, whereas any variations may indicate the presence of impurities or degradation. In this research, the melting points of three pesticides were accurately measured using the capillary fusion method, resulting in the following findings: Abamectin: 151–153°C, chlorantraniliprole: 199–201°C, and deltamethrin: 96–99°C. These measurements are within the standard ranges, affirming the integrity of the compounds and their suitability for further use [32].

3.1.3 Determination of λmax

The absorbance peaks of abamectin, chlorantraniliprole, and deltamethrin were measured using a UV-visible

spectrophotometer. The maximum absorbance for these pesticides was observed at 245, 212, and 213 nm respectively, which led to the selection of λmax for subsequent studies (Figure 4, 5).

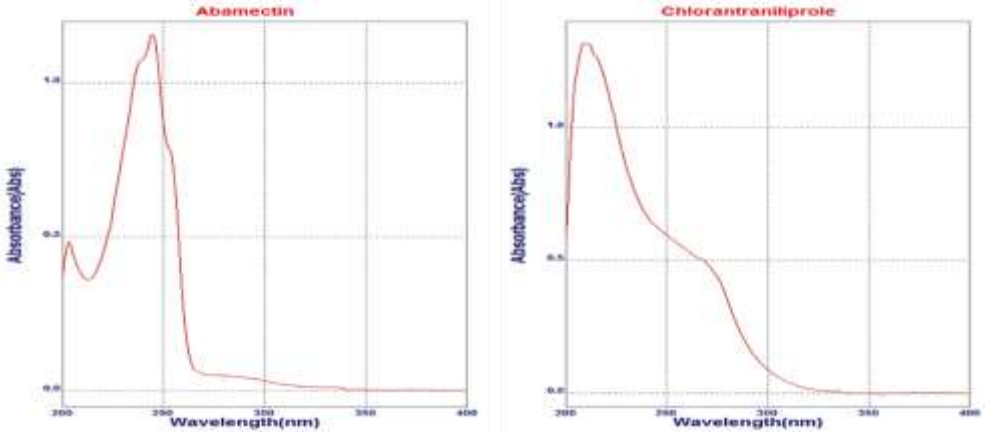


Figure 4. Absorbance Maxima of Abamectin, Chlorantraniliprole.

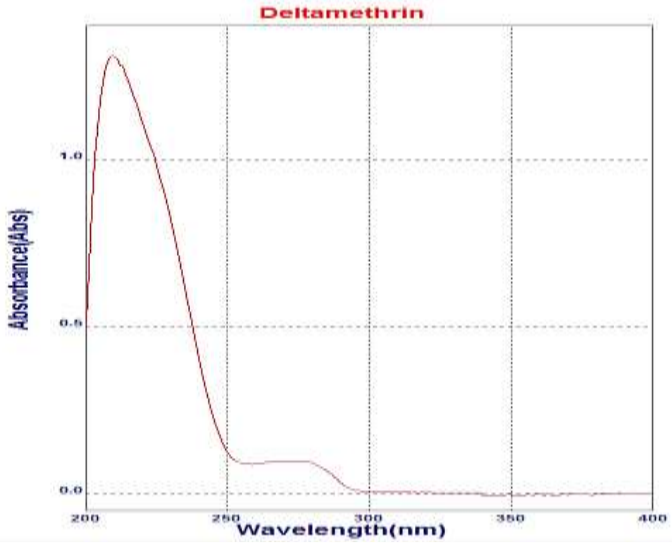


Figure 5. Absorbance Maxima of Deltamethrin.

3.1.4 Fourier Transform-infrared Spectroscopy

The FTIR spectrum for all three pesticides was captured within the range of 4000-400 cm-1. The peaks identified and their corresponding interpretations derived from this analysis are illustrated in Tables 3-5 and Figures 6-8.

Figure 6. IR Spectrum of Abamectin.

Table 3. Interpretation of Abamectine Spectra.

Observed Peak (cm^{-1})	Functional Group	Interpretation of chemical group
2965, 2933	C–H Stretch (Alkane)	Terminal methyl/methylene
2119	C≡C or C≡N Stretch	Alkyne or nitrile region (less likely for abamectin)
1707	C=O Stretch (Ester/Lactone)	Strong, characteristic for carbonyl
1457, 1375, 1338	C–H Bend (Alkane/CH_3)	Methylene/methyl bending
1294, 1263	C–O Stretch (Ester, Ether)	Ester/ether group vibrations
1158, 1107, 1050	C–O–C Stretch	Ether or cyclic ether functionalities
1011, 987	C–O or ring breathing	Possible cyclic ether vibrations
936, 907, 875, 832	C–H out-of-plane bend	Substituted alkene/alkane bending
761, 729, 698	C–H bend (aromatic ring)	Typically seen in aromatic compounds or cycloalkanes

