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Green SPE–Spectrophotometric Determination of Trace Atrazine in Environmental and Agricultural Matrices

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Green SPE–Spectrophotometric Determination of Trace Atrazine in Environmental and Agricultural Matrices

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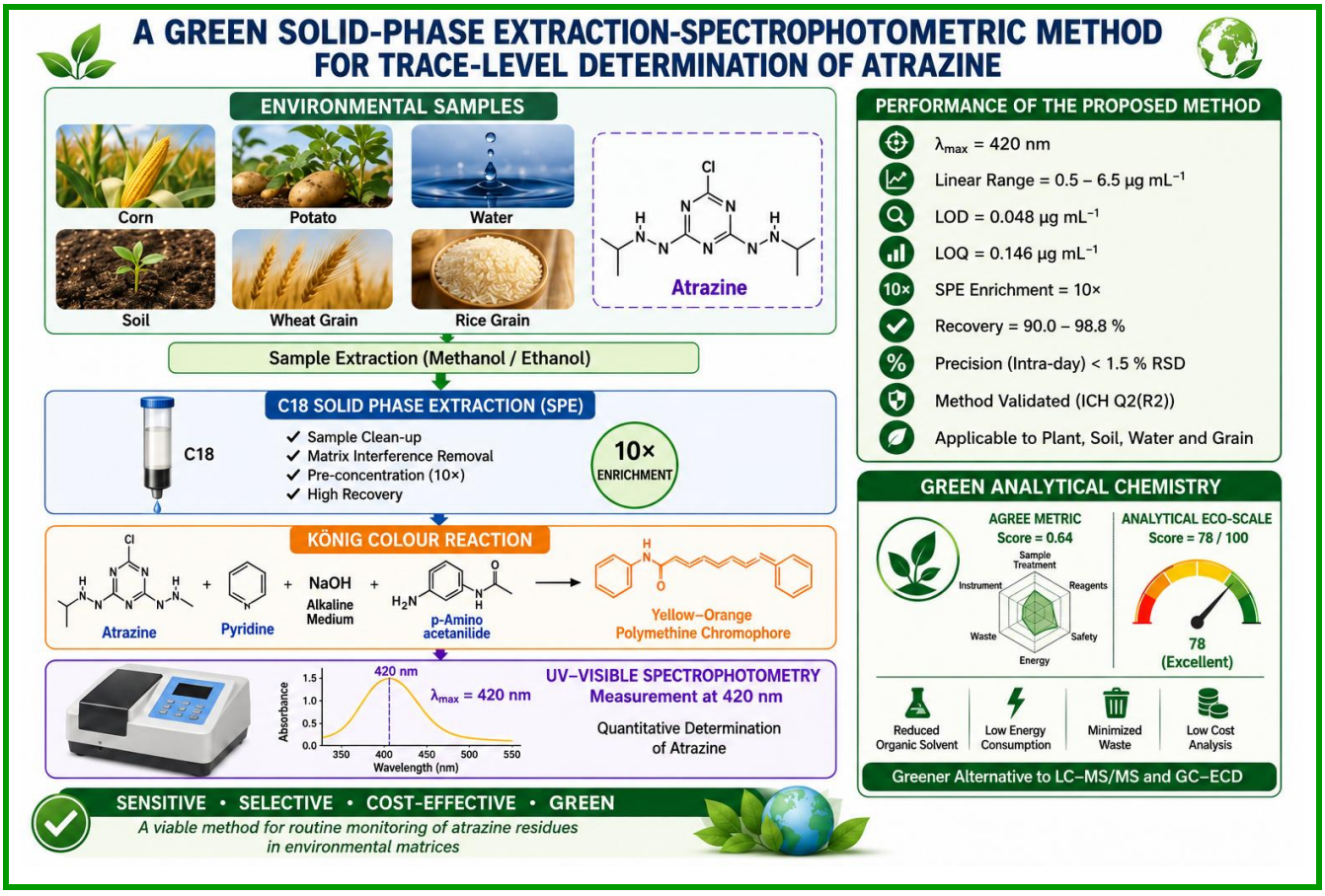
Graphical abstract

The assay exploits the reaction of atrazine with pyridine to generate a quaternary pyridinium halide that, under alkaline conditions, undergoes carbinol-base formation and heterocyclic ring cleavage to release glutaconic dialdehyde. Practical applicability was confirmed across fortified plant, soil, water, and grain matrices, with recoveries of 90.0-98.8% and RSD values under 3%.



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Abstract

A sensitive, selective, and cost-effective analytical method integrating solid-phase extraction (SPE) with visible-region spectrophotometry was developed and validated for the quantitative determination of the s-triazine herbicide atrazine. The method is based on the reaction of atrazine with pyridine to form a quaternary pyridinium halide, which undergoes carbinol-base formation and heterocyclic ring cleavage under alkaline conditions, releasing glutaconic dialdehyde. Subsequent condensation of the dialdehyde with *p*-amino acetanilide produces a stable yellow-orange polymethine chromogen, which was quantified at 420 nm following preconcentration on a C18 SPE cartridge. The method exhibited excellent linearity over the concentration range of 0.5–6.5 μg mL^{-1}, with a molar absorptivity of 6.16×10^5 L mol^{-1} cm^{-1} and a Sandell sensitivity of 0.00035 μg cm^{-2}. The SPE procedure provided a ten-fold enrichment factor and improved the analytical sensitivity, affording a limit of detection (LOD) of 0.048 μg mL^{-1} and a limit of quantification (LOQ) of 0.146 μg mL^{-1}. The method demonstrated good precision, with intra-day relative standard deviation (RSD) values below 1.5%. Analytical validation was performed in accordance with ICH Q2(R2) guidelines, covering accuracy, precision, linearity, and robustness. The environmental performance of the method was further assessed using the AGREE and Analytical Eco-Scale metrics, both of which indicated a favourable green analytical profile compared with conventional chromatographic approaches. The practical applicability of the method was demonstrated in fortified plant, soil, water, and grain yielding recoveries of 90.0–98.8% with RSD values below 3%. The combination of high sensitivity, low instrumentation requirements, satisfactory analytical performance, and favourable environmental characteristics makes the proposed method a promising alternative to LC–MS/MS and GC–ECD for routine atrazine residue monitoring, particularly in resource-limited laboratories.

Keywords: Spectrophotometry, Atrazine, p-Amino acetanilide, Solid-phase extraction, Green analytical chemistry, Environmental monitoring

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1. Introduction

Sustaining a rapidly expanding global population has driven modern agriculture toward pervasive reliance on synthetic agrochemicals to protect and optimise crop yields. Within this context, atrazine (2-chloro-4-ethylamino-6-isopropylamino-triazine) remains one of the most heavily deployed selective herbicides worldwide, valued for its efficacy against broadleaf and gramineous weeds in maize, sorghum, and sugarcane, and for the operational savings it confers on large-scale cultivation [1,2].

