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A New Spectrophotometric Method for the Determination of Imidacloprid in Real Samples by Diazotization-Coupling Reaction

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In this study, a novel azo dye with unique sensing properties, named IMID-P, was synthesized and comprehensively characterized. The synthesis involved the diazotization of the widely used insecticide Imidacloprid, followed by a coupling reaction with 5-amino-2-hydroxybenzoic acid in an alkaline medium (pH ~11.3), yielding a stable yellow azo compound. The dye exhibited a maximum absorbance (λmax) at 414.5 nm. Its successful synthesis and structural confirmation were achieved through ultraviolet–visible (UV-vis) spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), and carbon nuclear magnetic resonance (^{13}C-NMR) spectroscopy. IMID-P demonstrated remarkable sensitivity, as evidenced by its low limit of detection (LOD), and showed excellent linearity, adhering to the Beer-Lambert law within a concentration range of 5–60 μg/mL. Furthermore, the binding mechanism between Imidacloprid and 5-amino-2-hydroxybenzoic acid was investigated, providing insights into the dye's formation. These findings emphasize IMID-P's potential for quantifying Imidacloprid in environmental samples like water and soil, and its applicability in commercial insecticides formulations.

Keywords: UV spectrophotometry, imidacloprid, diazotization-coupling reaction

Introduction

Imidacloprid (IMID) is a widely used neonicotinoid insecticide known for its effectiveness against a broad range of pests, including potato beetles, locusts, bugs, and fleas [1]. It is commonly applied as a foliar treatment on various crops such as cereals, maize, pome fruit, citrus fruit, and vegetables [2]. However, because of its high toxicity and potential to accumulate in the environment, reliable analytical methods are necessary for its detection and quantification in different matrices, including soil, water, and food products [3].

IMID belongs to the nitroguanidine class of neonicotinoids and is considered a significant technogenic pollutant of soil and water [4, 5]. Its presence in agricultural and environmental samples necessitates accurate and sensitive analytical techniques to ensure food safety and environmental protection [6]. Many analytical studies have been conducted to determine IMID residues in real, biological, and environmental samples. For instance, a factorial design was applied to optimize IMID residue determination in stingless bees [7], while an HPLC-tandem mass spectrometry method was developed for IMID extraction in fruit juices [8]. Additionally, trace analysis methods were explored to study IMID metabolism in wheat [9], and surface-enhanced Raman spectroscopy (SERS) was used for its detection in drinking water [10]. While these methods provide high accuracy, they often require expensive instrumentation, complex sample preparation, and long analysis times [11].

Spectrophotometry, on the other hand, is a widely used analytical technique due to its simplicity, cost-effectiveness, and high sensitivity [12, 13]. The diazotization-coupling reaction is a well-established method in analytical chemistry, allowing the conversion of amine compounds into diazonium salts, which serve as intermediates in organic synthesis and various analytical applications [14]. Their high chemoselectivity and sensitivity make them valuable for IMID detection in both environmental monitoring and food safety analysis [15]. However, due to their instability, diazonium salts require careful handling and controlled reaction conditions [13-16].

In this study, we propose a new spectrophotometric method based on the diazotization-coupling reaction for the quantification of IMID. This method utilizes the reaction between IMID and 5-amino-2-hydroxybenzoic acid, forming a colored complex that can be easily measured. The proposed technique is fast, simple, cost-effective, and highly sensitive, making it a promising tool for quality control, environmental safety assessments, and regulatory compliance. This study aims to (1) optimize the reaction conditions for IMID determination, (2) evaluate the sensitivity and accuracy of the method, and (3) compare the results with existing analytical techniques. The development of this method contributes to enhanced monitoring and management of IMID usage, ensuring its safe application in agriculture and minimizing its environmental impact.

Experimental part

Materials

The following chemicals were used in this study without further purification: Imidacloprid (IMID) (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, (20%, Ascot); 5-amino-2-hydroxybenzoic acid (98%, MERCK); sodium hydroxide (98%, LOBAChemie); hydrochloric acid (35–38%, SDFCL); sodium nitrite (98%, CDH); and sulfamic acid ([H_3NSO_3], 99%, Eisen-Golden Laboratories).

