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A simple RP-HPLC method for determining imidacloprid residues in goat tissues.

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Abstract

The present work is aimed to develop a rapid, simple, sensitive and reliable reverse phase high performance liquid chromatography (RP-HPLC) method of imidacloprid and its residues in different substrates of goat tissues. Separation was achieved using C-18 column with mobile phase consisting of acetonitrile and purified HPLC grade water. The method consists of extracting with acetonitrile from different tissues and cleans up with acetonitrile and hexane (50%) mixture. Quantification was performed by reverse phase (RP) HPLC with PDA detector. The chromatogram showed a well-resolved peak of imidacloprid, and the retention time was 3.30 minutes. The limit of detection and limit of quantification were 0.01 µg/mland 0.04 µg/ml respectively and recovery from different substrates was 92-98%. Therefore, the method is satisfactory and proved to be very precise.

Keywords: High performance liquid chromatography (HPLC), Imidacloprid Residue, Goat, Tissues.

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Introduction

Imidacloprid belongs to a new chemical class of active ingredient. It has a new mode of action, outstanding biological efficacy, a broad spectrum of activities, low toxicity to warm-blooded animals [1]. Imidacloprid is a systemic insecticide, chemically belonging to the neonicotinoid group and acts on the nervous system. It acts by disrupting nicotinic acetylcholine receptors in the central nervous system [2]. The selective toxicity of imidacloprid to insects and not to mammals is attributed to differences in the binding affinity or potency at nicotinic acetylcholine receptors [3]. Since being introduced in the insecticide market in 1992, the use of imidacloprid has increased over the years. It is a category II acute toxicant and thus, is classified as a General Use Pesticide [4]. In veterinary medicine, it is used as a flea control agent on dogs and cats [5]. Indiscriminate usage of pesticides in agriculture is leading to contamination of the environment and natural resources. It is being greatly criticized due to their persistence in the environment and their accumulation in the living tissues of organisms and thereby, producing an adverse impact on animal and human health [6]. Repeated exposure to the insecticide may leave some residue in tissues. Therefore, it is essential to validate a method for estimation of residues of imidacloprid in animal tissues. The literature on determination of imidacloprid residues in plants and vegetables are many but regarding residue estimation of imidacloprid in animal is scarcely available. Therefore, the present work was undertaken to find out an easy and simple method for determination of imidacloprid residues in animal tissues. The method consists of reversed phase by high performance liquid chromatography with photo diode array (PDA) detector.

Materials and Methods

Analytical reference standard of imidacloprid was obtained from M/s United Phosphorus Limited Mumbai, India. The purity of imidacloprid was checked to be of ≥ 98.5% purity. All other chemicals and solvents used in the study was analytical and HPLC grade.

Collection of samples

Three adult goats weighing 10-12 kg were used for the experiment. Three samples of each tissue viz. blood, skeletal muscle, liver and kidney were collected from three animals. Blood samples (5 ml each) were collected from the left jugular vein in heparinized test tubes; whereas 1 gm of tissue from skeletal muscle, liver, kidney were collected following slaughter of animals.

This study was approved by the Institutional Animal Ethics Committee, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata, India.

Fortification of samples

Three numbers of samples of each tissue from three different animals was collected for analysis of imidacloprid. Recovery percentage of imidacloprid from different tissues was carried out to ascertain the reliability of the analytical method after fortifying the different tissues namely, blood plasma, liver, kidney and skeletal muscle with technical grade imidacloprid of different spike concentration e.g., 0.01, 0.1, 1.0, 5.0, 10.0, 12.5 μg/ml. After necessary work up, the concentrations of imidacloprid from different tissues was analyzed by high performance liquid chromatography (HPLC) [7-8] equipped with PDA detector.