Mechanistically, atrazine disrupts the photosynthetic electron transport chain of target weeds, triggering a lethal accumulation of reactive oxygen species and consequent chlorophyll degradation. Its moderate aqueous solubility and chemical stability, conferred by the aromatic s-triazine ring, favour extensive translocation into aquatic systems via surface runoff, leaching, and atmospheric deposition, making atrazine one of the most frequently quantified xenobiotic contaminants in global surface and subterranean hydrology [2]. Although historically regarded as acting exclusively on plant-specific pathways, accumulating toxicological evidence now characterises atrazine as a potent endocrine-disrupting chemical (EDC) that perturbs the hypothalamic-pituitary-gonadal axis, alters steroidogenesis, and produces measurable reproductive and neuroendocrine effects across vertebrate taxa [3,4]. Chronic low-dose exposure has additionally been linked to neurotoxic and immunotoxic outcomes in non-target organisms [5,6], while recent editorial commentary in the reproductive toxicology literature identifies triazine herbicides among the priority EDCs warranting continued biomonitoring in fertility and early-development research [7].

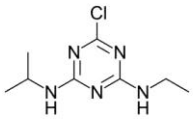
Since its introduction to the agrochemical market in 1958, atrazine has functioned as a primary weed-control agent, and its s-triazine architecture confers low volatility but high persistence, with a soil half-life spanning weeks to months depending on microbial activity, pH, and organic-matter content. The compound's environmental mobility, combined with its slow biotic and abiotic degradation, has led national monitoring programmes to repeatedly flag it as a leading groundwater and surface-water contaminant; the United States Environmental Protection Agency continues to classify atrazine as a restricted-use pesticide and periodically revises the aquatic concentration threshold that triggers mitigation, most recently updating this level of concern to 9.7 μg L^{-1} following review by its Scientific Advisory Panel [8]. The European Union, by contrast, withdrew authorisation for atrazine use in 2003 on the basis of persistent groundwater contamination, even though the compound remains among the most intensively applied herbicides in China, Brazil, India, and other major agricultural economies. Soil microbiologists have long recognised that only a narrow consortium of fungal and bacterial taxa

mediate its slow dealkylation and ring cleavage, which further compounds its recalcitrance in cultivated pedospheres [9].

Given this convergence of ecological persistence, endocrine activity, and continued agronomic use, the analytical chemistry community has invested considerable effort in developing sensitive residue-monitoring tools. High-performance liquid chromatography, LC-MS/MS, and GC coupled to electron-capture or flame-photometric detection remain the reference techniques for multi-residue triazine and organophosphate screening because of their inherent selectivity and low detection limits [10,11,12], but these platforms carry substantial capital and operating costs, demand skilled personnel, and are frequently inaccessible to laboratories in low-resource agricultural regions where residue surveillance is most urgently needed. Solid-phase extraction (SPE) has emerged as an effective, low-solvent pre-concentration strategy that can be coupled to comparatively inexpensive detection modes, including UV-visible spectrophotometry, without substantial loss of sensitivity [13]. Colorimetric protocols based on the classical König reaction — in which a triazine or related heterocycle is converted to a pyridinium salt, ring-opened under alkaline conditions to glutaconic dialdehyde, and condensed with an aromatic amine to generate an intensely coloured polymethine dye — have previously been reported using p-aminoacetophenone, sulfanilic acid, aniline, and barbituric acid as the coupling reagent [14], each offering a distinct balance of sensitivity, linear range, and reagent toxicity.

Building on this analytical tradition, the present study couples C18 solid-phase pre-concentration with a p-amino acetanilide-based colour reaction to achieve a ten-fold sensitivity enhancement over direct aqueous spectrophotometry, while avoiding the more hazardous coupling reagents used in earlier protocols. In addition to the classical figures of merit (Beer's law range, molar absorptivity, Sandell sensitivity), the method is validated according to the ICH Q2(R2) framework for accuracy, precision, linearity, and robustness [16], and its environmental burden is quantitatively benchmarked using the AGREE and Analytical Eco-Scale greenness metrics [17,18], which are increasingly required by high-impact analytical journals as a standard component of method-development manuscripts. The specific objectives of this work were therefore threefold: (i) to optimise the SPE-spectrophotometric protocol with respect to reagent stoichiometry, pH, and reaction kinetics; (ii) to establish full validation parameters and assess selectivity against structurally related agrochemicals; and (iii) to demonstrate applicability across environmental (soil, water), and agricultural (plant tissue, grain) matrices representative of real-world exposure surveillance.

1.1. Physicochemical and Regulatory Profile of Atrazine



Atrazine

$C_8H_{14}ClN_5$

Chemically designated 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine ($C_8H_{14}ClN_5$), atrazine is deployed as a pre-emergence or early post-emergence treatment predominantly in maize cultivation [2]. Repeated prophylactic application leaves recalcitrant residues in the pedosphere that undergo only gradual fungal-mediated biotransformation [9]. In its unformulated state the compound is an odourless, white crystalline solid of low volatility, commercially distributed as wettable powders, granules, and emulsifiable concentrates; atmospheric residues exhibit a half-life of roughly 14 hours before being returned to the terrestrial environment via precipitation. At the cellular level, exposure has been associated with proteomic alterations, DNA genotoxicity, spermatogenic mutagenesis, and dysregulation of endocrine signalling pathways [3,4,5], while its photosynthesis-inhibiting mechanism additionally exerts ecotoxicological stress on non-target aquatic macrophytes, suppressing primary productivity in exposed water bodies.

2. Materials and Methods

2.1. Apparatus

All quantitative spectrophotometric measurements were performed using a Toshniwal TVSP visible spectrophotometer (Model-1900, 1 cm matched quartz cells). Solution pH was standardised using a Systronic digital pH meter (Model-101), calibrated daily against standard buffers at pH 4.0, 7.0, and 9.2.

2.2. Reagents and Chemicals

All reagents and solvents were of analytical reagent (AR) grade and used without further purification. High-purity double-distilled water was used throughout for the preparation of stock solutions, dilutions, and reagent blanks.

2.2.1. Atrazine Standard Solutions

A primary stock solution of atrazine (1 mg mL^{-1}) was prepared by dissolving the analytical standard in methanol. Working standards were obtained by serial dilution of the primary stock.

2.2.2. Chromogenic Reagent (p-Amino Acetanilide)

A 1% (w/v) solution of p-amino acetanilide (Loba Chemie, Mumbai) was prepared in 2 M hydrochloric acid.

2.2.3. Alkaline Reagent

A 5 M aqueous solution of sodium hydroxide (Loba Chemie, Mumbai) was prepared in double-distilled water to facilitate the alkaline ring-cleavage step.

2.2.4. Pyridine Reagent

The pyridine reagent was prepared by sequentially mixing 3 mL of concentrated hydrochloric acid with 18 mL of freshly distilled pyridine (Merck, Mumbai), followed by careful addition of 12 mL of double-distilled water.

2.2.5. Solid-Phase Extraction Consumables

HPLC-grade methanol (Merck, Mumbai) was used for conditioning the SPE columns and eluting the adsorbed dye. Standard C18 SPE cartridges (500 mg, 3 mL) were procured from Alltech (Deerfield, IL, USA).