Apparatus

The products were characterized by comparing them with reference samples and spectroscopic data (FT-IR, UV, C-NMR, and H-NMR). The IR spectra were recorded using a Shimadzu FTIR-8400S spectrometer. UV-visible spectra were obtained with a Shimadzu UV-1800 Double Beam UV-Visible Spectrophotometer. C-NMR and H-NMR spectra were recorded on a BRUKER 400 MHz spectrometer (Germany). Melting points were determined using a Stuart SMP30 apparatus (UK). The pH was measured with a WTW 720 pH meter (Germany). Weighing was done using a high-precision Sartorius BP 3015 digital balance (Germany).

Preparation and characterization of azo compounds

Step 1. A volume of 2.5 mL of 5-amino-2-hydroxybenzoic acid (10^{-3} mol/L) was combined with 1 mL of 1 mol/L HCl and 1.25 mL of 0.5 mol/L $NaNO_2$ solution in an ice bath (0–5°C). The reaction mixture was allowed to stand for six minutes. Then, 0.3 mL of 0.2 mol/L sulfamic acid was added to eliminate excess sodium nitrite. Step 2. Afterward, 3 mL of the prepared IMID solution (10^{-3} mol/L, made by dissolving 0.025 g in 100 mL of distilled water) was mixed with 0.5 mL of 1.00 mol/L NaOH solution to adjust the pH to approximately 11–11.5. The optimal absorbance was determined by preparing multiple solutions with varying pH values, with the highest absorbance observed within this pH range. Finally, the mixture from Step 2 was added to the mixture from Step 1, stirred briefly, and the volume was adjusted to 25 mL. The formed precipitate was collected as a pure solid-colored material with a melting point of 147.7 – 149.0°C. Structural characterization of the product was performed using FT-IR, H-NMR, and C-NMR spectroscopy to confirm the functional groups and molecular structure. The absorbance of IMID-P was analyzed in the visible region, with a maximum absorption wavelength (λ_{max}) at 414.5 nm, which was used to determine IMID.

Results and discussion

Reaction mechanism

The synthesis of the azo compound involves a diazotization-coupling reaction. Initially, 5-amino-2-hydroxybenzoic acid undergoes diazotization in the presence of sodium nitrite ($NaNO_2$) and hydrochloric acid (HCl) at low temperatures (0–5°C), resulting in the formation of the diazonium salt. This highly reactive intermediate then participates in an electrophilic coupling reaction with IMID, leading to the formation of the azo bond (–N=N–) and the final colored product. The reaction follows a classical electrophilic substitution mechanism, where the diazonium ion acts as an electrophile, attacking the activated aromatic system of IMID. Scheme 1 illustrates the expected initial reaction, as well as the formation of the azo compound.

Characterization of the product

The structure of the synthesized azo compound was confirmed through spectroscopic techniques, including FT-IR, H-NMR, C-NMR, and UV-Vis spectroscopy. The absorbance of IMID-P was analyzed in the visible region, with a maximum absorption wavelength (λ_{max}) at 414.5 nm, which was used for the determination of IMID (Fig. 1). The IR spectrum showed characteristic peaks confirming the presence of key functional groups, including N-H (3427 cm^{-1}), C-H (2881 cm^{-1}), N=N (1705 cm^{-1}), N=O (1589 cm^{-1}), C-N (1282 cm^{-1}), and aromatic C-Cl (680 cm^{-1}) (Fig. S1). The proton NMR analysis in DMSO-d_6 (400 MHz) revealed signals corresponding to aromatic protons (7.5–8.5 ppm), NH-CH_2-CH_2-NH (3.5–4.5 ppm), and NH–C=N (9.0 ppm), confirming the expected structure (Fig. S2). The DMSO-d_6 (100 MHz) spectrum exhibited chemical shifts at 120–140 ppm (aromatic carbons) and 160–180 ppm (N–C=N group), further verifying the formation of the azo compound (Fig. S3).

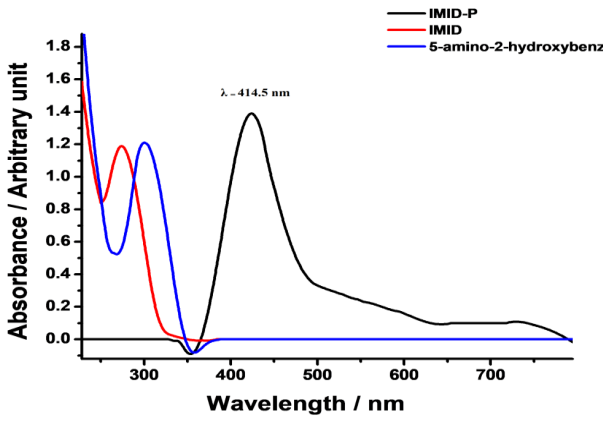


Figure 1. Absorption spectra of IMID insecticide, 5-amino-2-hydroxy benzoic acid and IMID-P at λ_{max} 414.5 nm.

