Research Article

Analysis Of Imidacloprid And Diafenthiuron Pesticides Residue In Cauliflower Using Rp-Hplc

Priya Dharshini M*[1], Umamaheswari D[2], Kavinesh[3], Keerthana. B[3], Kaviya. R[3]

[1]* Assistant Professor, Department of Pharmaceutical Chemistry, Vinayaka Missions College of Pharmacy VMRF (DU), Salem, Tamil Nadu, India.
[2]Professor, Department of Pharmaceutical Chemistry, Vinayaka Missions College of Pharmacy VMRF (DU), Salem, Tamil Nadu, India.
[3]Vinayaka Missions College of Pharmacy VMRF (DU), Salem, Tamil Nadu, India.

Abstract

This study developed and optimized a reverse phase performance liquid chromatography (RP-HPLC) method to measure residues of the insecticides imidacloprid and diafenthiuron in cauliflower. Chromatographic separation was achieved using a C18 column. For imidacloprid, the mobile phase was acetonitrile: water (35:65), while diafenthiuron was analyzed using methanol: water (80:20). The method was validated to quantify pesticide levels down to the relevant regulatory thresholds. Measured values were compared with the maximum residue limits (MRLs): 0.4 ppm for imidacloprid and 0.02 ppm for diafenthiuron. In all tested cauliflower samples, both pesticide residues were found to be within these MRLs. The RP-HPLC procedure proved to be straightforward, reliable and accurate for routine monitoring of these compounds in cauliflower. By delivering sensitive and reproducible measurements, the method supports quality control and food- safety assessments and helps ensure that cauliflower entering the market meets established safety standards. Overall, the optimized RP-HPLC approach offers a practical tool for laboratories tasked with surveillance of pesticide residues, contributing to consumer protection and regulatory compliance in vegetable production and distribution.

Keywords: Imidacloprid, Diafenthiuron, Pesticides Residue, RP-HPLC

1. Introduction

The use of pesticides is unavoidable in agricultural practices. This class of chemicals is highly toxic for the environment as well as for humans. The present work was carried out to assess the presence of pesticide.[1]

The monitoring of proper investigation of pesticide residues in fruits and vegetables have great importance to consumer health protection, and the development or usage of robust and economical analytical methods is of great interest.[2] Pesticides polluting the earth and causing problem in human beings and wildlife, the quantity of pesticides being consumed becomes necessary knowledge. Analytical quality requirements like trueness, precision, sensitivity and selectivity have been met suit the need for any analysis.[3]

Chromatography is separation of a mixture into individual components using a stationary phase and a mobile phase. The stationary phase may be gaseous or liquid. Chromatographic separation relies on relative movement of two phases. In chromatography one phase is fixed (stationary phase) and other is mobile (mobile phase) the mobile phase passes over the stationary phase. The separation of components is a result of the differential affinity of the components for the mobile phase and a stationary phase.[4]

Pesticides have been linked with wide range of health hazards ranging from short term effects such as headaches, and nausea to chronic ailments like cancer, reproductive harm and endocrine disruptions. Acute dangers include nerves, skin eye irritation and damage, dizziness, fatigue, and systematic poisoning. Chronic health effects may occur years after even exposure to pesticides in environment or result from pesticides residues which we take through food and water. It has been linked to all kind of diseases and conditions like Parkinsons syndrome to cancer. Impacts on environment have been well known. Too much pesticide use can damage agricultural land by harming beneficial insects' species, soil microorganisms and worms which naturally limit pest populations and maintain soil health. It will also reduce concentration of essential nutrients in soil like nitrogen and phosphorous. Pesticides can also reach surface water through runoff from treated plants and soils. Contamination of water by pesticides is widespread.[5]

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. The technique of High-Performance liquid chromatography is so called because of its improved performance when compared to column chromatography: Advances in column chromatography into high speed, efficient, accurate and highly resolved method of separation [6-7]

The HPLC is the method of choice in the field of analytical chemistry.[8]

Most laboratories that deal with a need for analyzing chemical mixtures are equipped for HPLC; it is often the first choice of technique.[9]

2. Materials And Methods:

2.1. Chemicals and Reagents Used:

The study requires only those chemicals that are HPLC grade. HPLC grade water, Methanol, acetonitrile. The study also includes other chemicals sodium sulfate and n-hexane.

