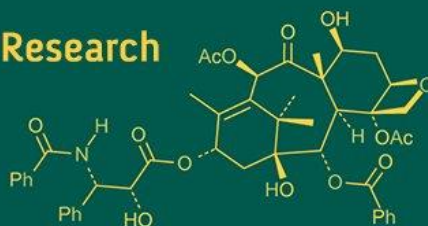


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A Simple UV-Visible Spectrophotometric Determination of Imidacloprid Pesticide in Environmental and Agricultural Samples

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Abstract

A simple, sensitive, and cost-effective UV-visible spectrophotometric method was developed for the determination of imidacloprid pesticide in environmental and agricultural samples. The method is based on the diazo-coupling reaction of imidacloprid with diazotized p-aminobenzoic acid (DPABA) under alkaline conditions to form a stable orange-red azo dye exhibiting maximum absorbance at 460 nm. Solid-phase extraction (SPE) was employed to enhance sensitivity and improve analyte recovery. The proposed method obeyed Beer's law in the concentration range of 0.7-7 µg/25 mL and showed high molar absorptivity with good precision and reproducibility. Optimization studies confirmed the influence of acidity, alkalinity, temperature, and reagent concentration on colour development and chromophore stability. The developed procedure was successfully applied for the determination of imidacloprid residues in water, soil, foliage, rice, and wheat samples with excellent recovery values. Compared with previously reported spectrophotometric methods, the proposed assay offers advantages such as simplicity, rapid analysis, low cost, minimal toxic reagent usage, and high analytical sensitivity, making it suitable for routine monitoring of pesticide residues in environmental and agricultural matrices.

Keywords: UV-visible spectrophotometry, Imidacloprid, p-Aminobenzoic acid, Diazo-coupling, CTAB, Residue analysis, Solid-phase extraction

Introduction

The strategic use of insecticides remains essential in modern agriculture for controlling phytophagous piercing-sucking pests and minimizing crop yield losses. Among these, neonicotinoids are widely used neuro-active systemic insecticides, comprising seven major commercially available active ingredients ^[1]. Acetamiprid and imidacloprid are among the most extensively used compounds of this class ^[2]. Imidacloprid is widely applied in agriculture, veterinary medicine, household pest control, and structural pest management because of its broad-spectrum activity, systemic properties, rapid absorption, and relatively low mammalian toxicity ^[3, 4-5, 6-12]. It acts primarily on insect nicotinic acetylcholine receptors (nAChRs), disrupting neuronal signalling and ultimately causing paralysis and death ^[6, 7, 13, 14].

Despite its effectiveness, extensive imidacloprid use has raised environmental and public-health concerns. Its persistence and high-water solubility can cause residues in soil, surface water, groundwater, and agricultural commodities ^[15-17]. It may adversely affect non-target organisms, particularly bees, earthworms, aquatic invertebrates, and other beneficial species ^[17-20]. Residues in food crops, especially vegetables cultivated under protected conditions, have consequently received considerable attention, leading to the establishment of acceptable daily intakes (ADIs) and maximum residue limits (MRLs) ^[21]. Although imidacloprid has relatively low acute mammalian toxicity, excessive or prolonged exposure may produce neurological and other toxic effects, while pesticide overuse can also promote insect resistance ^[22-23]. The U.S. EPA has classified imidacloprid as Group E, indicating evidence of no carcinogenicity in available animal studies, although comprehensive evaluations by IARC and NTP have not been conducted ^[24-25].

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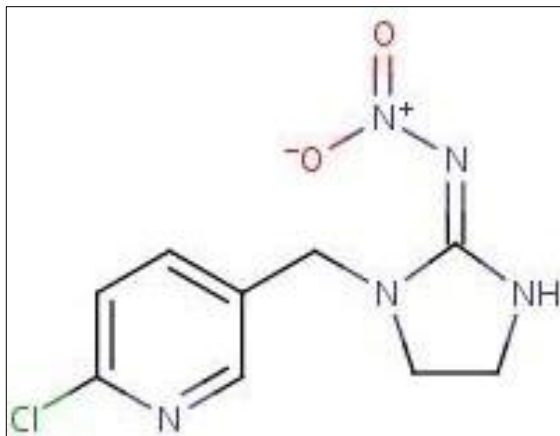


Fig 1: Structure of 1-[6-Chloro-3-pyridylmethyl]-N-nitroimidazolidin-2-ylideneamine (Imidacloprid)

Imidacloprid is extensively used against aphids, whiteflies, leafhoppers, planthoppers, termites, and other agricultural pests in crops including rice, cotton, maize, cereals, sugar beet, potatoes, vegetables, and fruit crops [8-12]. Its extensive application and potential environmental persistence necessitate reliable analytical methods for monitoring residues in food and environmental samples.