Optimization of reaction conditions

Various experimental parameters influencing the formation of the azo compound were systematically optimized. The effects of reagent volumes, reaction time, and temperature on absorbance were investigated to identify the most favorable conditions for the diazotization and coupling reactions.

Optimization of reagent volumes. The influence of various reagent volumes on the formation of the azo compound was studied to determine the conditions

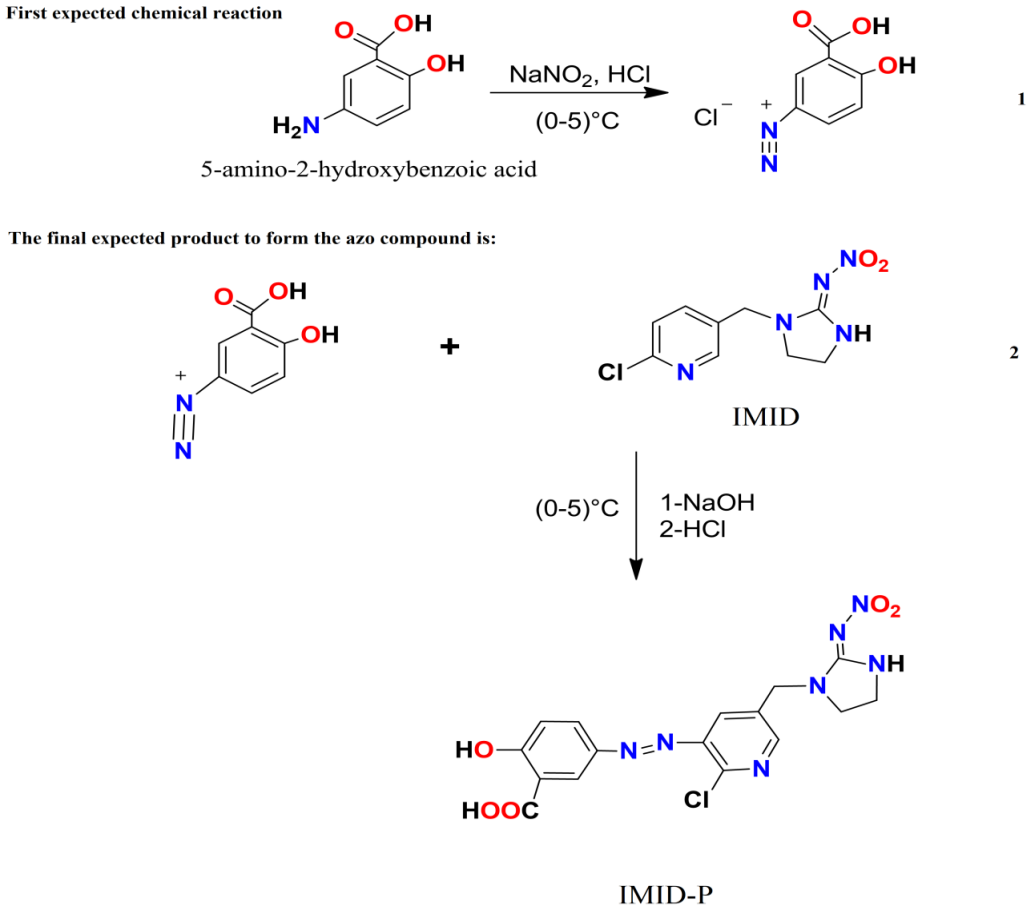
that yield the highest absorbance. Different volumes of IMID (1.0–5.0 mL, 10^{-3} mol/L) were tested, and the highest absorbance was observed at 3.0 mL. Varying the volume of 5-amino-2-hydroxybenzoic acid (10^{-3} mol/L) from 0.5 to 4.0 mL indicated that the optimal absorbance was achieved at 2.5 mL. The volume of 1.00 mol/L NaOH varied between 0.1 and 0.9 mL, with the best absorbance occurring at 0.5 mL. Different volumes of 1.00 mol/L HCl (0.25–1.5 mL) were tested, and the highest absorbance was observed at 1.0 mL. The acidic medium is essential for protonating the amine group, making it more reactive to nitrous acid. The volume of 0.50 mol/L $NaNO_2$ was varied from 0.25 to 2.0 mL, and the best absorbance was recorded at 1.25 mL. Sodium nitrite facilitates the diazotization reaction, converting primary aromatic amines into diazonium salts. Different volumes of 1.00 mol/L sulfamic acid (0.10–0.60 mL) were tested. Sulfamic acid plays a crucial role in diazo coupling reactions, maintaining the acidic environment necessary for diazonium salt formation and acting as a stabilizing agent in azo compound synthesis. The optimum volume was 0.40 mL. The effects of these reagent volumes on the absorbance of the newly formed azo compound are illustrated in Figure S4.