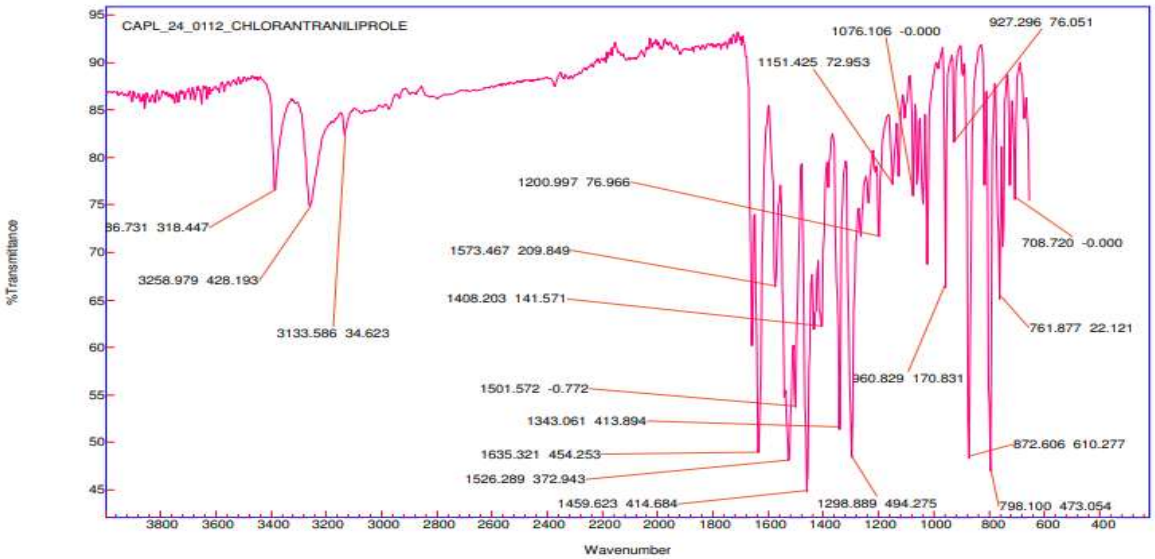


Figure 7. IR Spectrum of Chlorantleranilipro.

Table 4. IR Spectrum of Chlorantleranilipro.

Observed Peak (cm^{-1})	Functional Group	Interpretation of chemical group
3387	N–H stretching	Amide/secondary amide N–H
3259	N–H stretching	Amide/secondary amide N–H
3134	C–H aromatic stretching	Aromatic C–H
1635	C=O stretching	Amide carbonyl (CONH)
1573, 1526	C=C, C=N, N–O stretching	Aromatic ring or pyrazole C=N/C=C
1502, 1459	C=C stretching	Aromatic ring
1408, 1343	C–N stretching, C–H bending	Amide linkage, methyl bending
1299, 1201	C–N stretching	Amide, carbamate
1151, 1076	C–O, C–N stretching	Carbamate C–O/C–N
961, 927	C–Cl stretching, ring modes	Aryl–Cl, aromatic ring deformation
873, 798, 762, 709	Aromatic C–H bending	Monosubstituted/chloro-aromatics

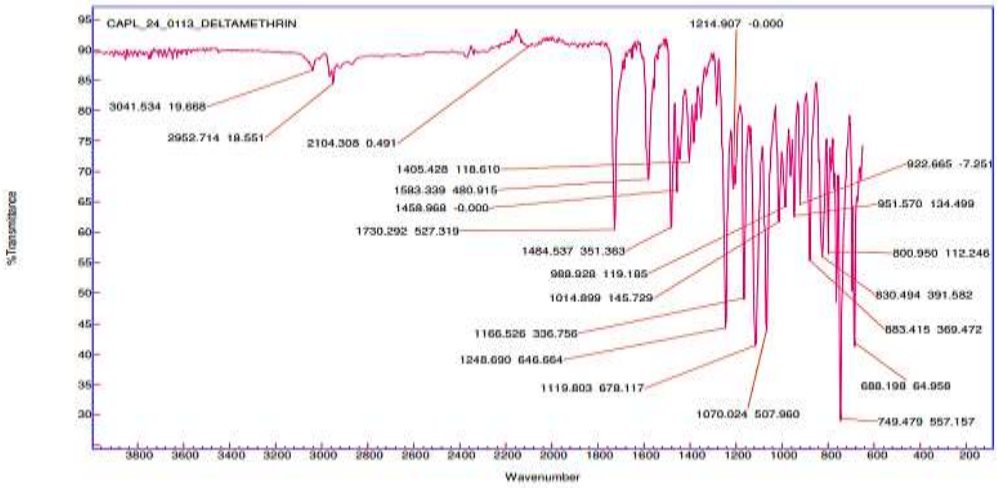


Figure 8. IR Spectrum of Deltamethrin.