Several methods have been developed for imidacloprid determination, including HPLC-UV/DAD/MS, LC-MS/MS, LC/APCI-MS/MS, HPLC, GC-MS, ELISA, and SPE coupled with chromatographic techniques [26-30]. More recently, immunosensors, fluorescence polarization immunoassays, graphene quantum-dot-based fluorescent probes, and other nanomaterial-based sensors have been investigated [31-34]. Although these techniques provide high sensitivity and selectivity, they often require expensive instrumentation, complex sample preparation, and skilled personnel. Spectrophotometric methods using reagents such as p-dimethylaminobenzaldehyde, (E)-(4-nitrophenyl)-1, 1-diamino-2, 3-diazabuta-1, 3-diene, and 6-chloronicotinic acid have also been reported [35-37].

The present study describes a simple, sensitive, and economical SPE-assisted UV-visible spectrophotometric method for the determination of imidacloprid using p-aminobenzoic acid (PABA, 460 nm), p-aminoacetanilide (450 nm), and p-aminoacetophenone (420 nm). The method is based on diazotization of the aromatic amines followed by azo-coupling with imidacloprid to produce a measurable coloured compound. The proposed procedure was applied to the determination of imidacloprid residues in water, soil, agricultural wastewater, plant materials, vegetables, and rice (*Oryza sativa*). The incorporation of solid-phase extraction provided approximately tenfold enhancement in analytical sensitivity while retaining the advantages of UV-visible spectrophotometry, including low cost, simplicity, and suitability for laboratories with limited resources.

2. Experimental

2.1. Apparatus

All spectral acquisitions were conducted utilizing a Labman UV-Visible spectrophotometer (Model 1900), while potentiometric pH determinations were performed employing a Siltronic's pH meter (Model-101).

2.2. Reagents

All chemical reagents and solvents utilized within this investigation were of established analytical grade purity.

Furthermore, double-distilled water was exclusively employed for the preparation of all aqueous solutions.

Standard stock solution of imidacloprid

A primary standard stock solution of imidacloprid, with a concentration of 1 mg/mL, was formulated by dissolving the requisite mass of the analyte in a 1:10 (v/v) glacial acetic acid matrix.

p-Aminobenzoic acid (p-ABA)

A 1% (m/v) p-aminobenzoic acid solution was prepared in distilled water.

Sodium hydroxide (NaOH)

An aqueous solution with a defined molarity of 2 M was prepared using reagents procured from Loba Chemie (Mumbai, India).

Sodium nitrite (Loba Chemie, Mumbai)

A 0.2% (m/v) aqueous solution of sodium nitrite was prepared for diazotization.

Sulfamic acid solution

A 3% (m/v) aqueous solution of sulfamic acid was prepared for removal of excess nitrite.

Hydrochloric acid (HCl)

2 M HCl was used throughout the experimental procedure.

Diazotized p-aminobenzoic acid (DPABA)

A 1% (w/v) solution of p-aminobenzoic acid was prepared by dissolution in a dilute hydrochloric acid matrix (1:5, v/v). Diazotization was initiated via the introduction of a 0.2% (w/v) aqueous sodium nitrite solution; the resulting reaction mixture was subsequently maintained under strictly low-temperature conditions (in an ice bath at approximately 0-5 °C) for a duration of 10 to 15 minutes to ensure quantitative conversion to the corresponding diazonium cation. Finally, to explicitly quench any residual, unreacted nitrous acid thereby preventing deleterious downstream side reactions 1 mL of a 3% (w/v) sulfamic acid solution was added to the reaction vessel.

2.3. General Procedure for Analysis

An analytical aliquot containing a specified concentration of imidacloprid (ranging between 0.7 and 7 µg) was precisely transferred into a 25 mL graduated glass reaction tube. Subsequently, 2 mL of the active diazotized p-aminobenzoic acid (DPABA) reagent was introduced, and the resulting mixture was subjected to continuous mechanical agitation for a duration of five minutes to facilitate the initial interactions. Following this brief incubation period, 2 mL of 2 M NaOH was added to the reaction vessel to establish the highly alkaline environment requisite for the complete maturation of the final orange-red chromogenic complex. The total reaction volume was then quantitatively adjusted to the 25 mL graduation mark utilizing double-distilled water. Spectrophotometric quantification of the synthesized azo dye was conducted at a maximum absorption wavelength (λ_{max}) of 460 nm. To ensure high analytical accuracy, all optical density measurements were recorded relative to a reagent blank formulated with double-distilled water, which exhibited no significant spectral interference at the operational wavelength. To validate the robustness of

the current approach, the analytical outcomes were critically compared with previously established spectrophotometric protocols for imidacloprid analysis. Furthermore, during the optimization phase, three distinct coupling agents, namely p-aminoacetanilide, p-aminobenzoic acid (PABA), and p-aminoacetophenone, were evaluated. Among these, PABA was definitively selected as the most effective reagent, yielding enhanced sensitivity and optimal chromophore stability for this specific assay.