Optimization of reaction time. The time required for the complete formation of the diazonium salt for IMID was determined to be 6.0 minutes. The effect of reaction time on the removal of excess nitrous acid after diazotization was also investigated over different time intervals (1.0–5.0 minutes). The results indicate that 2.0 minutes is sufficient for the complete elimination of excess nitrous acid. Additionally, the stability of the IMID-P azo compound was assessed through its absorbance over time, confirming that it remains stable for extended periods. The results obtained are presented in Figure S5.

Effect of temperature. The effect of temperature on the spectrophotometric determination of IMID was evaluated to determine the optimal reaction conditions. The results show that absorbance is highest at 5°C, followed by a slight decrease of about 0.029 units at 10°C. After this point, the absorbance remains relatively stable, indicating that the reaction is not significantly influenced by higher temperatures.

Determination of reaction stoichiometry

Molar ratio method. The molar ratio method is essential for determining the stoichiometry of the reaction between 5-amino-2-hydroxybenzoic acid and IMID. This method confirms the suitability of an analytical approach for analyzing the newly formed compound (IMID-P). The results, illustrated in Figure S6, provide insights into the reaction's stoichiometry.



Scheme 1. Schematic representation of the reaction between 5-amino-2-hydroxybenzoic acid and diazotized IMID.

Job's method. In Job's method, equimolar concentrations of IMID and 5-amino-2-hydroxybenzoic acid are used to determine the stoichiometry of the reaction. This technique is crucial for verifying the reliability of the method in analyzing the formation of azo compounds, helping to clarify the composition of the new compound. The results are presented in Figure S7.

Analytical parameters and validation of the proposed method

A series of IMID solutions with concentrations ranging from 5 to 60 μg/mL were prepared in the presence of 5-amino-2-hydroxybenzoic acid under optimal conditions. The absorbance of the newly formed compound was measured at 414.5 nm, and a calibration curve was plotted by plotting absorbance against concentration, as shown in Figure S8. The limit of detection (LOD) and limit of quantification (LOQ) were determined from the intercept. Table 1 summarizes the analytical parameters of the proposed method.

Table 1. Analytical parameters of the proposed method.

Parameter	Value
λ_{exc}(nm)	384.4
λ_{em}(nm)	752.5
Linear range (μg/mL)	5-60
Slope	0.0032
Determination coefficient (r^2)	0.9779
Correlation coefficient (r)	0.9888
Intercept	0.0315
Molar absorptivity (L/mol.cm)	0.818×10^3
Limit of detection (μg/mL)	0.216
Limit of quantitation (μg/mL)	0.72

Accuracy

To evaluate the accuracy of the proposed method, three different concentrations of the sample solution (20, 30, and 40 μg/mL) were analyzed. Each concentration was tested in triplicate, and the experiment was repeated four times to minimize errors and ensure reproducibility.

The recovery percentage (Rec%) values confirm the high accuracy of the method. However, at 40 μg/mL, a lower recovery rate was observed, which may be attributed to the breakdown or evaporation of IMID during the analytical process [17]. The accuracy results are validated [18], summarized in Table 2.

Table 2. Accuracy of the proposed method.

Sample	Conc., μg/mL	R.S.D%	Recovery%*± SD
1	20	1.76	97.270 ± 0.0020
2	30	0.72	94.530 ± 0.0010
3	40	0.33	87.500 ± 0.0006

*Mean of four determinations.

Precision

To assess the precision of the proposed method, three IMID concentrations (25, 30, and 35 μg/mL) were prepared and analyzed. Each concentration was measured four times, and precision was evaluated using intra-day and inter-day measurements. The results indicate that the method has satisfactory precision, as the relative standard deviation (R.S.D.%) values are all below 2%. The precision details are presented in Table 3.