Table 5. IR Spectrum of Chlorantleranilipro.

Observed Peak (cm^{-1})	Functional Group	Interpretation of chemical group
3042	=C–H Stretch (Aromatic)	Aromatic/alkene ring vibration
2953	C–H Stretch (Alkane)	Alkyl/methyl/methylene stretch
2104	C≡C or C≡N Stretch	Alkyne/nitrile region, rare for deltamethrin
1730	C=O Stretch (Ester)	Ester carboxyl carbonyl, strong peak
1583, 1485	C=C Stretch (Aromatic)	Benzene/C=C region
1459, 1405	C–H Bend (Alkane/Aromatic)	Aromatic and methyl bending
1249, 1215, 1167	C–O Stretch (Ester/Ether)	Ester/ether stretching
1120, 1070	C–O/C–Cl Stretch	Ether and halogen (chlorine) stretching
1015, 989, 952	C–H wag/bend	Alkyl/aryl out-of-plane bending
923, 883, 831, 801	C–H out-of-plane bend	Aromatic/alkene bending
749, 688	C–Cl Stretch	Halogen assignment, prominent for deltamethrin

3.2 System Suitability Parameters

These parameters serve as the benchmarks for comparing the results against the approximate standard values. They encompass resolution, theoretical plates, and asymmetry factors. In this study, the resolution was determined to be greater than 1.75, which guarantees that the pesticide peaks were sufficiently separated. The theoretical plates surpassed 2000, indicating satisfactory column efficiency. Furthermore, the asymmetry factor stayed below 2, denoting acceptable peak symmetry. These findings validate that the method fulfills the necessary suitability criteria for accurate and dependable analysis.

3.2.1 Validation Parameter

The drug analysis was performed utilizing HPLC. The results acquired were employed to validate the analytical method concerning linearity, accuracy, precision, recovery, and sensitivity (LOD & LOQ) in accordance with FDA guidelines.

3.2.2 Linearity

The linearity was performed using 6 different dilutions using different concentrations. Slope, regression equation, and R^2 was calculated. The findings of this study for all pesticides are mentioned in following Table 6.

Table 6. Linearity Results of Abamectin, Chlorantraniliprole and Deltamethrin.

Solutions	Abamectin		Chlorantraniliprole		Deltamethrin	
	Conc. (ppm)	Area	Conc. (ppm)	Area	Conc. (ppm)	Area
Std. A	0.015	21192	0.5	41381	0.05	41724
Std. B	0.015	21408	0.5	42629	0.05	42499
Std. C	0.03	23447	1	63538	0.1	70620
Std. D	0.09	27961	3	169487	0.3	138732
Std. E	0.18	34529	6	315902	0.6	235630
Std. F	0.24	39265	8	426374	0.8	289399
ULOQ	0.3	44250	10	509690	1	359604

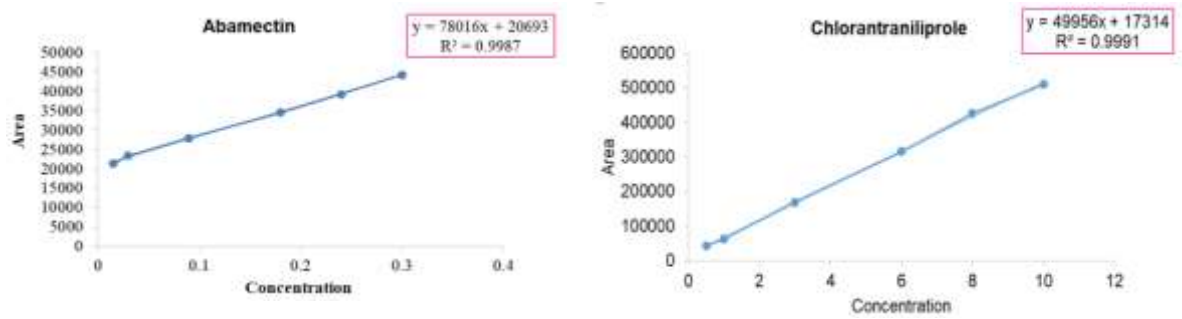


Figure 9. Linearity Graph of Pesticides.