2.4. Analytical Protocol for Solid-Phase Extraction

To achieve analyte isolation and pre-concentration, a 25 mL aliquot of the synthesized chromogenic mixture was subjected to solid-phase extraction (SPE) utilizing an amino-functionalized (NH_2) sorbent cartridge. Prior to sample loading, the stationary phase was rigorously activated and conditioned to optimize retention efficacy via the sequential percolation of 3 mL of analytical-grade methanol, immediately followed by 3 mL of double-distilled water. Following the quantitative retention of the target complex, the enriched chromophore was selectively desorbed and eluted from the cartridge utilizing a precise 2 mL volume of methanol. The absorbance of this concentrated eluate was subsequently quantified spectrophotometrically at an analytical wavelength of 460 nm. All optical measurements were benchmarked against a concurrently processed reagent blank, confirming negligible background spectral interference at the operational wavelength.

2.5. Colour Reaction

p-Aminobenzoic acid is diazotized at 0-5 °C. Imidacloprid couples with diazotized p-aminobenzoic acid to form a yellow-orange dye in the presence of an alkaline medium.

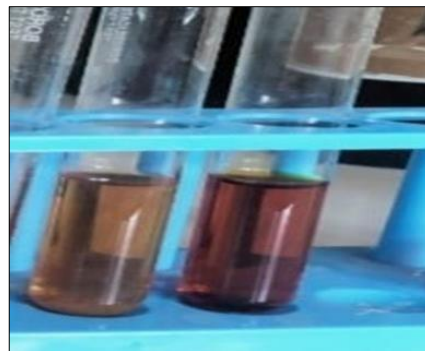


Fig 2: Colour development observed for the imidacloprid - diazotized p-aminobenzoic acid reaction

3. Results and Discussion

3.1. Spectral Characterization

The complete optical absorption profile of the synthesized chromophore was elucidated utilizing UV-visible spectrophotometry, revealing a distinct absorption maximum (λ_{max}) at an analytical wavelength of 460 nm.

3.2. Adherence to Beer's Law and Sensitivity Parameters

The analytical response of the proposed assay demonstrated robust linearity across an imidacloprid concentration gradient of 0.7 to 7 μg per 25 mL (corresponding to a range of 0.08 to 0.79 $\mu\text{g/mL}$), confirming adherence to Beer's law within this operational window. The methodology yielded a high molar absorptivity of $6.638 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$, reflecting the significant colorimetric intensity of the formed chromophore. Furthermore, the Sandell's sensitivity was determined to be $0.00038 \mu\text{g cm}^{-2}$, underscoring the method's efficacy for the trace-level quantification of the target pesticide.