Extraction

Blood

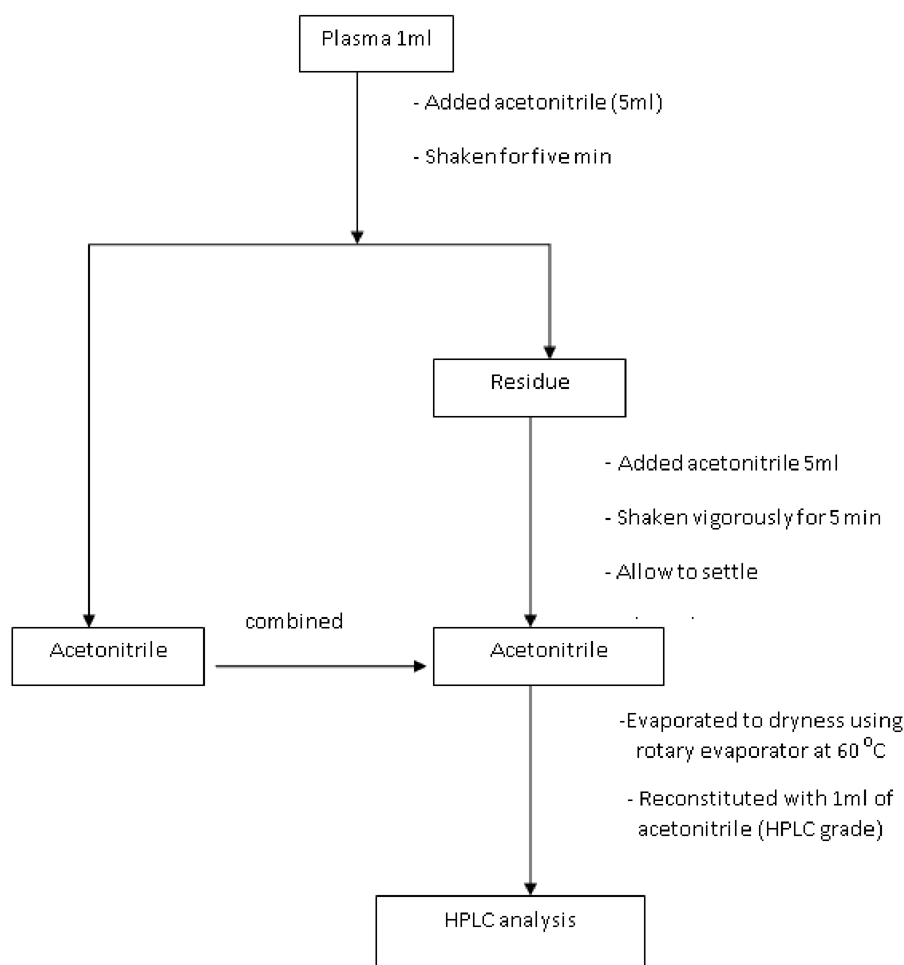
Blood samples (5 ml each) were collected from the left jugular vein in heparinized test tubes. Plasma was separated by centrifugation at 3000 rpm for 20 minutes. The blood samples were subjected to liquid-phase extraction [9]. To a sample of plasma (1 ml), acetonitrile (5 ml) was added, shaken for 5 min, filtered through filter paper no. 1. This process was repeated thrice. The total filtrate was evaporated to dryness using rotary vacuum evaporator. The final volume was made up by HPLC grade acetonitrile (1 ml) for subsequent HPLC analysis (Scheme I) [10].

Tissues: Liver, kidney, skeletal muscle.