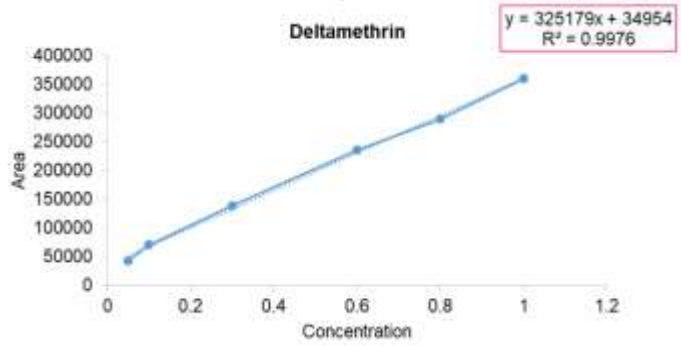


Figure 10. Linearity Graph of Deltamethrin.

The calibration data for abamectin, chlorantraniliprole, and deltamethrin exhibited outstanding linearity, as detailed in Table 6. The linear regression model effectively represented the data, achieving a maximum R^2 value (approaching 1), which signifies a linear relationship between concentration and response (area). The slopes derived from the linear regression analysis were 78016, 49956, and 325179, respectively, confirming that the method is suitable for quantitative analysis within this concentration range. The method's linearity guarantees its reliability for the routine analysis of these three drugs.

3.2.3 Accuracy

Accuracy and precision were calculated using a minimum of five determinations of each drug per 3 determinations (LQC, MQC, and HQC). The outcomes obtained from this study are arranged in Table 7-9.

Table 7. Calculated accuracy and precision of abamectin.

Abamectin			**Standard Deviation**		**Accuracy**	**Precision**
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
LQC	1	22143	22408.2	297.9793617	1.32977821	1.33
	2	22400				
	3	22462				
	4	22877				
	5	22159				
MQC	1	27978	27637.4	311.8666382	1.12842249	0.176712391
	2	27861				
	3	27528				
	4	27178				
	5	27642				
HQC	1	44916	44507.2	435.0835552	0.97755769	0.98
	2	44072				
	3	44616				
	4	44905				

	5	44027

Table 8. Findings of accuracy and precision of chlorantraniliprole.

Chlorantraniliprole			**Standard Deviation**		**Accuracy**	**Precision**
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
LQC	1	63161	63439.4	286.4005936	0.45145539	0.45145539
	2	63776				
	3	63559				
	4	63582				
	5	63119				
MQC	1	234131	234954.8	773.2281035	0.32909653	0.087222665
	2	235418				
	3	235029				
	4	234246				
	5	235950				
HQC	1	502607	506371	2521.495885	0.49795424	0.49795424
	2	508884				
	3	505071				
	4	507697				
	5	507596				

Table 9. Indicating of accuracy and precision of Deltamethrin.

Deltamethrin			Standard Deviation		Accuracy	Precision
Sr. No.	**Conc.**	**Area**	**Mean**	**SD**	**%SD**	**%RSD**
1	LQC 1	57563	59156.8	2275.854279	3.84715583	3.85
	LQC 2	56496				
	LQC 3	60406				
	LQC 4	62244				
	LQC 5	59075				
2	MQC 1	146215	139944.6	5710.416561	4.08048368	1.233900595
	MQC 2	144147				
	MQC 3	138928				
	MQC 4	131494				
	MQC 5	138939				
3	HQC 1	359299	362226.2	6651.25298	1.83621532	1.84
	HQC 2	365770				
	HQC 3	364454				
	HQC 4	352236				
	HQC 5	369372				

A total of three samples of LQC, MQC, and HQC were used to identify the accuracy and precision of the method discussed above. The LQC, MQC, and HQC results were very the low values of SD and RSD. The results are ideal as they indicate that there was very good precision and replicability during the repeated measurement of the pesticide quantification. For abamectin, at LQC, the mean area was 22408.2, with an SD of 297.98 and RSD of 1.33% identified. At MQC, the precision was even better, with an RSD of 0.18%. The HQC also recorded a good RSD of 0.98%. For chlorantraniliprole, the RSD was LQC (0.45%), MQC (0.08%) and HQC (0.49%). For deltamethrin, the RSD was LQC (3.85%), MQC (1.23%) and HQC (1.84%). It is evident that this method provides very good accuracy, more so when quantifying the analytes at varying concentrations.