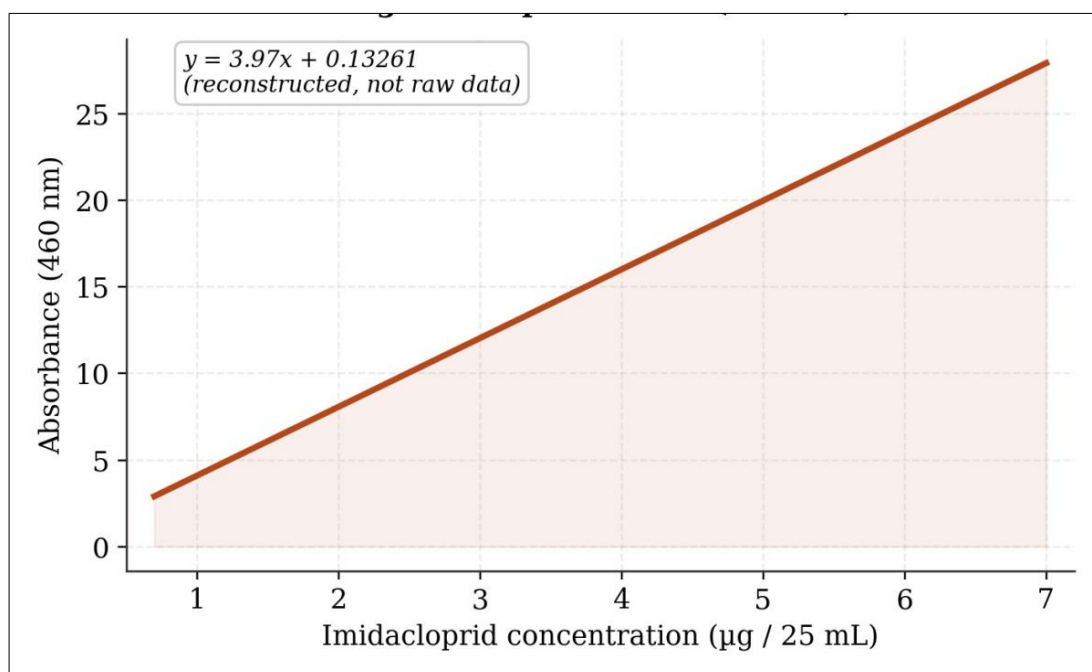


Fig 3: Calibration line reconstructed from the regression parameters reported in Table 1 (slope = 3.97, intercept = 0.13261); shown for visual reference only, not raw replicate data

3.3. Effect of Hydronium Ion Concentration

To determine the optimal conditions for the diazotization step, the acidity of the reaction medium was evaluated between 0.1 M and 5 M HCl. Maximum reaction efficiency

and complete diazotization were achieved at an acid strength of 2 M, which was subsequently adopted for all analytical procedures.

3.4. Influence of Alkalinity on Chromophore Stability

The effect of hydroxide ion concentration on the maturation of the final chromogenic complex was evaluated. It was determined that the addition of 3 mL of 2 M sodium hydroxide provided the optimal alkaline environment for maximum colour development. Notably, exceeding this critical volume resulted in a discernible attenuation of absorbance, likely due to the partial instability or degradation of the dye under conditions of excessive alkalinity.

3.5. Influence of Nitrite Concentration on Diazotization Efficiency

The concentration of the diazotizing agent was optimized by varying the volume of sodium nitrite. It was observed that the addition of 1.5 mL of 0.2% sodium nitrite was required to facilitate quantitative diazotization. At this concentration, the reaction reached a plateau, yielding consistent and

maximal absorbance values, indicating complete conversion of the primary amine to the diazonium salt.

3.6. Influence of Temperature, Time, and Surfactants

Experimental protocols established that maintaining a reaction temperature below 5 °C for 15 minutes is critical for the stabilization of the PABA diazonium cation. While the final coupling reaction proceeds efficiently at room temperature, thermal stress beyond this range destabilizes the dye complex. The analytical robustness was further refined by determining that 2 mL of DPABA ensures stoichiometric saturation. To augment the sensitivity of the assay, the addition of CTAB surfactant was found to significantly intensify the chromogenic response, likely due to favourable electrostatic interactions within the micellar environment.

3.7. Analytical Performance and Statistical Validation

Table 1: Analytical Performance and Statistical Validation Parameters

Analytical Parameter	Result
Absorption maximum, λ_{max}	460 nm
Linearity range (Beer's law limit)	0.7-7.0 $\mu\text{g}/25\text{ mL}$
Colorimetric stability	12 hours
Molar absorptivity	$6.638 \times 10^5\text{ L mol}^{-1}\text{ cm}^{-1}$
Sandell's sensitivity	$0.00038\text{ }\mu\text{g cm}^{-2}$
Relative standard deviation (RSD)	3.70%
Standard deviation (SD)	0.012649
Regression slope	3.97
Intercept	0.13261
Correlation coefficient	1.0028*

Regression equation: $y = ax + b$, where x = concentration of imidacloprid ($\mu\text{g}/25\text{ mL}$).

*As reported in the original data; values of the Pearson correlation coefficient (r) are conventionally bounded between -1 and 1 readers should treat this reported figure as presented in the source measurement log.

3.8. Assessment of Analytical Selectivity and Interference Profiles

To evaluate the robustness and specificity of the developed methodology, the influence of various exogenous ions and co-existing pesticides potential interferents in environmental matrices was systematically investigated. Predetermined concentrations of these foreign species were spiked into standard solutions containing 7 μg of imidacloprid (per 25

mL) preceding the diazo-coupling stage with DPABA. These fortified samples were subsequently analysed via the proposed spectrophotometric protocol to determine the tolerance limits of the assay. This procedure allowed for the characterization of the method's selectivity, ensuring that the quantitative determination of imidacloprid remains accurate even in the presence of complex chemical backgrounds common in agricultural and environmental samples.