2.2.Working Standard and Sample Used:

Equipment used:

Denver Instrument analytical and precision balance, waters 2998 HPLC, PCI Analytics ultrasonic processor and Weibfr PH meter.

2.3. Preparation Of Solutions

Preparation of mobile phase:

The formulation of the mobile phase was done in a clean and dry container into which acetonitrile and water were mixed in the ratio of 35:60 (v/v). The solvents were mixed thoroughly to obtain uniform mobile phase. The prepared mobile phase was subjected to sonification for 10-15 minutes using an ultrasonic bath to remove dissolved gases. After sonication, the mobile phase was filtered to eliminate particulate matter by using a 0.45 µm membrane filter. After the process of filtration, the mobile phase was transferred into HPLC reservoir and used for chromatographic analysis.

In the preparation of mobile phase of Diafenthiuron methanol and water were mixed in the ratio of 80:20 (v/v) in a clean and dry container. The solvents were mixed thoroughly to obtain uniform mobile phase. The prepared mobile phase was subjected to sonification for 10-15 minutes using an ultrasonic bath to remove dissolved gases. After sonication, the mobile phase was filtered to eliminate particulate matter by using a 0.45µm membrane filter. After the process of filtration, the mobile phase was transferred into HPLC reservoir and used for chromatographic analysis.

Standard preparation for Imidacloprid:

A clean and dry volumetric flask was used for standard preparation. A volumetric flask was filled with 0.16g of standard imidacloprid after it had been weighted. About 100ml of acetonitrile was added to the flask to dissolve the standard. Transfer 20ml of this solution via pipette to 100ml volumetric flask, then fill it off with acetonitrile. Shake well until homogeneous solution is obtained.

Standard preparation for Diafenthiuron:

Prepare a 100ml volumetric flask with precisely 0.16g of diafenthiuron reference standard of known purity. Use the methanol to dissolve and replenish the volume. Pour 20ml of this solution into 100ml volumetric flask using a pipette, then fill it over with methanol and water in the ratio of 80:20. To homogenize, give it a good shake.

Formulation of sample Imidacloprid solution:

To prepare the sample solution, weight out 5ml of the sample into a clean and dry 50ml volumetric flask for preparation of sample solution. Dissolve and makeup the volume with acetonitrile. Pipette out 1ml of this solution and transfer to 50ml volumetric flask and fill it over with acetonitrile. Shake well until homogeneous solution is formed.

Formulation of sample Diafenthiuron solution:

Fill a 100ml volumetric flask with the sample that weights 0.16g of diafenthiuron. Use the methanol to dissolve and replenish the volume. Fill a 100ml volumetric flask with 20ml of this solution using a pipette, then top it off with methanol.

Optimized chromatographic condition for Imidacloprid:

Column	**SPOLAR C_{18} column**
Pump mode	Isocratic
Flow rate	1.2ml/min
Detection	270nm
Volume of injection	20µL
Column Temperature	30℃

Optimized Chromatographic Condition for Diafenthiuron:

Column	NUCLEOSIL C_{18}
Pump mode	Isocratic
Flow rate	0.5ml/min
Detection	256nm
Volume of injection	10µl
Column Temperature	30℃