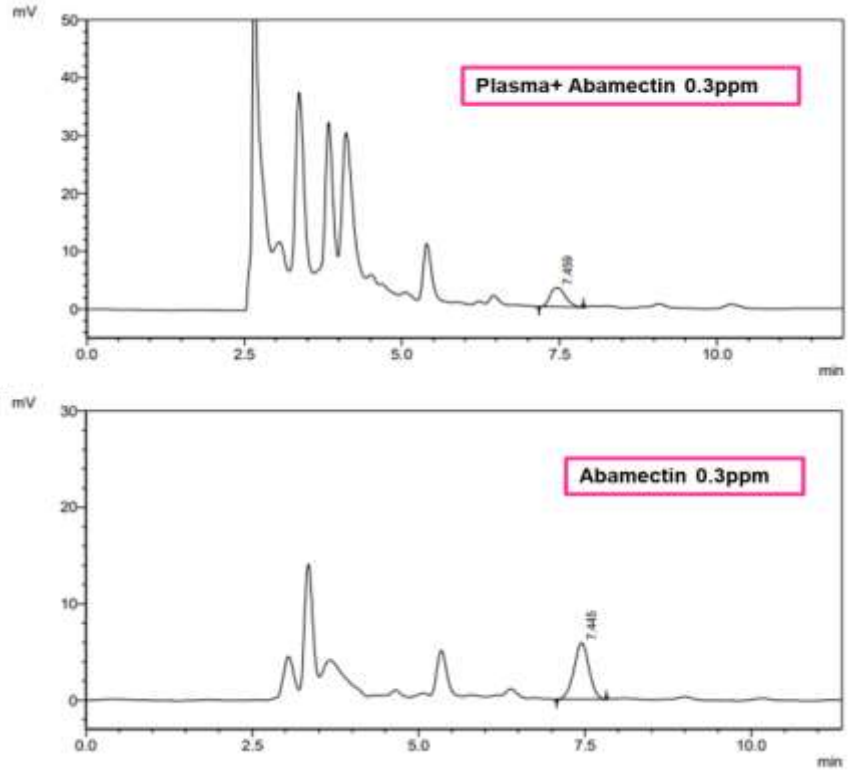
3.2.4 Percentage Recovery

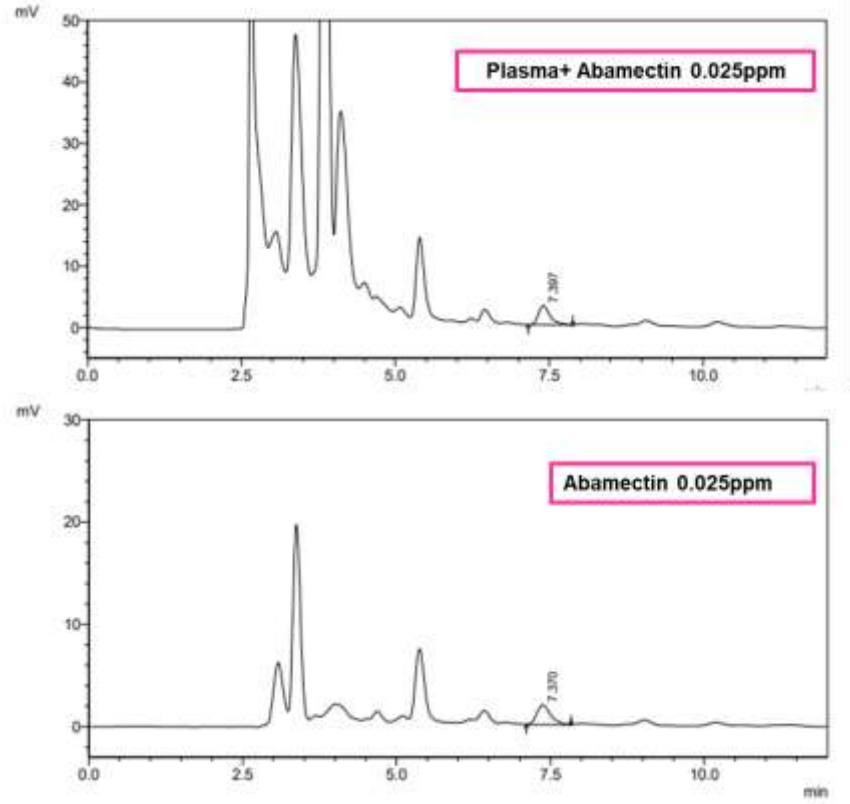
The recovery of all pesticides was assessed at different concentrations (LQC, & HQC level) by comparing the area of the standard to that of the extracted sample. The data obtained from this study are summarized in Table 10.

The recovery values of abamectin at 0.3 and 0.025 ppm were found to be 60.47% and 61.87% respectively. Whereas for chlorantraniliprole, the % recovery was 54.74% and 66.32% accordingly. For deltamethrin, it was shown at 53.86% and 49.87% at 1 and 0.025 ppm respectively. While these recovery rates are slightly lower than expected (typically close to 100%), they still suggest that the extraction process was reasonably efficient. The possible factors contributing to the lower recovery could include matrix effects, loss of analyte during sample preparation, or instrumental limitations.