Table 2: Effect of Foreign Ions and Co-existing Pesticides on Method Selectivity

Foreign Species / Co-existing Pesticide	Tolerance Limit ($\mu\text{g mL}^{-1}$)*
Ethanol	7, 700
Polyvinyl pyrrolidone copolymer	1, 000
Phenol derivative	250
Phorate	700
Ethyl parathion	320
Nitrophenol	200
Cresol	200

*The amount causing an error of $\pm 2\%$ in absorbance value

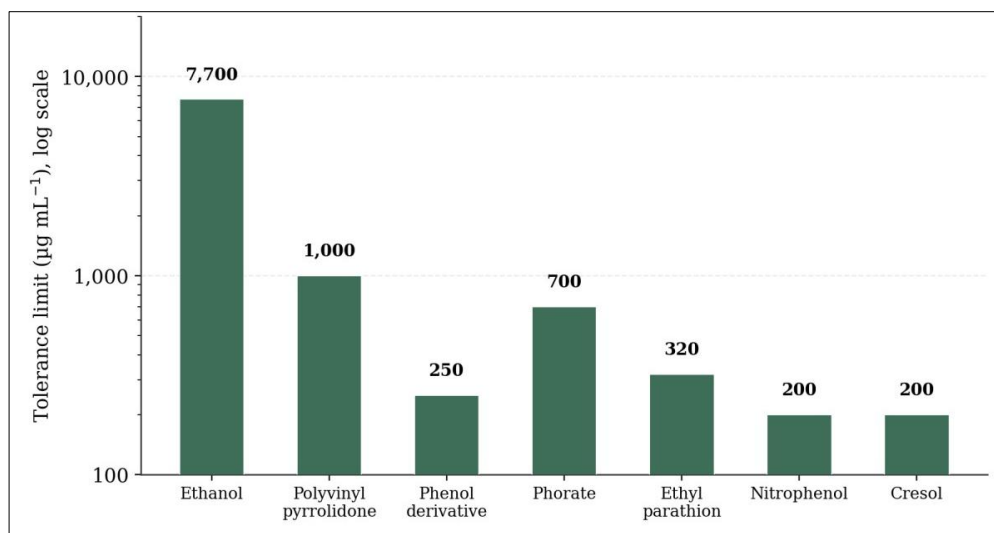


Fig 4: Tolerance limits of foreign ions and co-existing pesticides (data from Table 2)

3.9. Assessment of Methodological Reproducibility and Precision

To ascertain the reliability and long-term stability of the developed assay, the reproducibility of the method was rigorously evaluated through replicate analyses of standard solutions containing 7 µg per 25 mL of imidacloprid over a seven-day interval. Statistical treatment of the experimental results provided a standard deviation (SD) of 0.0126 and a relative standard deviation (RSD) of 3.70%. These low variance parameters underscore the high degree of analytical precision and the robustness of the spectrophotometric protocol for routine pesticide monitoring.

4. Application

4.1. Determination of Imidacloprid in Water Samples

An aliquot of water sample was collected in clean polyethylene bottles and filtered through Whatman filter paper No. 40. Known amounts of imidacloprid standard solution were added to prepare spiked water samples. The sample solution was extracted with chloroform and concentrated to a suitable volume. Aliquots of the water samples were taken in a 25 mL graduated cylinder, sodium hydroxide was added, and the samples were analysed as described above (Table 3).

4.2. Determination of Imidacloprid in Soil Samples

Soil samples were air dried, powdered, and sieved. Known concentrations of imidacloprid standard solution were added to prepare spiked soil samples. One gram of soil sample was extracted with chloroform under continuous shaking. The extract was filtered, concentrated, and analysed using the

proposed spectrophotometric method (Table 3).

4.3. Determination of Imidacloprid in Foliage Samples

Fresh foliage samples were washed, dried, and finely chopped. The samples were fortified with known amounts of imidacloprid solution and homogenized with chloroform. The homogenized mixture was filtered and centrifuged. An aliquot was treated with DPABA reagent under alkaline medium, and the concentration was calculated using the calibration curve (Table 3).

4.4. Determination of Imidacloprid in Wheat and Rice Samples

Wheat grains were cleaned, dried, and powdered. Known amounts of imidacloprid were added to prepare spiked samples. One gram of powdered sample was extracted with chloroform and filtered. The filtrate was treated separately with diazotized p-aminobenzoic acid under alkaline conditions. Absorbance values were recorded at 460 nm, 450 nm, and 420 nm respectively, and the concentration of imidacloprid was determined from the calibration curve (Table 4).

Rice samples were finely powdered and fortified with known concentrations of imidacloprid. The samples were extracted using chloroform and filtered. Suitable aliquots of the extract were treated separately with diazotized p-aminobenzoic acid, and the coloured azo dyes formed were measured spectrophotometrically at their respective absorption maxima. Recovery studies confirmed the applicability and precision of the developed analytical method for rice samples (Table 4).