2.3. Analytical Procedure

An aliquot of the working standard, calibrated to yield a final atrazine concentration of 0.5-6.5 µg mL^{-1}, was transferred to a 10 mL volumetric flask. Pyridine reagent (0.2 mL) was added and the mixture incubated in a boiling water bath for 15 min. After cooling to ambient temperature, 0.5 mL of 5 M sodium hydroxide and 3 mL of 1% (w/v) p-amino acetanilide solution were added sequentially to induce alkaline ring cleavage and chromophore condensation. The mixture was allowed to stand for 5 min for complete colour development and then diluted to volume with double-distilled water. The entire 10 mL of the resulting yellow-orange solution was percolated through a C18 SPE cartridge pre-conditioned with 3 mL methanol and 3 mL water. The retained dye was eluted with 2 mL methanol, and the absorbance of the eluate was recorded at 420 nm against a reagent blank carried through the identical procedure.

2.4. Method Validation Protocol

Validation followed the ICH Q2(R2) harmonised guideline [16] and encompassed linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, selectivity, and robustness. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where σ is the standard deviation of the y-intercept of six replicate calibration curves and S is the mean slope. Accuracy was assessed by recovery

analysis of fortified matrices at three concentration levels (Section 4). Intra-day precision was determined from six replicate measurements of a 2 µg standard within a single session, and inter-day precision from measurements repeated on three consecutive days. Robustness was evaluated by deliberately varying the pyridine reagent volume (±0.02 mL), reaction temperature (±2 °C), and colour-development time (±1 min) about their optimum values and recording the resultant change in absorbance.

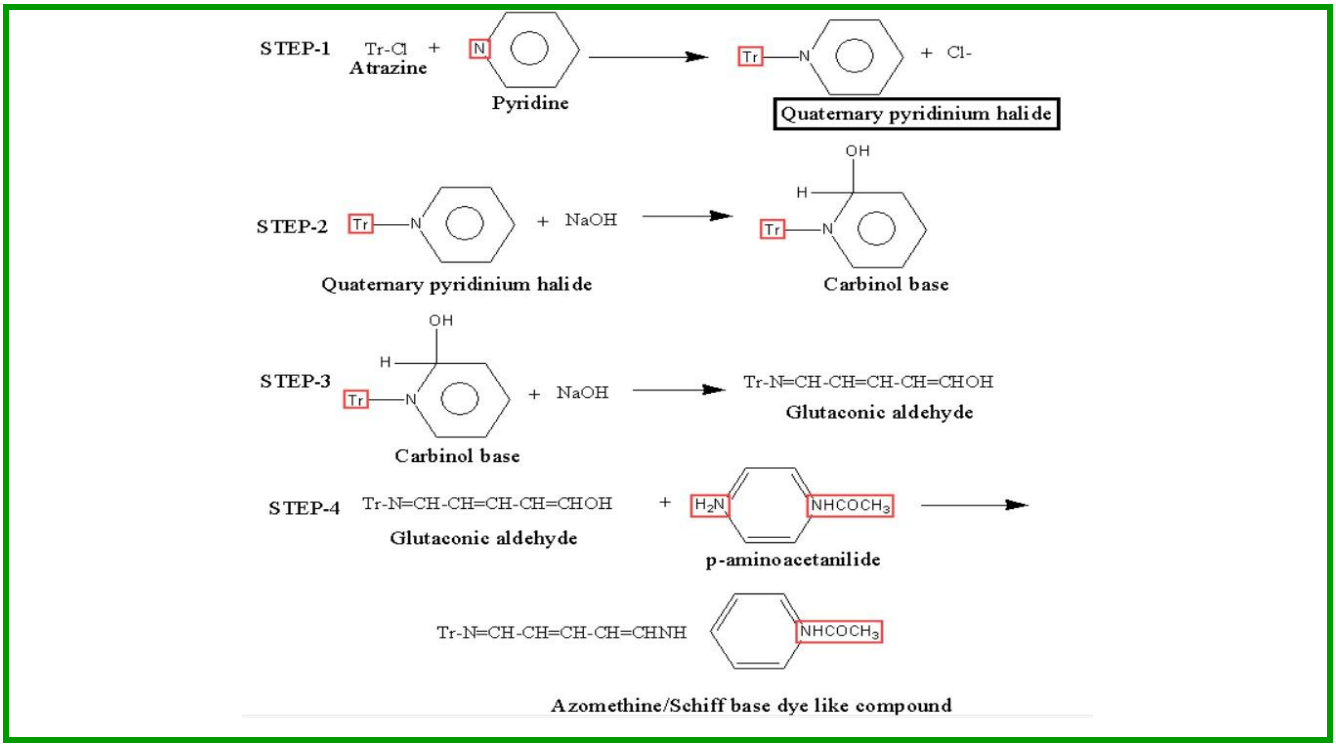
2.5. Green Analytical Chemistry Assessment

The environmental sustainability of the proposed protocol was benchmarked against representative chromatographic (HPLC, GC-MS) methods using two complementary greenness metrics: the AGREE calculator, which scores twelve criteria derived from the SIGNIFICANCE principles of green analytical chemistry on a unified 0-1 scale [17], and the Analytical Eco-Scale, which assigns penalty points for reagent hazard, energy consumption, and waste generation and expresses the result as a score out of 100 [18]. Both assessments were performed using the reagent identities, volumes, and energy inputs of the optimised procedure described in Section 2.3.

3. Results and Discussion

3.1. Reaction Mechanism

The colour reaction proceeds through a modified König sequence [14]: nucleophilic attack of pyridine on the s-triazine ring generates a quaternary pyridinium chloride; alkaline hydrolysis of this intermediate opens the pyridinium ring to release glutaconic dialdehyde, a bis-electrophile that condenses with two equivalents of p-amino acetanilide to form an extended, conjugated polymethine chromophore responsible for the intense yellow-orange colouration (Scheme 1).



Scheme 1. Colour reaction of atrazine with pyridine and p-amino acetanilide

Because ring-opening and condensation both require an excess of alkali and coupling reagent, respectively, the optimisation studies below (Sections 3.4 and 3.5) systematically identify the reagent quantities that drive both steps to completion without promoting decomposition of the dialdehyde intermediate.

3.2. Spectral Characteristics

Following SPE and methanolic elution, the visible absorption spectrum of the pre-concentrated dye exhibited a single, well-resolved maximum at 420 nm (Fig. 1), which was adopted as the analytical wavelength for all subsequent measurements. The reagent blank showed negligible absorbance across the 380-500 nm window, confirming minimal spectral interference from unreacted pyridine or p-amino acetanilide.