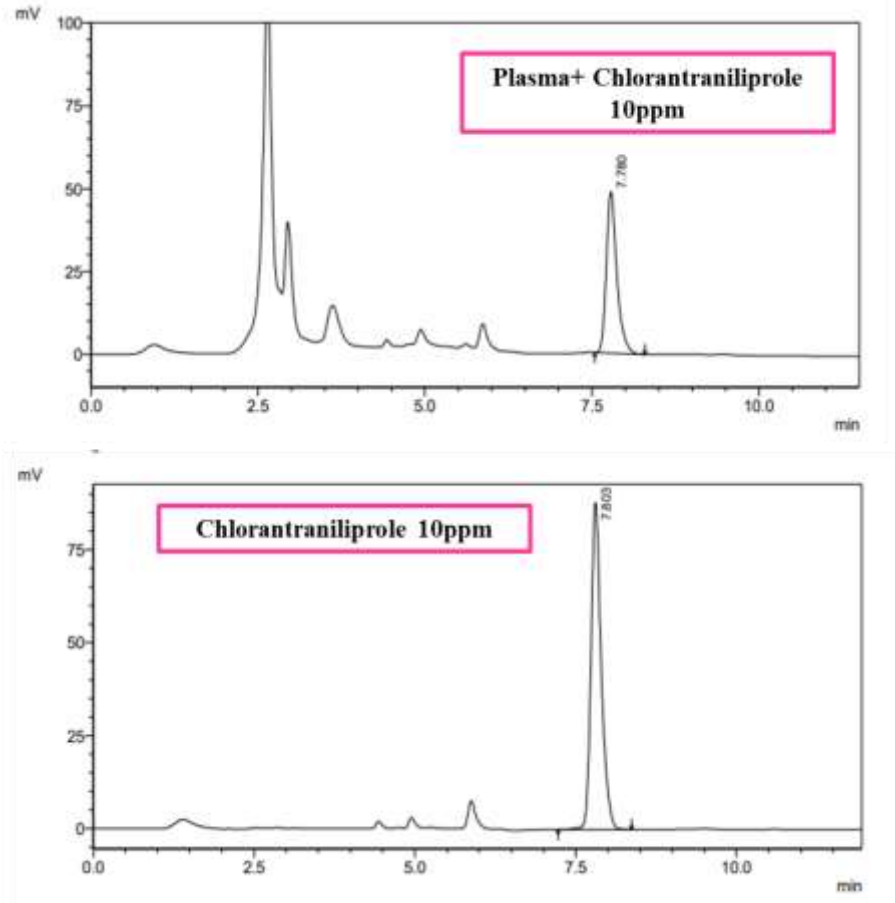
Table 10. Percentage recovery of pesticides.

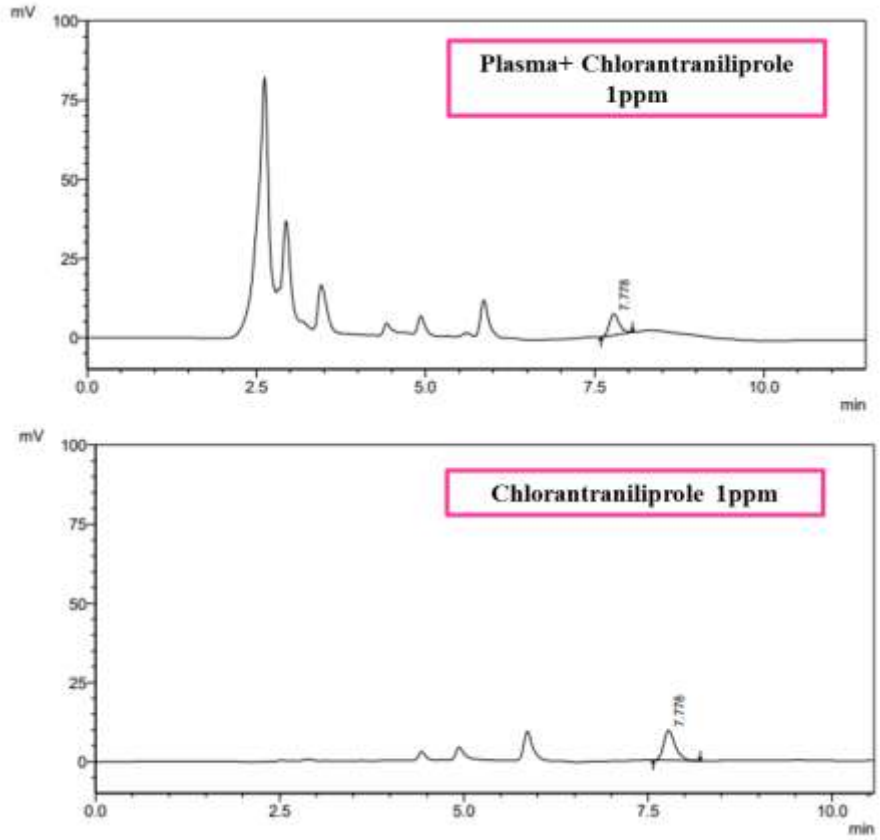
% Composition	Area of Standard	Area of Extracted Sample	% Recovery
Abamectin			
0.3ppm	73797	44628	60.47400301
0.025ppm	41827	25880	61.8739092
Chlorantraniliprole			
10ppm	973335	532804	54.74004325
1ppm	102259	67822	66.32374657
Deltamethrin			
1ppm	655270	352977	53.86741343
0.025ppm	46557	23221	49.87649548