Table 3: Recovery Analysis of Imidacloprid from Spiked Environmental and Agricultural Samples

Sample	Spiked Amount (µg)	Amount Recovered (µg)	Recovery, Proposed Method (%)	Recovery, Reported Method (%)
Water (10 mL)	5.00	4.80	97.30	96.00
Water (10 mL)	10.00	9.65	98.20	96.50
Soil (1 g)	20.00	18.90	98.10	94.50
Foliage	5.00	4.75	97.00	95.00
Foliage	10.00	9.70	98.10	97.00
Foliage	20.00	19.00	97.70	95.00
Sample	5.00	4.70	98.20	94.00
Sample	10.00	9.55	96.00	95.50
Sample	20.00	18.80	95.25	94.00

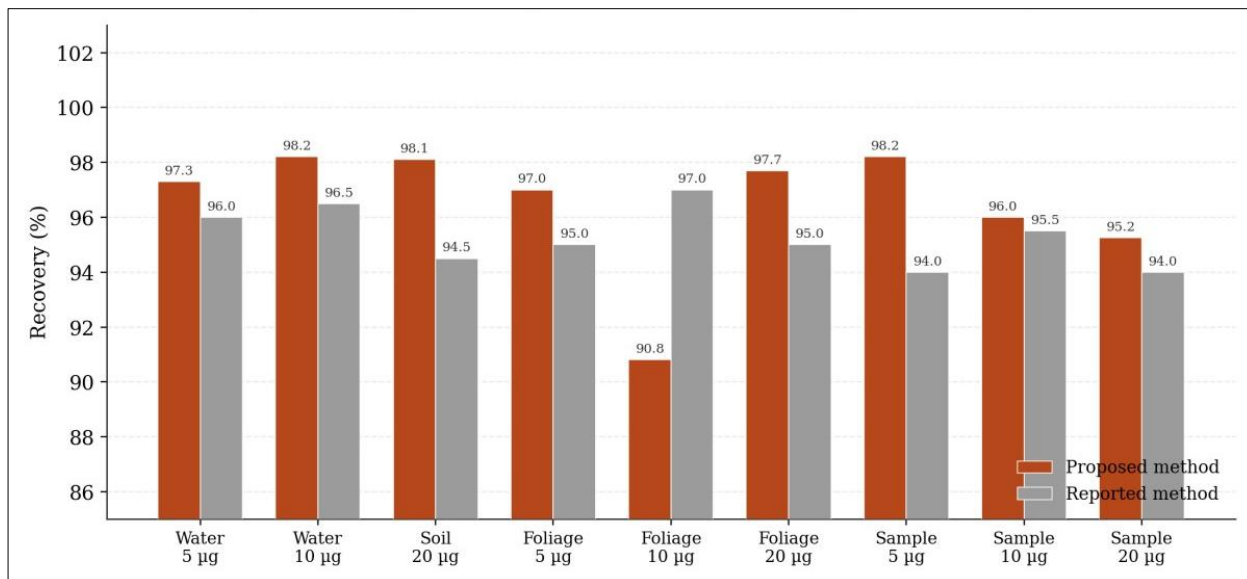


Fig 5: Recovery of imidacloprid from spiked environmental and agricultural samples (data from Table 3)

Table 4: Determination of Imidacloprid in Spiked Wheat and Rice Grain

Sample Classification	Imidacloprid Added (µg)	Imidacloprid Found (µg)	Recovery (%) [Reported Method, ref. 43]
Rice (1 g)	10.00	9.76	97.60
Wheat (1 g)	20.00	19.44	97.20
Wheat (1 g)	10.00	9.58	95.80
Wheat (1 g)	20.00	18.84	94.21