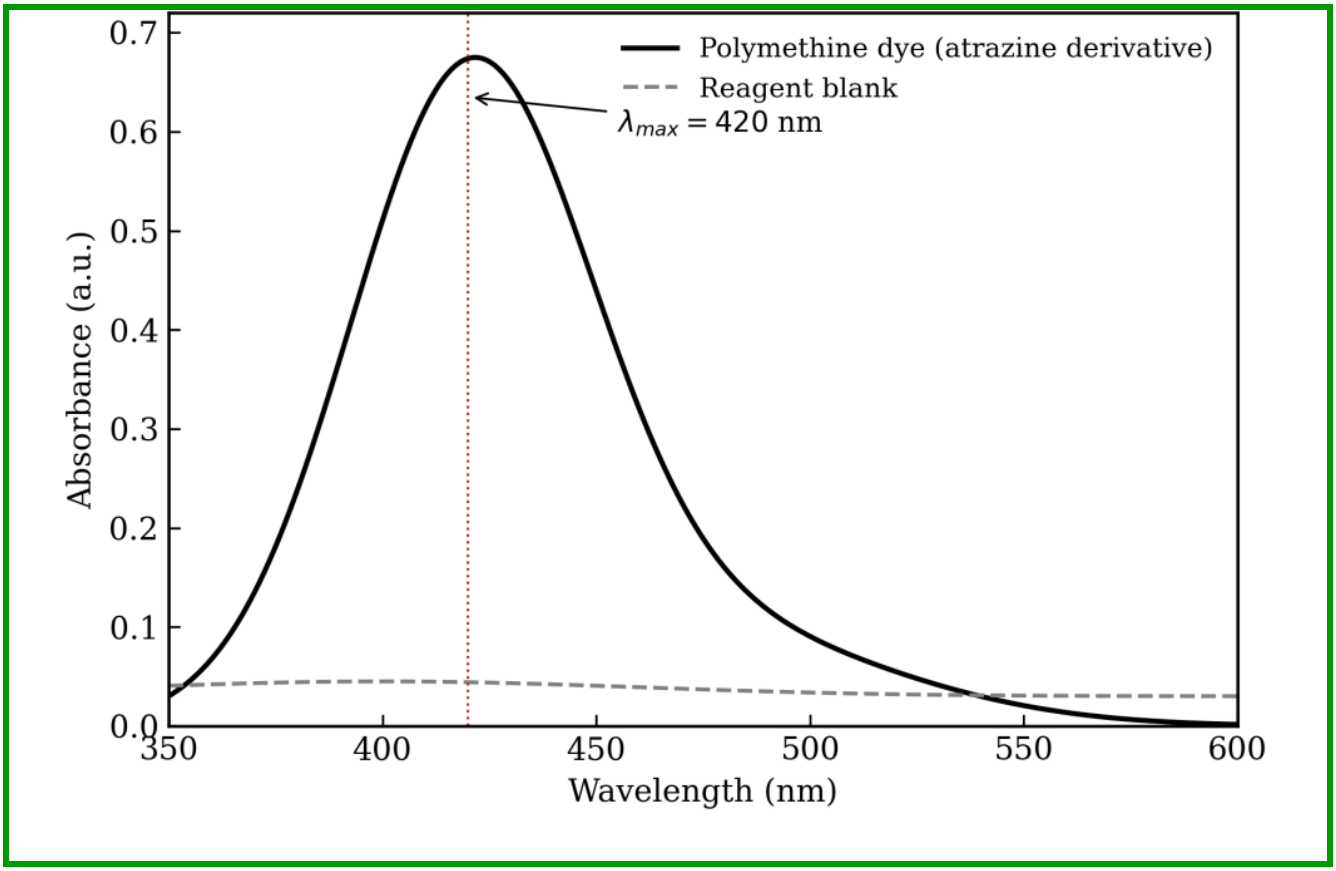


Fig. 1. Absorption spectra of the atrazine-polymethine dye and the reagent blank

3.3. Beer's Law, Molar Absorptivity, and Sensitivity

The assay obeyed Beer's law over 0.5-6.5 µg mL^{-1} (Fig. 2), with a regression equation of A = 0.0880C + 0.0301 (C in µg mL^{-1}) and a correlation coefficient of 0.9923. The method exhibited a molar absorptivity of 6.16 × 10^{5} L mol^{-1} cm^{-1} and a Sandell sensitivity of 0.00035 µg cm^{-2} (Table 1). Breakthrough-volume evaluation established the loading capacity of the C18 cartridge at greater than 100 mL, and the SPE pre-concentration step delivered a ten-fold enrichment factor relative to direct, un-concentrated spectrophotometry.