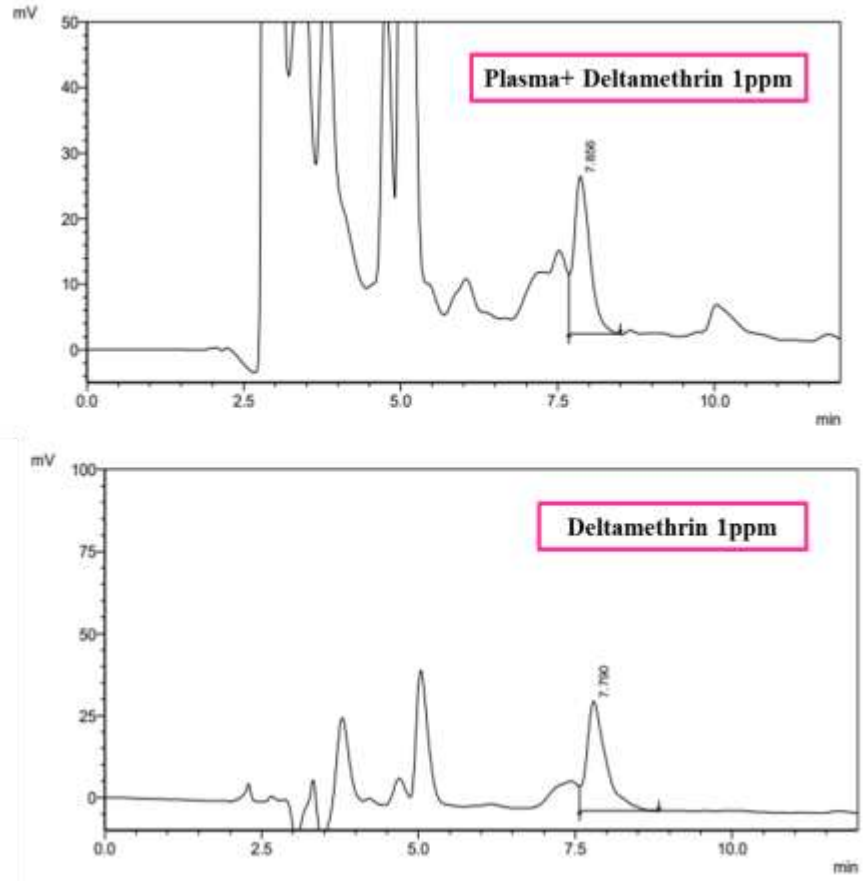

mV
Plasma+ Abamectin 0.025ppm
7.397
mV
Abamectin 0.025ppm
7.370
min

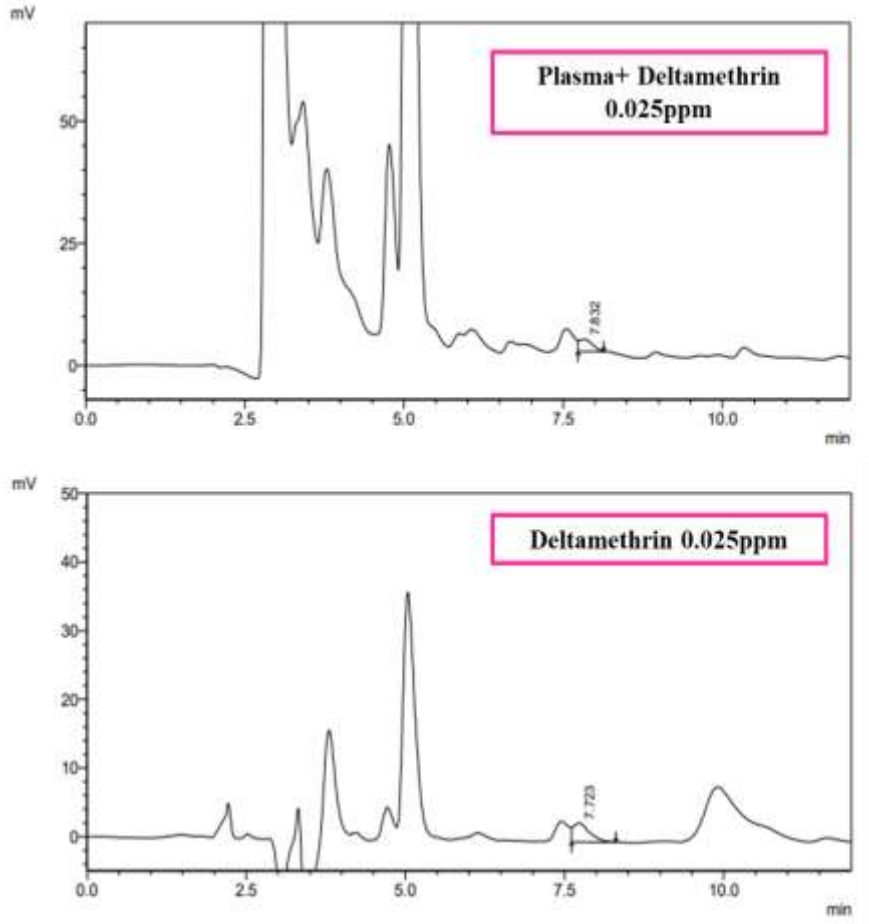
(a)