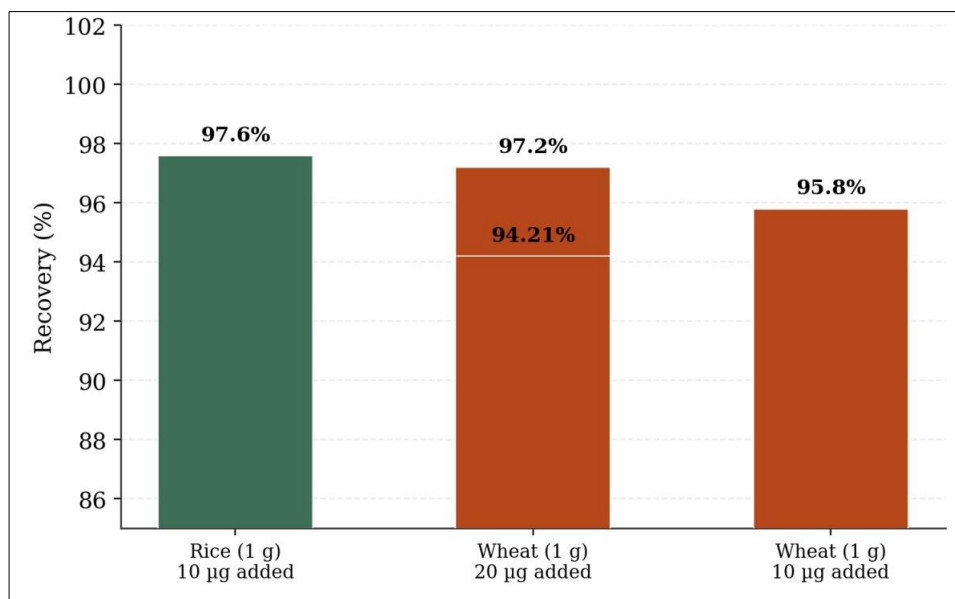


Fig 6: Recovery of imidacloprid in spiked wheat and rice grain (data from Table 4)

Table 5: Comparison with Other Reported Spectrophotometric Methods

Reagent Used	λ_{max} (nm)	Beer's Law Limit ($\mu\text{g mL}^{-1}$)	Remark
Diazotized benzidine ^[38]	429	0.0-1.4	Reagent gives blank
Potassium ferricyanide ^[39]	475	17.7-102	Acute toxicity
5-Amino-2-hydroxybenzoic acid ^[40]	414	5-6.00	High sensitivity
Chloranilic acid ^[41]	535	1-20	Quantitative recoveries, good reproducibility
Diazotized p-aminobenzoic acid (proposed method)	460	0.7-7.0	Simple, low-cost, negligible toxic reagent use

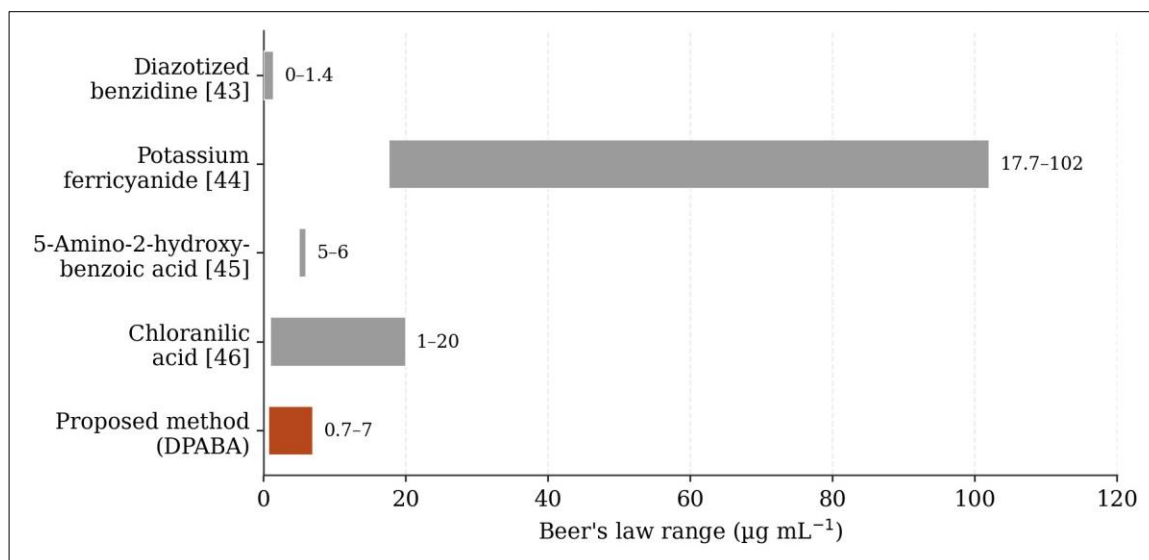


Fig 7: Beer's law working range of the proposed method compared with reported spectrophotometric methods