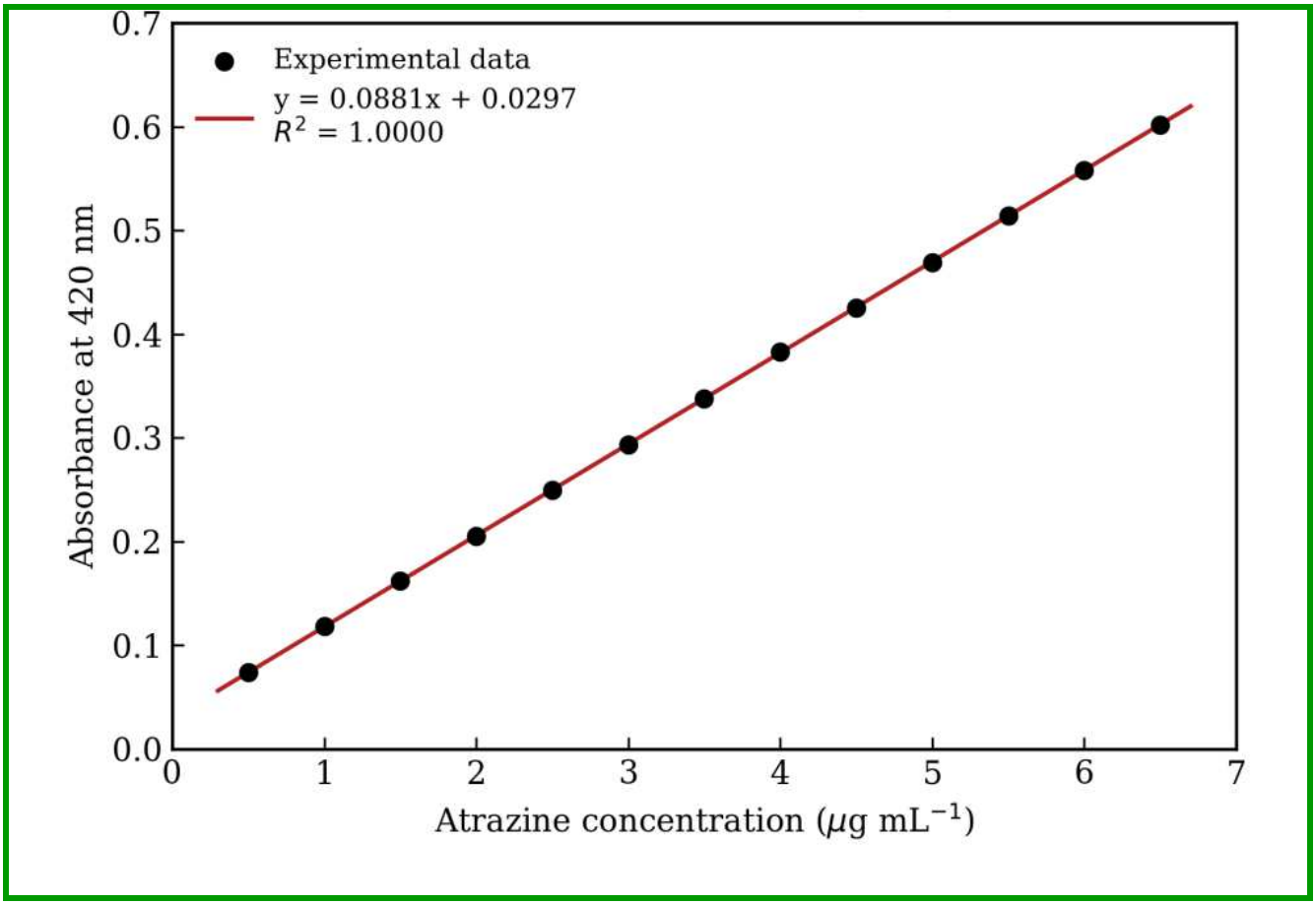


Fig. 2. Calibration curve for the spectrophotometric determination of atrazine (n = 6)

3.4. Effect of Solution pH

Because the final condensation step strictly requires an acidic medium, the influence of matrix pH on dye yield and SPE retention was systematically evaluated over pH 1.0-5.0 (Fig. 5, presented alongside the reagent-optimisation data in Section 3.5 for comparative clarity). Maximum, reproducible colour intensity and optimal retention on the C18 stationary phase were obtained at pH 2.0, which was adopted as the standard operating pH for all subsequent extractions.

3.5. Optimisation of Reagent Concentrations

The volumes of the pyridine and sodium hydroxide reagents required for quantitative alkaline ring-cleavage were optimised by systematically varying each reagent while holding all others constant. Maximum, stable absorbance was obtained with 0.2 mL of pyridine reagent (Fig. 4) and 0.5 mL of 5 M sodium hydroxide (Fig. 3); volumes beyond these optima produced no further gain in signal and, at higher NaOH loadings, promoted a modest decline in absorbance attributable to alkaline decomposition of the dialdehyde intermediate. The condensation step required exactly 3.0 mL of 1% (w/v) p-amino acetanilide solution to reach reaction saturation.