mV
Plasma+ Chlorantraniliprole 10ppm
7.780
min
mV
Chlorantraniliprole 10ppm
7.803
min


mV
Plasma+ Chlorantraniliprole 1ppm
7.778
min
mV
Chlorantraniliprole 1ppm
7.776
min

(b)


mV
Plasma+ Deltamethrin 1ppm
7.856
min
mV
Deltamethrin 1ppm
7.790
min



(c)

Figure 11. % Recovery of Abamectin (a), Chlorantraniliprole (b), and Deltamethrin (c)

3.2.5 Limits of Detection and Quantification

The LOD and LOQ for 3 pesticides were calculated on the basis of the SD and slope from the linearity data. The findings from this analysis is mentioned in Table 11.

Table 11. LOD & LOQ Values of Pesticides.

Drug	SD	Slope	LOD (ppm)	LOQ (ppm)
Abamectin	297	78016	0.012562808	0.038069114
Chlorantraniliprole	286	49956	0.018892626	0.05725038
Deltamethrin	2275	325179	0.023095953	0.069987736

The LOD and LOQ of abamectin were observed at 0.0126 and 0.0381 ppm, whereas for chlorantraniliprole, 0.019 and 0.057, and deltamethrin, it was revealed at 0.023 and 0.07 ppm, respectively. These values demonstrate that the method is highly sensitive and capable of detecting and quantifying these pesticides at very low concentrations.

3.3 DISCUSSION

The identification study for the abamectin, chlorantraniliprole, and deltamethrin confirmed the identity of the compound using the physicochemical characterization process. The HPLC quantification identifies that the method is very reliable, precise, and has high sensitivity. The method is highly linear L.R^2 that is near 1 confirmed a high relationship between concentration and detector response. Low SD and RSD show how the LQC is accurate while low SD and RSD The low RSD shows high precision and high similarities while from the LOD and LOQ results, the method provides a traceable outcome. The pretreatment did not cause a lot of lost for that can be accounted for by the values for the recovery study. The quantities are slightly below because of the data above showing that the accuracy. There is no deviation for the System suitability shows that the method will produce quality results with the given case from the system suitability study. The recovery values and system suitability is the best alternative to validate. The linearity, precision, and accuracy have been established from the RSD value and SD respectively.

4. CONCLUSION

This study presents a HPLC method developed and validated according to the strict FDA requirements for bioanalytical validation. Upon successful validation, the method can be considered reliable and reproducible. The linearity occurred within a wide range of concentrations as indicated by R^2 values close to 1; this establishes confidence in the method's ability to accurately quantify the concentrations. The precision was strong, as demonstrated through quality control tests with low variability between runs, indicating that the method is robust. Recovery tests showed that the complex plasma matrixes were efficient in extracting the 3 pesticides in sufficient amounts. Moreover, the detection and quantification limits were in parts per million, pointing to the high sensitivity of the method even in identifying trace amounts of the pesticides. Complementary tests, such as UV and FTIR spectroscopy further confirm the identity and purity of the substances, hence increasing specificity. In general, this method provides high confidence in the results of measurements, which is feasible for regular, non-demanding toxicology, exposure, and pharmacokinetic studies with the 3 pesticides. Consequently, the sensitivity and specificity enable a more accurate identification of residues, increasing regulatory compliance, and protection of the public.

Statements and Declarations

Ethical Approval: Not applicable.

Informed Consent: Not applicable.

Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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