IMID residue analysis in soil

The impact of IMID on soil was evaluated by applying different concentrations (40, 50, and 60 μg/mL) using the recommended analytical method. Four samples were analyzed for each concentration. The IMID solutions were sprayed onto the soil, which was then placed in a sealed plastic container and left undisturbed for two weeks to simulate environmental exposure. After the incubation period, 1 gram of surface soil was collected, washed with 10 mL of distilled water, filtered by using filter paper, and analyzed for absorbance. The results, summarized in Table 4, indicate that a significant portion of IMID remains in the soil, with an average weight-to-volume retention ratio of approximately 30%, suggesting a high potential for leaching.

A comparison between the proposed method and other established techniques for IMID determination was conducted. The results, shown in Table 5, confirm that the suggested method provides reliable relative standard deviation (RSD) values and an acceptable recovery range, demonstrating its effectiveness in detecting even trace amounts of IMID in soil samples.

Conclusions

This study proposes a new, fast, and simple technique for measuring IMID based on the diazotization-coupling reaction mechanism. The method allows for the efficient conversion of amine compounds into aryl diazonium derivatives, facilitating the identification and characterization of IMID's

Table 3. Precision of the proposed method.

Sample	Concentration, μg/mL	Intra-day precision		Inter-day precision	
		Recovery (%)	R.S.D%	Recovery (%)	R.S.D%
1	25	99.00	1.17	97.00	0.72
2	30	94.00	0.97	92.00	1.26
3	35	91.00	1.19	92.00	0.99

*Mean of four determinations.

Table 4. IMID recovery in soil samples.

Sample	Taken Conc., µg/mL	Found Conc., µg/mL	Error %	Recovery %*
1	40	26.09	-34.77	65.23
2	50	28.91	-42.19	57.81
3	60	31.72	-47.14	52.87

*Mean of four determinations.

Table 5. Comparative analysis of IMID determination methods.

Technique	Linear range	RSD %	Recovery %	LOD	LOQ	Ref.
HPLC	1–6 mg/L	1.23	85.20 -93.15	0.12 mg/L	0.37 mg/L	18
TLC– Densitometric	10–60 mg/L	1.70	92.57 -96.31	1.39 mg/L	4.18 mg/L	18
Fluorimetric	1–20 mg/L	1.09	96.16- 99.83	0.32 mg/L	0.97 mg/L	18
HPLC- DAD	----	---	82.6- 109	0.08+ 0.06 mg kg^{-1}	---	19
HPLC-MS/MS	0.001- 0.25 mg/kg	6.0	68- 118	0.0015 mg/kg	0.005 mg/kg	20
HPLC in soil	0.02- 5.00 mg/kg	1.21- 3.37	94.66- 95.27	0.006 mg/kg	0.02 mg/kg	21
HPLC in water	0.02–5.00 mg/L	1.66- 3.23	92.34- 96.86	0.006 mg/L	0.02 mg/L	21
Chronopotentiometric Method	(0.8-30.0) + (7.0-70.0) mg/L	3.73	97.3- 98.1	0.17+0.93 mg/L	---	22
QuEChERS Methods	10–100 µg/Kg	< 20	---	---	400 µg/Kg	23
Modified QuEChERS +LC-MS	1-100 µg/Kg	< 28	---	---	0.04 µg/ Kg	24
QuEChERS and LC-hybrid LTQ/Orbitrap-MS	---	2.7- 3.2	87.2- 96.0	15.3 µg/Kg	51.0 µg/Kg	25
QuEChERS and LC-hybrid LTQ/ Orbitrap-MS	---	3.0- 5.7	73.3- 83.0	15.5 µg/Kg	52.0 µg/Kg	25
UV-Visible	5-60 µg/mL	0.967- 1.186	90.63- 98.75	0.216 µg/mL	0.72 µg/mL	current study

chemical structure. The formation of a diazonium salt can help confirm the presence of specific functional groups in the IMID molecule. The coupling reaction exhibits high chemoselectivity and sensitivity, making it highly suitable for quality control and environmental monitoring applications. An important toxicological aspect to consider is that diazonium salts may have increased insecticidal efficacy compared to the parent molecule, potentially altering IMID's toxicity profile. The proposed method offers several advantages, including being rapid, sensitive, cost-effective, and easy to implement, making it a valuable tool for IMID determination in various applications.

Recommendations

Further research is encouraged to explore the application of this method in biological and environmental samples. Additionally, the newly synthesized diazonium derivative of IMID may serve as a more effective insecticide compared to the original compound, warranting further investigation into its potential practical applications.

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