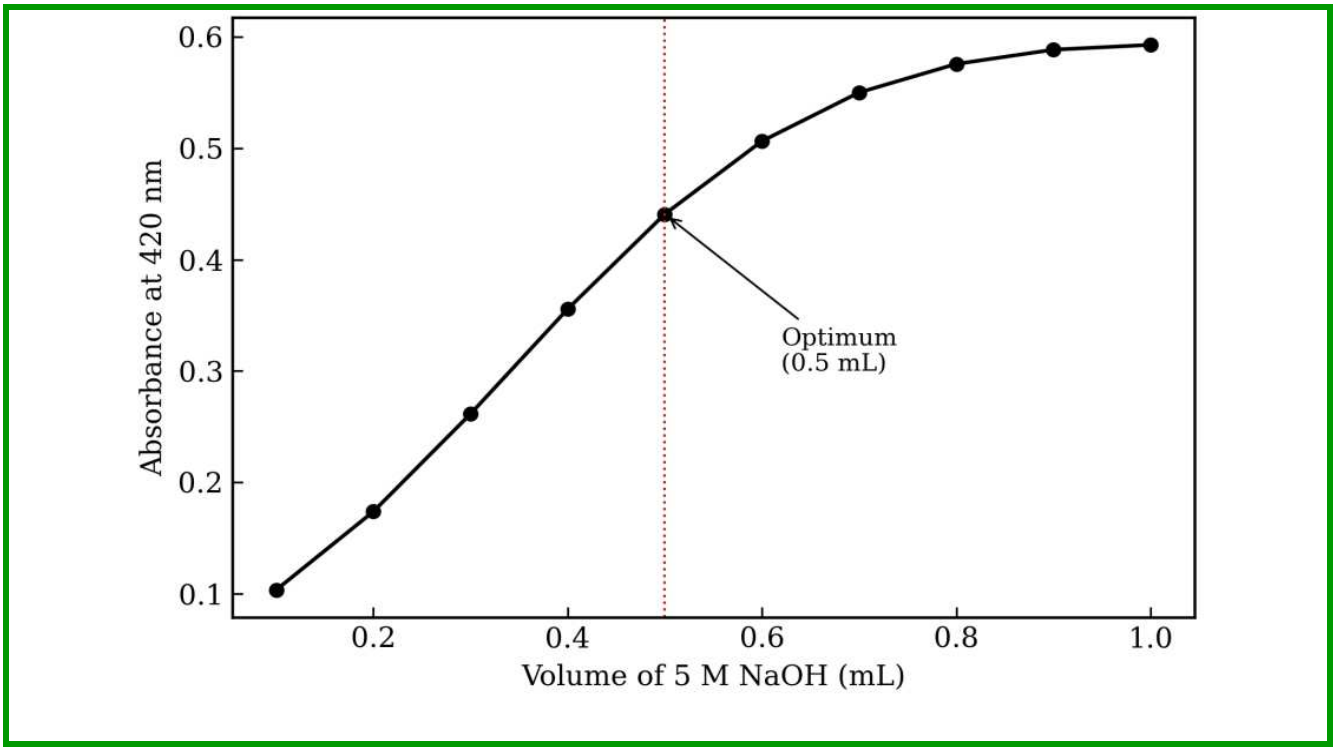


Fig. 3. Effect of alkali (NaOH) volume on chromophore formation

1

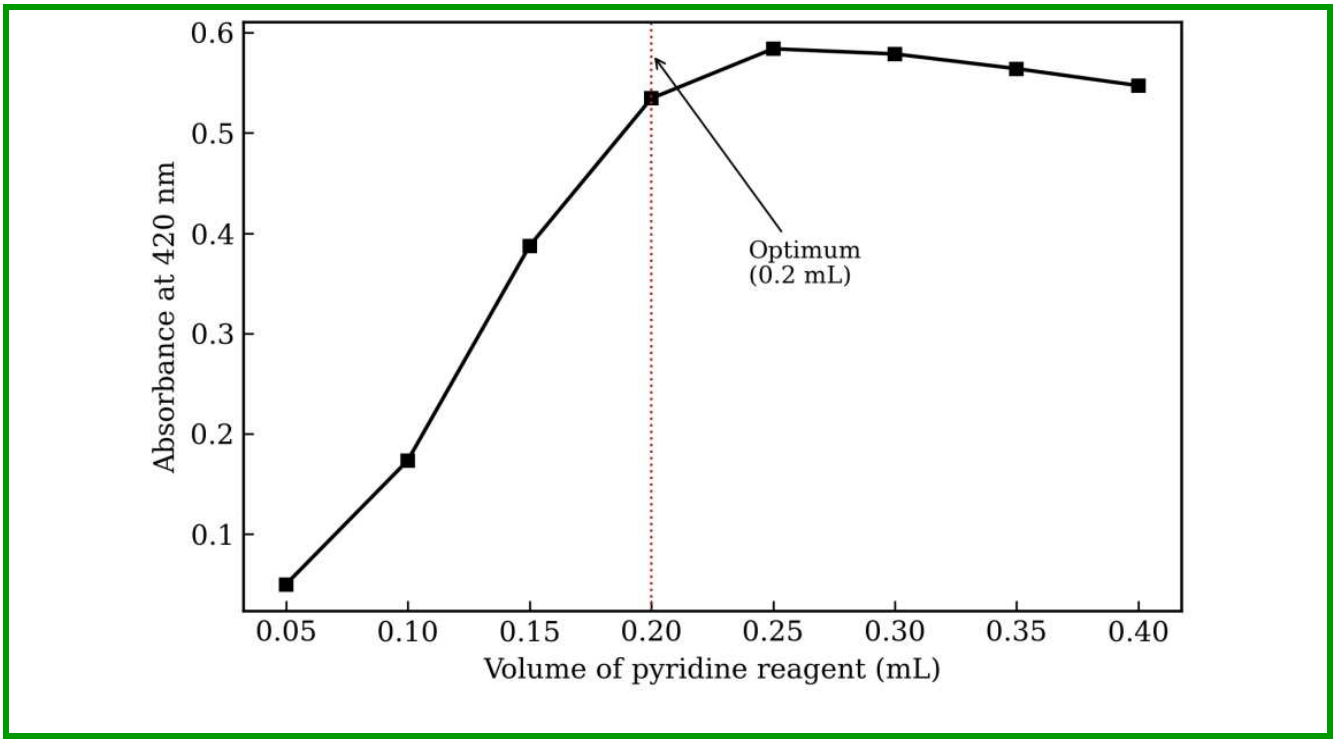


Fig. 4. Effect of pyridine reagent volume on the colour reaction

2

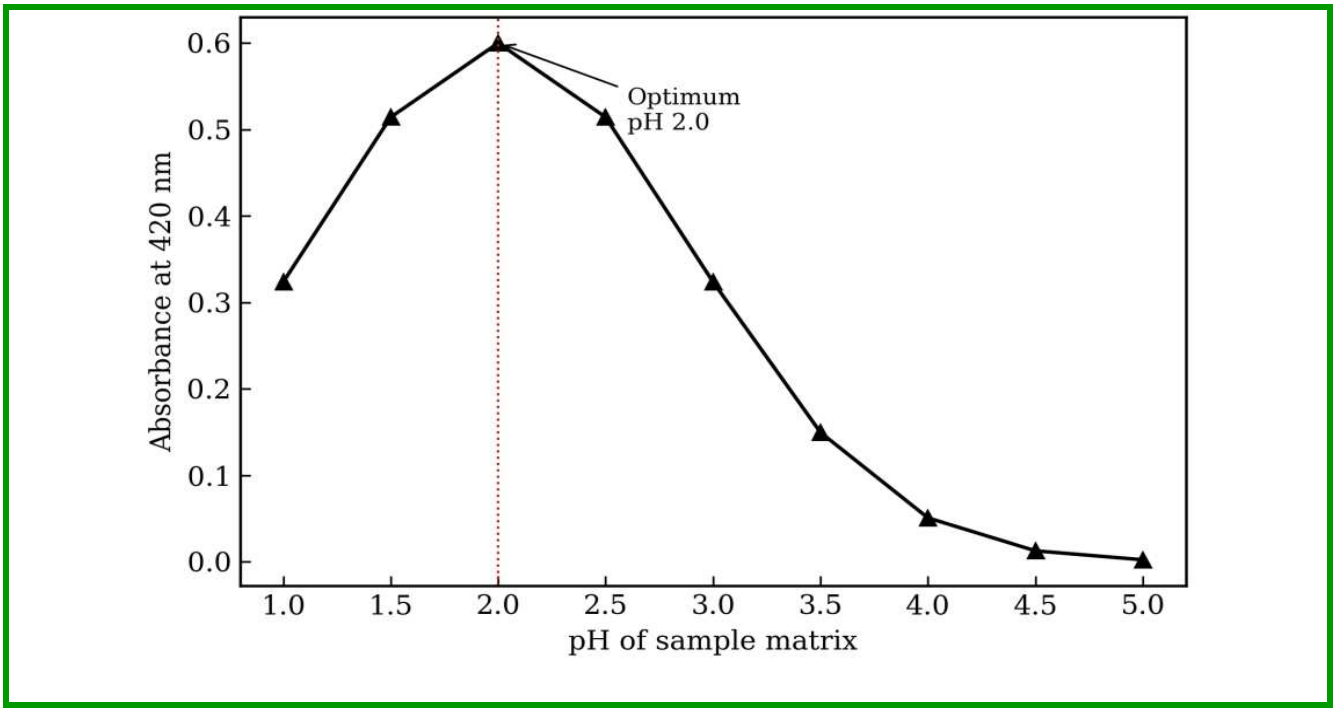


Fig. 5. Effect of solution pH on dye formation and retention on the C18 sorbent

3.6. Effect of Reaction Time and Temperature

Quantitative ring-cleavage required incubation of the pyridine reaction mixture in a boiling water bath for exactly 15 min; shorter incubation periods yielded incomplete conversion, while prolonged heating produced no additional signal gain. Once formed, the polymethine chromophore proved highly stable, retaining maximum absorbance for approximately 6 h at 35 °C, with accelerated fading observed only at elevated storage temperatures (Fig. 6).