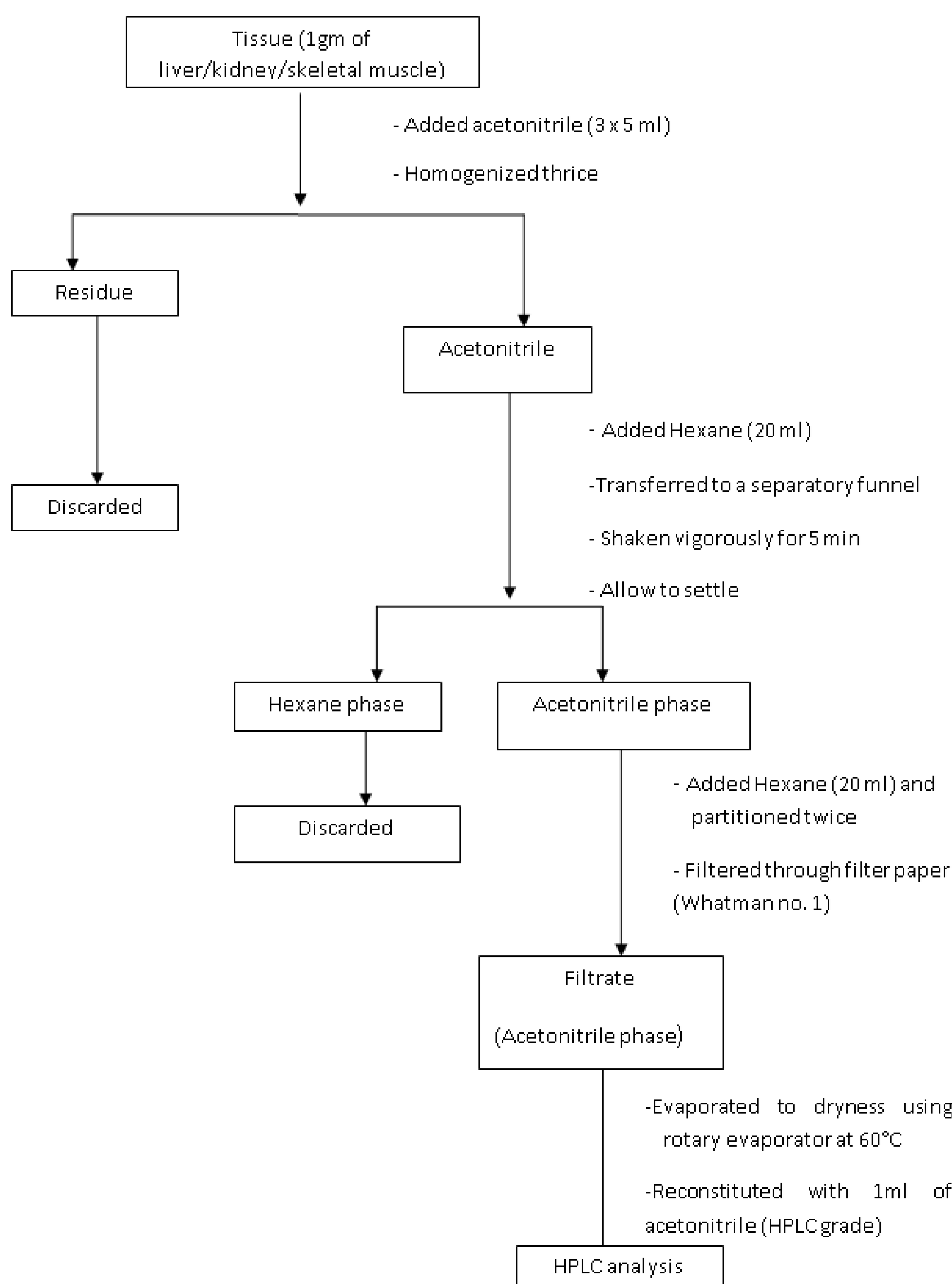
The tissue (1 gm) was first minced with scissors and then homogenized in Remi-tissue homogenizer (RQ-127A) for 4 minutes with acetonitrile (5 ml). The homogenate was filtered through filter paper (Whatman No. 1) and the remnants of tissue were re-homogenised and re-extracted twice with acetonitrile (5 × 2 ml) and filtered through above filter paper (Scheme II) [10].

Clean up

The acetonitrile filtrates were transferred to a clean, dry separatory funnel and n-hexane was added to it (1:1). The mixture was shaken vigorously for 2 min and allowed to settle (2 min) till the two phases were distinctly separated. The lower acetonitrile phase was collected in another separatory funnel and re-partitioned twice with n-hexane. The acetonitrile phase was then collected in another conical flask and evaporated to dryness using rotary vacuum evaporator. Final volume was made to 1 ml with acetonitrile (HPLC grade). The sample was filtered through 0.20μ membrane filter before injection to HPLC (Scheme II) [10].



Scheme 1: *Extration and analysis of imidacloprid from plasma.*



***Scheme II:** Extraction and analysis of imidacloprid from tissues.*

Estimation of Concentration of insecticide

Apparatus: SHIMADZU LC-20 AT liquid chromatogram coupled with Photo Diode-Array detector attached with computer SPD-MXA 10 software.

HPLC condition for Imidacloprid

- Mobile phase: Acetonitrile and Water (90:10). This mixture was subjected to membrane filtration.
- Column : 5µ Luna C_{18} (2); 250 × 4.6 mm (RP)
- Flow rate : 1 ml/min
- Wave length : 270 nm
- Retention time: 3.30 min

Estimation of Imidacloprid

Standard and sample (20 µl each) were injected by Hamilton Syringe into the injector port of liquid chromatography with the first and last being the standard. The residues were estimated after comparing with external standard.