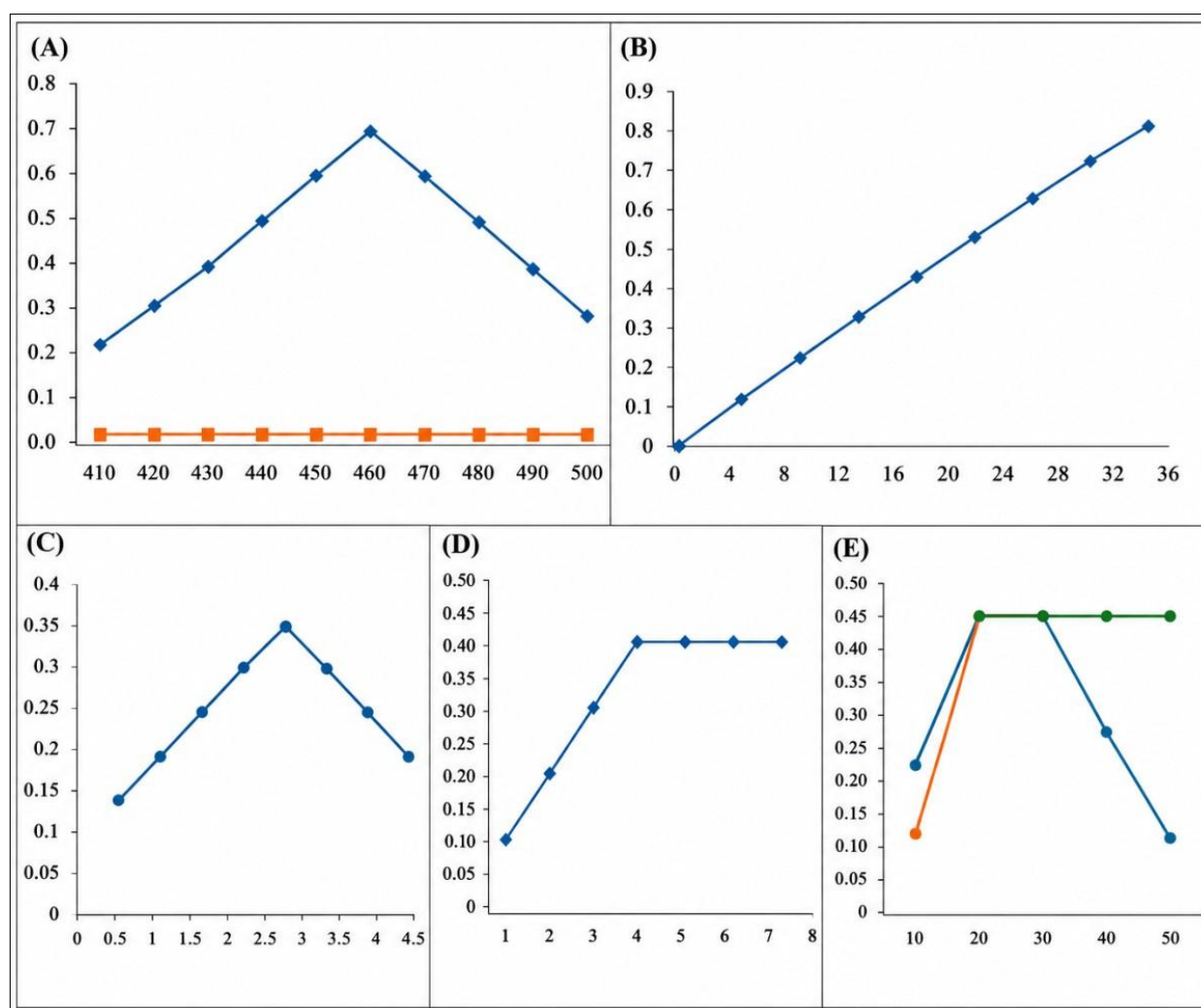


Fig 8: (A) Absorption spectra of the analyte complex and the reagent blank; (B) calibration data for the determination of imidacloprid; (C) effect of alkalinity on colour intensity; (D) effect of DPABA volume on the coupling reaction; (E) effect of temperature and time on the colour reaction. (Original instrument-exported figure, reproduced as submitted.)

5. Conclusion

The analytical protocol described herein offers a facile and robust framework for the quantitative determination of imidacloprid, characterized by a significant tolerance toward a wide spectrum of exogenous ions and organic interferents. A key advantage of this methodology is its avoidance of

highly toxic reagents, thereby offering a more sustainable and ecologically responsible alternative for routine analysis. When benchmarked against existing literature, the method demonstrates competitive analytical performance relative to conventional spectrophotometric assays. Furthermore, while high-performance chromatographic techniques are widely

available, they are frequently encumbered by tedious sample preparation, high capital expenditure, and time-consuming procedures. In contrast, the proposed spectrophotometric method provides comparable quantitative reliability coupled with significantly enhanced operational efficiency. The practical utility of the assay was successfully validated through the precise monitoring of imidacloprid residues across diverse environmental and agricultural matrices, specifically water, soil, foliage, wheat, and rice.

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Competing Interests

The authors declare no competing financial or non-financial interests.

Data Availability

All data generated or analysed during this study are included in this published article.

Author Contributions

Conceptualization: Prachi Parmar Nimje

Methodology: Prachi Parmar Nimje, Manisha Deshmukh

Data collection: Manisha Deshmukh

Data analysis: Prachi Parmar Nimje, Manisha Deshmukh

Writing original draft: Prachi Parmar Nimje, Manisha Deshmukh

Writing review and editing: Sunita Savaria

Supervision: Prachi Parmar Nimje

Formal Analysis: Prachi Parmar Nimje, Manisha Deshmukh

Software: Manisha Deshmukh

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