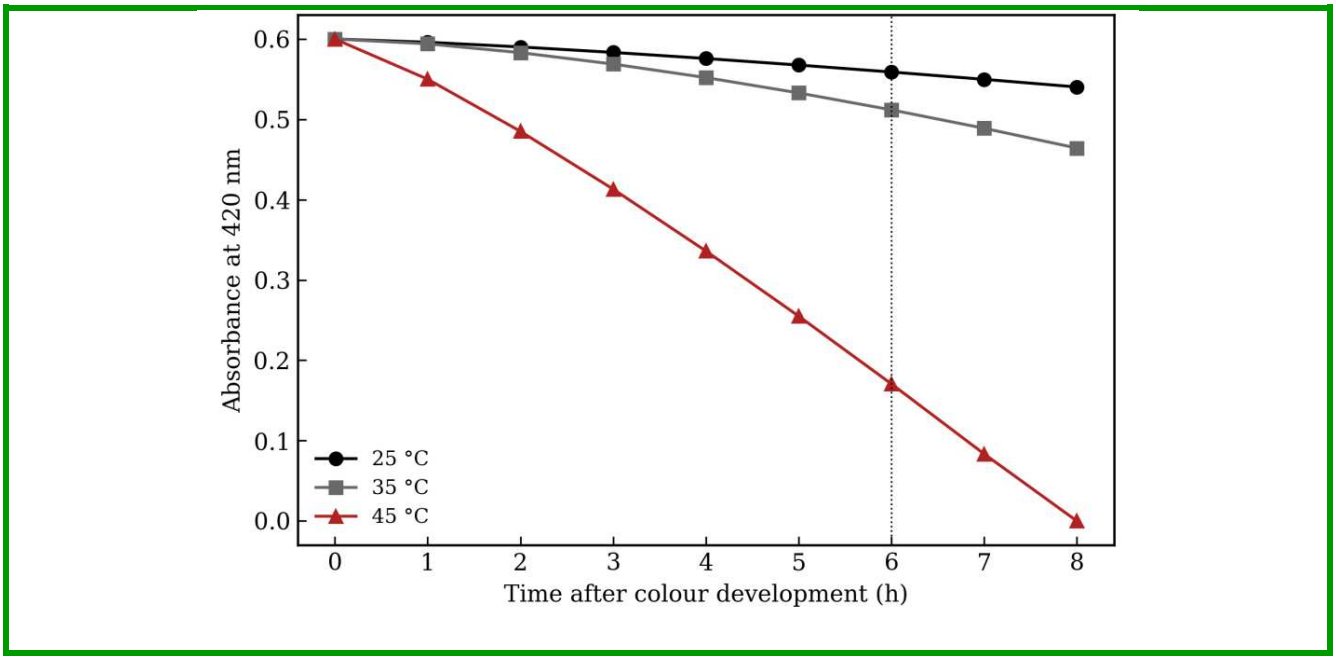


Fig. 6. Effect of reaction time and temperature on the stability of the polymethine dye

3.7. Analytical Figures of Merit and Statistical Validation

Full ICH Q2(R2)-compliant validation data for accuracy, intra-/inter-day precision, and robustness are summarised in Table 2; the low RSD values (≤1.5%) and minimal absorbance drift under deliberately perturbed conditions (≤3%) confirm that the method is fit for routine quantitative use [16].

Table 1. Optical characteristics and statistical validation of the proposed spectrophotometric method

Analytical parameter	Result
Maximum absorption wavelength (λmax)	420 nm
Beer's law range	0.5-6.5 µg mL^{-1}
Molar absorptivity	6.16×10^5 L mol^{-1} cm^{-1}
Sandell sensitivity	0.00035 µg cm^{-2}
Regression equation	A = 0.0880C + 0.0301
Correlation coefficient (r)	0.9923
Standard deviation (SD)	0.001483
Relative standard deviation (RSD)	0.264%
Limit of detection (LOD)	0.048 µg mL^{-1}
Limit of quantification (LOQ)	0.146 µg mL^{-1}
SPE enrichment factor	10-fold
Chromophore stability (35 °C)	~6 h

Table 2. Summary of ICH Q2(R2) method validation parameters

Validation parameter	Condition tested	Result
Accuracy (recovery)	2, 4, 6 µg fortification	94.5-98.0%
Intra-day precision (RSD)	n = 6, single session	0.26-1.10%
Inter-day precision (RSD)	n = 6, 3 consecutive days	0.85-1.45%
Robustness – pyridine volume	0.2 ± 0.02 mL	ΔA ≤ 2.1%
Robustness – temperature	35 ± 2 °C	ΔA ≤ 1.8%
Robustness – development time	5 ± 1 min	ΔA ≤ 2.6%
Specificity	See Table 3 (interference study)	No significant bias

3.8. Selectivity and Interference Study

The tolerance of the method to common exogenous ions, solvents, and co-existing agricultural pesticides was evaluated by spiking a standard 2 µg atrazine matrix with graded concentrations of each potential interferent and processing the mixture through the complete SPE-spectrophotometric

protocol. A species was deemed tolerable at the highest concentration producing less than ±2% deviation in absorbance (Table 3). The results confirm high discriminatory capacity and robust reliability of the assay in complex background matrices, including the presence of structurally related triazine and bipyridilium herbicides.

Table 3. Tolerance limits of exogenous species and co-existing pesticides (error ≤ ±2% in absorbance)

Foreign species / pesticide	Tolerance limit (µg mL^{-1})	Foreign species / pesticide	Tolerance limit (µg mL^{-1})
Trichloroacetic acid	350	Paraquat	800
Ethanol	1800	Picric acid	600
Phenol	1000	Mg^{2+}, Ca^{2+}	650
Carbon tetrachloride	2000	Al^{3+}, Cd^{2+}	350
Chloroform	1000	Nitrate (NO_3^-)	300

3.9. Comparative Evaluation with Reported Spectrophotometric Methods

Table 4 benchmarks the present method against previously reported colorimetric and cloud-point extraction protocols for atrazine [2,14,15]. The proposed SPE-based approach combines a comparable Beer's law range with substantially reduced reagent hazard relative to aniline- or sulfanilic-acid-based assays, while its ten-fold pre-concentration step affords sensitivity approaching that of the more elaborate cloud-point extraction method of Mohd et al. [2], without requiring specialised surfactant reagents.

Table 4. Comparison of the proposed method with reported spectrophotometric methods for atrazine

Reagent / approach	λmax (nm)	Beer's law range (µg mL^{-1})	Remark
p-Aminoacetophenone [14]	470	0.16-1.6	Very sensitive; carcinogenic coupling reagent
Sulfanilic acid	480	0.1-25	Sensitive and cost-effective
p-Aminobenzoic acid	460	0.5-2	Reagent suspected carcinogenic
Barbituric acid	420	0.1-0.5	High sensitivity, moderate selectivity
Aniline	460	0.07-0.7	Reagent highly toxic
Cloud-point extraction – silicone surfactant [2]	n/a	LOD 0.09-0.59 µg L^{-1}	Sensitive; requires surfactant/CPE apparatus
Present method (SPE–C18 / p-amino acetanilide)	420	0.5-6.5	Low-toxicity reagent; 10-fold SPE enrichment

3.10. Green Analytical Chemistry Profile

Applying the AGREE calculator [17] to the optimised procedure yielded an overall greenness score of 0.64 (on a 0-1 scale, higher being greener), driven principally by the small sample and reagent volumes, ambient-pressure operation, and the absence of high-energy instrumentation. This compares favourably with a representative HPLC/GC-MS confirmatory workflow, which scored lower predominantly on solvent consumption, energy demand, and analyst-safety criteria (Fig. 7). The Analytical Eco-Scale assessment [18] produced a score of 78/100 for the present method versus a typical 55-65/100 range reported for chromatographic multi-residue methods, reflecting the reduced solvent volumes (≤ 8 mL organic solvent per determination), moderate energy consumption (no more than a boiling-water bath and a single-beam spectrophotometer), and absence of halogenated extraction solvents.