The concentration of Imidacloprid in blood and tissue was calculated using the following equation:

Concentration of Imidacloprid in blood (µg/ml) = $a_2 \times v_2 \times C / a_1 \times v_1$

Residue of Imidacloprid in tissues (µg/gm) = $a_2 \times v_2 \times C / a_1 \times g$

a_1=area of standard chromatogram

a_2=area of sample chromatogram

V_2=Final volume of sample after processing (ml)

V_1=Volume of plasma and urine taken for processing (ml)

C=concentration of standard

g=amount of tissue taken for processing

Concentration (µg/ml)	Mean peak area*
0.01	1008.33
0.1	11228.66
1	115554.33
5	566457
10	1113334
12.5	1456101.66

*Mean of three replicates

Table 1: *Data for linearity Imidacloprid in solvent.*

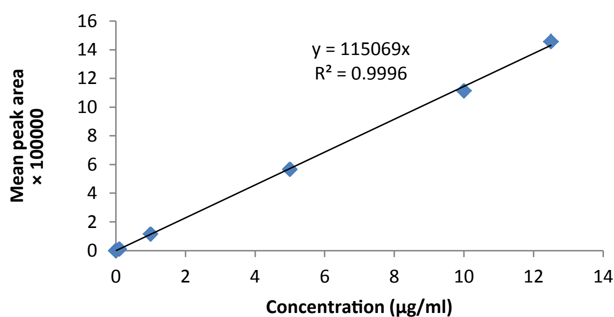


Figure 1: *Detector linearity Imidacloprid in solvent.*

Results and Discussion

The linearity of the detector response was tested for imidacloprid in solvent over the range of 0.01 µg/mlto 12.50 µg/ml (Table 1). A very precise linear relation between the injected amount and the resulting peak area was observed over the entire range with correlation coefficient between 0.9958 and 0.9996 (Figure 1). Imidacloprid estimated by reverse phase high performance liquid chromatography (RP-HPLC). The chromatogram (Figures 2 and 3) showed a well resolved peak of imidacloprid and the retention time was at 3.30 minutes under the operating condition of the chromatogram. The method is satisfactory and subsequently used for extraction, clean up and estimation of imidacloprid from blood, and other tissues of goat (Schemes I and II). A summary of the obtained recovery values is given in Table 2. The repeatability of the method was determined by running a set of three recoveries. The resulting mean recovery rates ranged from 93 to 97% with relative standard deviations (RSD) between 0.52% and 0.88% (Table 2). Limit of detection (LOD) was 0.01 µg/ml and limit of quantification (LOQ) was found to be 0.04 µg/ml. These data demonstrate the excellent sensitivity, selectivity and precision of the method. Therefore, this method may be adapted to determine the imidacloprid residue level in animal tissues. The study reveals that the extraction of samples with acetonitrile and hexane are suitable for imidacloprid analysis. The separation and quantification of imidacloprid by RP-HPLC is better at