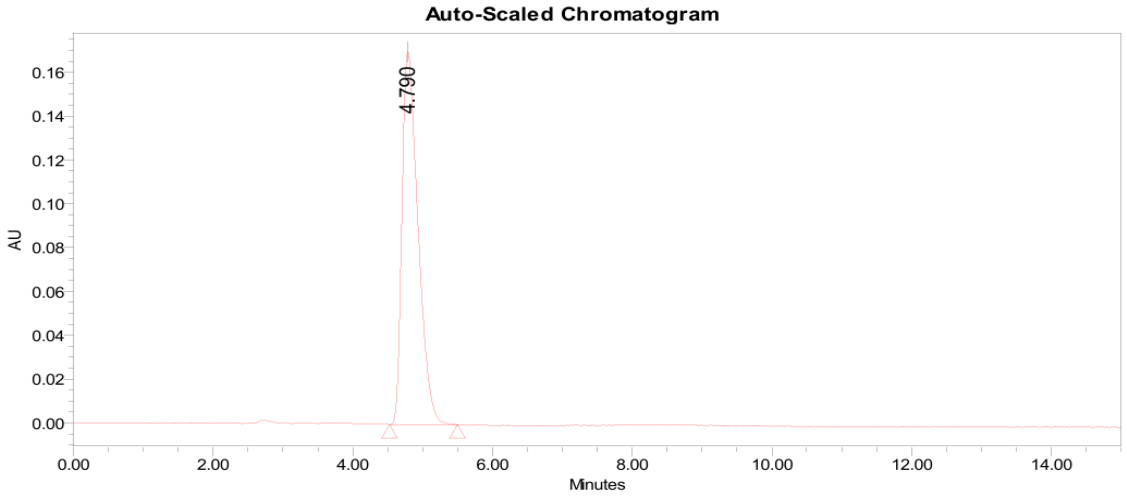

Figure no 1: Chromatogram of standard imidacloprid

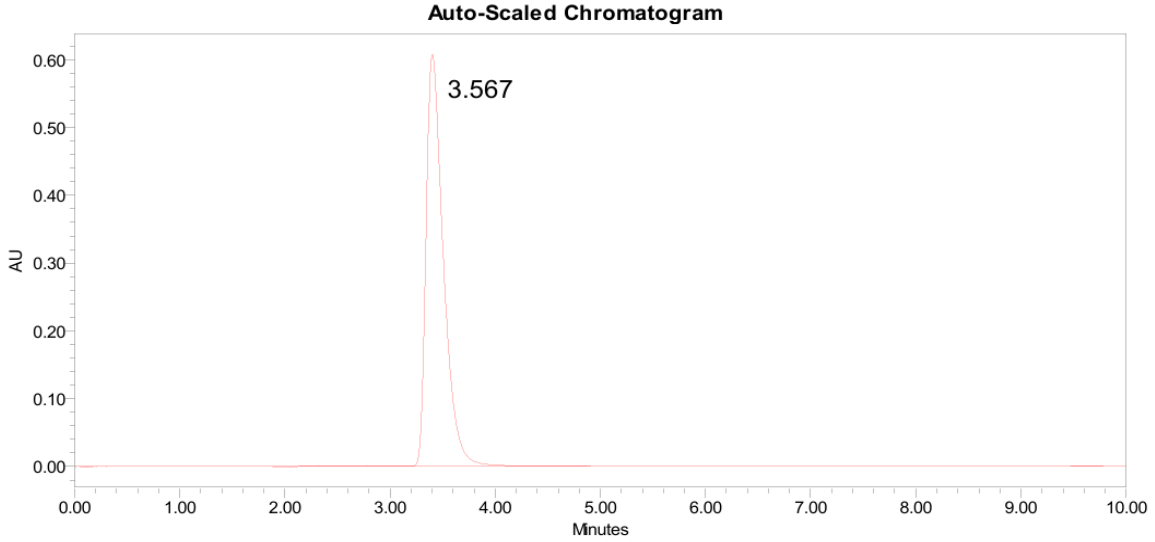

Figure no 2: Chromatogram of sample solution

Calculation:

Imidacloprid:

$$\textbf{Imidacloprid content}\ \% = \frac{\textbf{Weight of std}}{\textbf{Weight of sample}} \times \frac{\textbf{Area of Sample}}{\textbf{Area of standard}} \times \textbf{Purity of Std}$$

$$\textbf{Imidacloprid content}\ \% = \frac{\mathbf{0.16 \times 1800000 \times 98}}{\mathbf{0.26 \times 2706622}}$$

= 40.1

= 40 %

Formula (External Standard Method)

$$\text{Concentration of sample} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{Concentration of standard}$$

Step 1: Calculate concentration in test solution

$$= \frac{180000}{2706622} \times 0.32$$

$$= 0.02\ \mu g/mL$$

Step 2: Calculate imidacloprid content in cauliflower

- Weight of cauliflower taken = **25 g**
- Final volume of extract = **50 mL**

$$\text{Amount of imidacloprid} = \frac{0.02 \times 50}{25}$$

$$= 0.04\ \mu g/g$$

$$= 0.04\ mg/kg\ (ppm)$$

DIAFENTHIURON:

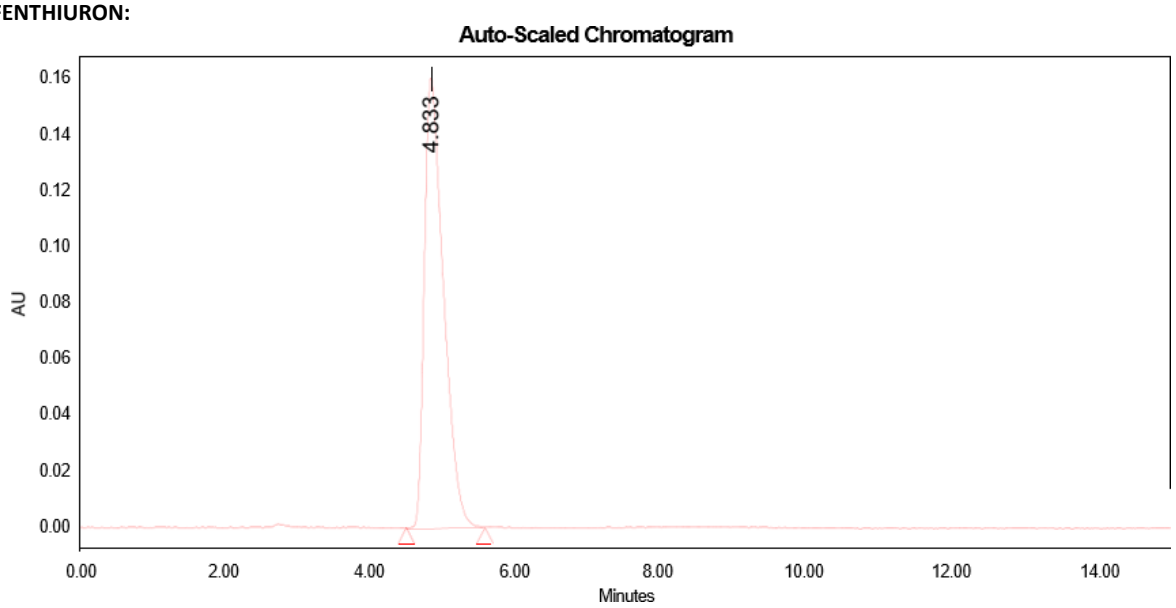

Figure no 3: Chromatogram of standard diafenthiuron

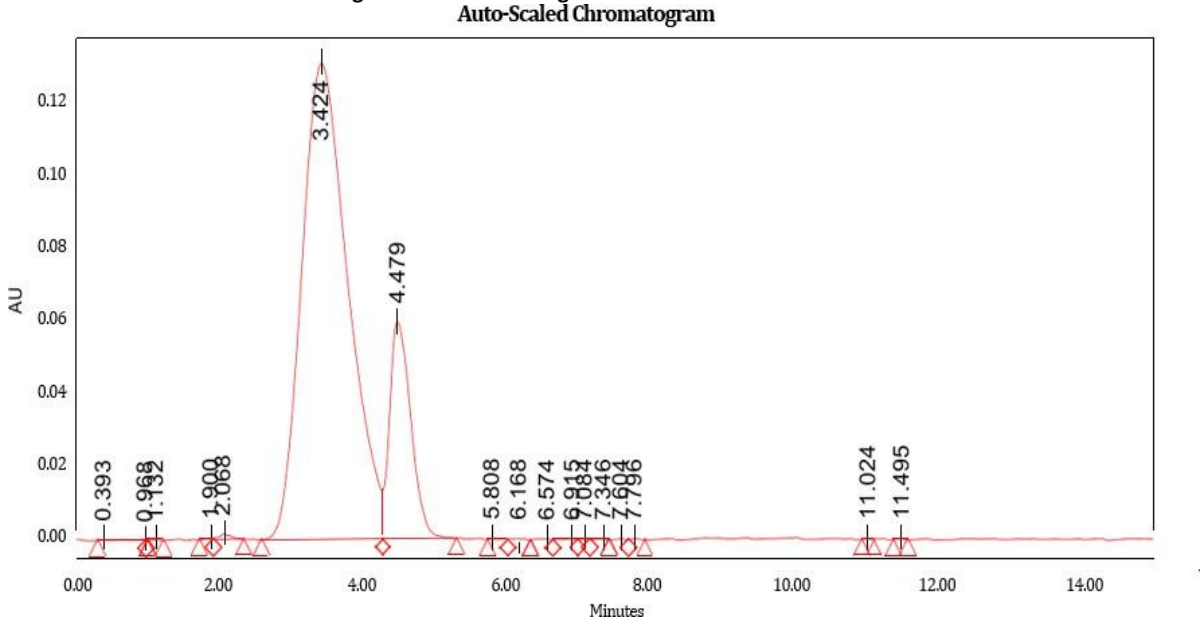

Figure no 4: Chromatogram of sample solution

Calculation:
Diafenthiuron:

$$\textbf{Diafenthiuron content}\ \% = \frac{\textbf{Weight of std}}{\textbf{Weight of sample}} \times \frac{\textbf{Area of Sample}}{\textbf{Area of standard}} \times \textbf{Purity of Std}$$

$$\textbf{Imidacloprid content}\ \% = \frac{0.16 \times 124209 \times 98}{0.25 \times 2920651}$$

= 26.6

= 27 %

Formula (External Standard Method)

$$\textbf{Concentration of sample} = \frac{\textbf{Peak area of sample}}{\textbf{Peak area of standard}} \times \textbf{Concentration of standard}$$

Step 1: Calculate concentration in test solution

$$= \frac{124209}{2920651} \times 0.32 = 0.0136$$

$$= 0.01\ \mu g/mL$$

Step 2: Calculate imidacloprid content in cauliflower

- Weight of cauliflower taken = **25 g**
- Final volume of extract = **50 mL**

$$\text{Amount of imidacloprid} = \frac{0.01 \times 50}{25}$$

$$= 0.02 \frac{\mu g}{g}$$

$$= 0.02 \frac{mg}{kg}$$

3. Result And Discussion:

Cauliflower samples were dried and extracted, and analyzed by RP-HPLC. Residue identification and quantification were performed by comparing the sample chromatograms with those of known standard using external standard method. The method produced well- resolved chromatograms with consistent retention times for both standards and samples, indicating good specificity and reproducibility.

Imidacloprid Residue

Imidacloprid was detected at 0.04mg/kg in the analyzed cauliflower sample. This concentration is substantially below the established MRL of 0.4 mg/kg, indicating that the measured imidacloprid level falls within the permissible regulatory limit.

Diafenthiuron Residue

Diafenthiuron was detected at 0.02 mg/kg (0.02µg/g). This value is at the reported MRL of 0.02 mg/Kg, indicating compliance with the applicable maximum residue limit.

The RP-HPLC procedure demonstrated satisfactory resolution, repeatability and specificity for the simultaneous determination of imidacloprid and diafenthiuron in cauliflower.

Quantification by the external standard method yielded reliable residue estimates for both pesticides, supporting the method's suitability for routine monitoring of pesticide residues in vegetable samples.

4. Reference:

1. Mujahid M, Latif S, Ahmed M, Shehzadi W, Asari A, Jehangir M, et al. Modified matrix solid phase dispersion-HPLC method for determination of pesticide residue in vegetables and their impact on human health: a risk assessment. Front Chem. 2022;10:1084350. doi:10.3389/fchem.2022.1084350.
2. Oellig C, Schmid S. Polyethyleneimine as weak anionic exchanger adsorbent for clean-up in pesticide residue analysis of fruits and vegetables. Journal of Chromatography A. 2019 Jul 19;1597:9-17.
3. Ng B, Quinete N, Gardinali PR. Assessing accuracy, precision and selectivity using quality controls for non-targeted analysis. Science of the Total Environment. 2020 Apr 15;713:136568.
4. Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations. Unique Publishers; 1985.

5. Ahmad MF, Ahmad FA, Alsayegh AA, Zeyaullah M, AlShahrani AM, Muzammil K, Saati AA, Wahab S, Elbendary EY, Kambal N, Abdelrahman MH. Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon. 2024 Apr 15;10(7).
6. Sharma BK. *Instrumental methods of chemical analysis*. 16th ed. Meerut: Goel Publishing House; 1999. p. 145-148.
7. Cazes J, Scott RPW. *Chromatography: theory and practice*. New York: Marcel Dekker Inc.; 2002. p. 4-5.
8. Swadesh JK. *HPLC: Practical and Industrial Applications*. Boca Raton (FL): CRC Press; 1997. p. 20-22.
9. Kumar N, Narayanan N, Gupta S. Ultrasonication assisted extraction of chlorpyrifos from honey and brinjal using magnetic molecularly imprinted polymers followed by GLC-ECD analysis. *React Funct Polym*. 2019;135:103-112. doi:10.1016/j.reactfunctpolym.2018.12.012