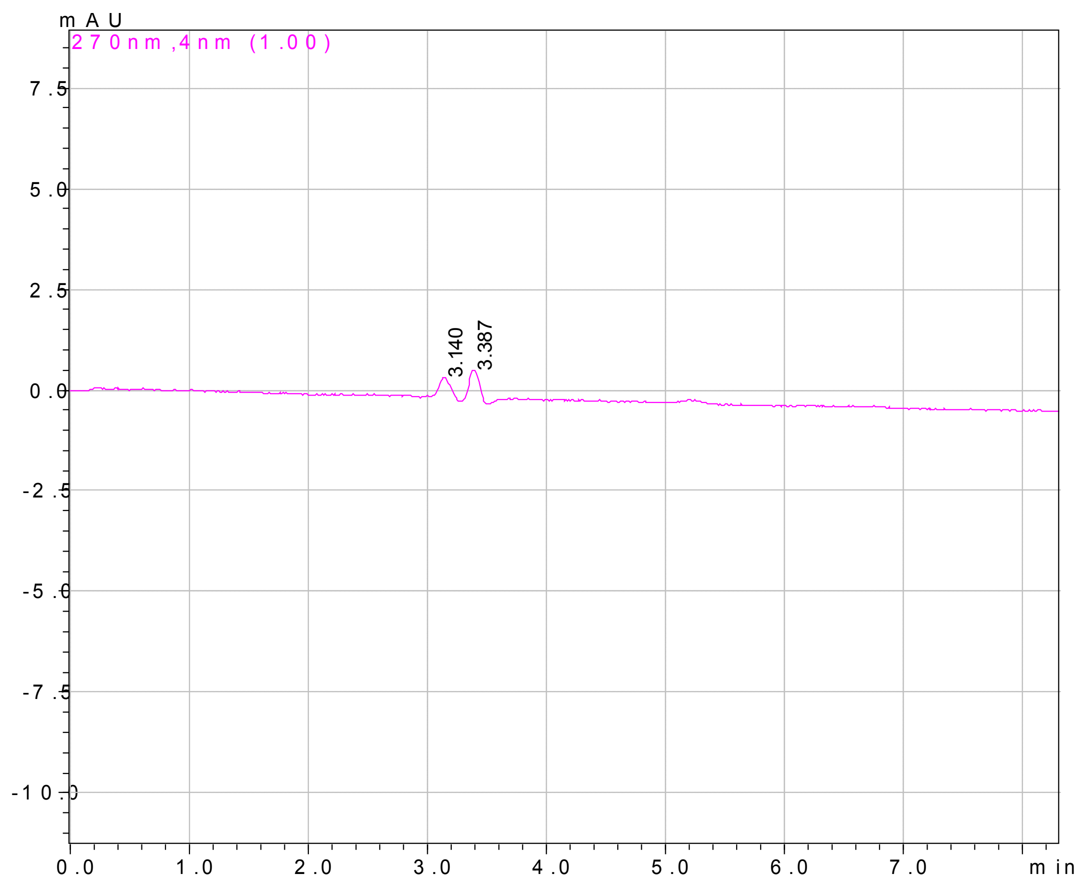


Figure 2: *Typical Chromatogram of standard Imidacloprid (analytical grade, 0.1µg/ml).*

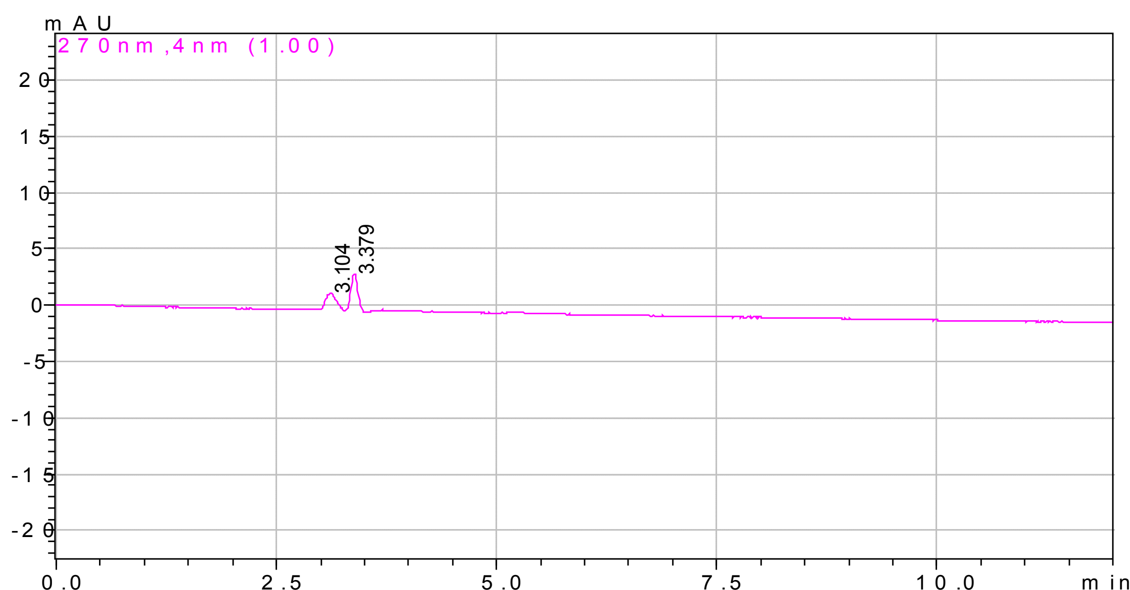


Figure 3: *Typical Chromatogram of standard Imidacloprid (analytical grade, 1 µg/ml).*

Substrates	**Percentage recovery (%)**			**Mean percentage recovery (%)**	**SD**	**SE**	**% RSD**
	I	**II**	**III**				
Blood	97.8	96.8	97.2	97.27	0.50	0.28	0.52
Liver	95.8	97.0	96	96.27	0.64	0.36	0.64
	93.7						
	94.6						
Kidney	93.7	95.5	94.6	94.6	0.90	0.52	0.95
	0.52						
	0.95						
Skeletal muscle	93.2	94.6	93.1	93.63	0.83	0.48	0.88

Table 2: *Recovery of Imidacloprid from different substrates of goat following fortification at 0.1 µg/ml.*

wavelength of 270 nm with mobile phase of acetonitrile and water (90:10 v/v). The method is most suitable for rapid precise analysis of imidacloprid in animal tissues.

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