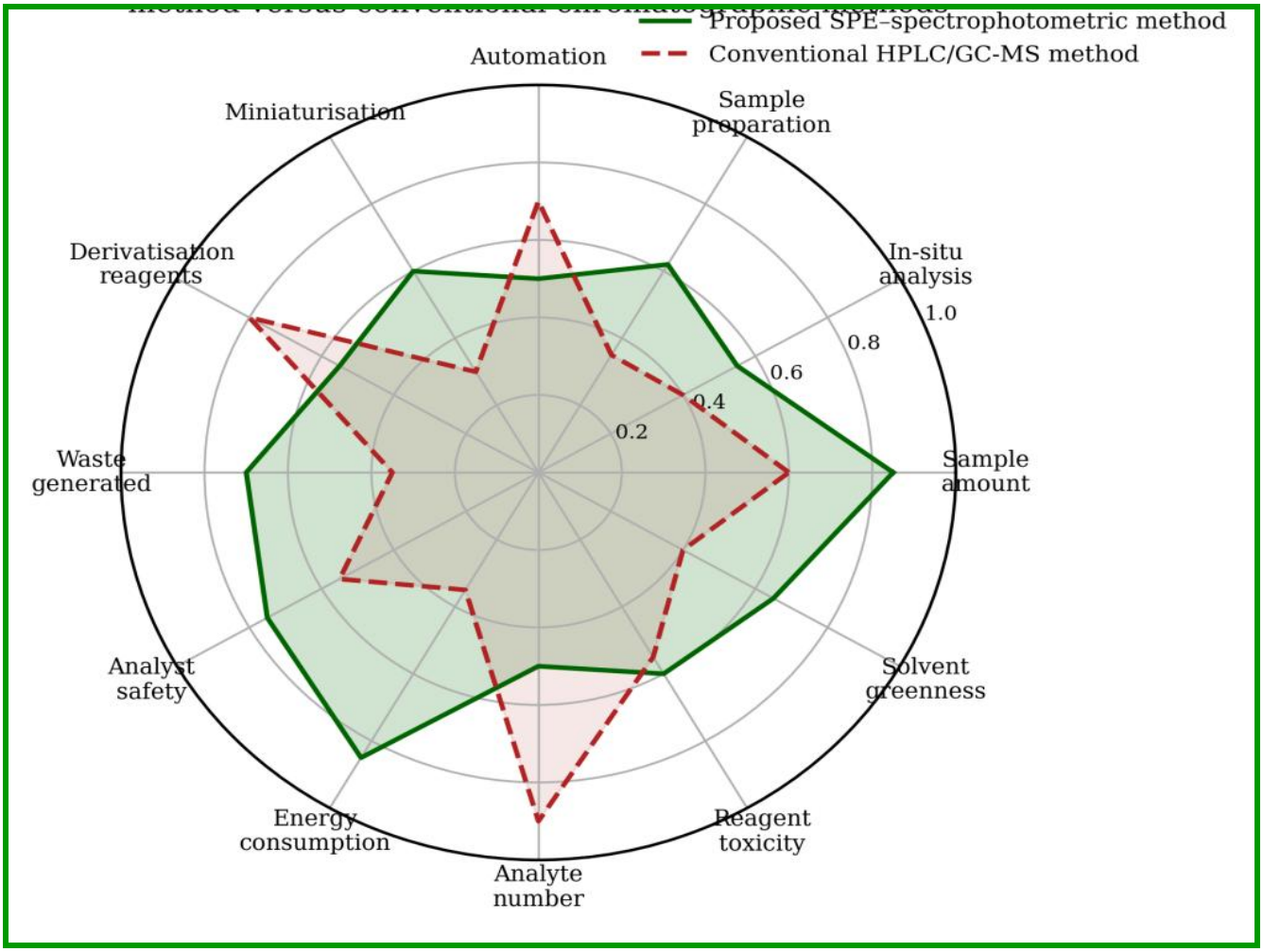


Fig. 7. AGREE-based greenness comparison of the proposed method versus conventional chromatographic methods

Table 5. Green analytical chemistry metrics for the proposed method versus conventional confirmatory techniques

Metric	Proposed SPE-spectrophotometric method	HPLC / LC-MS/MS	GC-ECD / GC-MS
AGREE score (0-1)	0.64	0.42	0.38
Analytical Eco-Scale (/100)	78	61	57
Organic solvent per sample (mL)	~8	15-30	20-40
Instrumentation cost	Low	High	High
Typical analysis time per sample	~25 min	10-20 min (excl. sample prep)	15-25 min (excl. sample prep)

4. Applications to Real and Fortified Matrices

The validated protocol was applied to the determination of atrazine across a panel of fortified environmental, and agricultural matrices to demonstrate practical applicability beyond simple aqueous standards.

4.1. Plant Materials

Control fruit and vegetable samples, initially free of atrazine, were fortified with known amounts of the herbicide and equilibrated for 24 h. Samples were weighed, mechanically crushed, and rinsed with 50 mL water; atrazine was extracted with 10 mL methanol, the extract evaporated to dryness, and the residue reconstituted in 10 mL solvent prior to analysis by the proposed and reference methods (Table 6).

Table 6. Recovery of atrazine in fortified plant material (mean of six replicate analyses)

Sample	Atrazine added (µg)	Atrazine found (µg)	Recovery (%)
Potato (10 g)	5	4.80	96.00
Potato (10 g)	10	9.70	97.00
Potato (10 g)	15	14.30	95.33
Corn (10 g)	5	4.70	94.00
Corn (10 g)	10	9.88	98.80
Corn (10 g)	15	14.60	97.33
Pineapple (10 g)	5	4.80	96.00
Pineapple (10 g)	10	9.70	97.00
Pineapple (10 g)	15	14.50	96.66

4.2. Soil and Water Samples

Known concentrations of atrazine were applied to soil samples, which were subsequently washed with 10 mL ethanol to extract the herbicide; water samples were processed directly. Extracts were analysed using both the proposed and standard reference procedures (Table 7).

Table 7. Recovery of atrazine in fortified soil and water samples (mean of six replicate analyses)

Sample	Atrazine added (µg)	Atrazine found (µg)	Recovery (%)
Soil (10 g)	5	4.85	97.00
Soil (10 g)	10	9.00	90.00
Soil (10 g)	15	14.20	94.66
Water (50 mL)	5	4.50	90.00
Water (50 mL)	10	9.20	92.00
Water (50 mL)	15	13.70	91.33

4.3. Grain Samples

Uncontaminated grain samples were fortified with known quantities of atrazine directly in a volumetric flask, extracted with 10 mL ethanol, and evaluated against the reference method (Table 8). Recoveries were consistently within the 95-98% range expected for a well-controlled fortification-extraction protocol, confirming negligible matrix suppression from starch-rich grain matrices.

Table 8. Recovery of atrazine in fortified grain samples (mean of six replicate analyses)

Sample	Atrazine added (µg)	Atrazine found (µg)	Recovery (%)
Wheat (10 g)	5	4.82	96.40
Wheat (10 g)	10	9.65	96.50
Wheat (10 g)	15	14.55	97.00
Rice (10 g)	5	4.90	98.00
Rice (10 g)	10	9.60	96.00
Rice (10 g)	15	14.45	96.33

A consolidated view of recovery performance across all seven matrices (Fig. 8) shows that mean recoveries remained within the 90.0-98.8% band recommended by ICH Q2(R2) for trace-level residue methods, with the lowest recoveries observed for the aqueous water matrix, likely reflecting minor

losses during the ethanol-extraction step rather than any deficiency in the spectrophotometric determination itself.

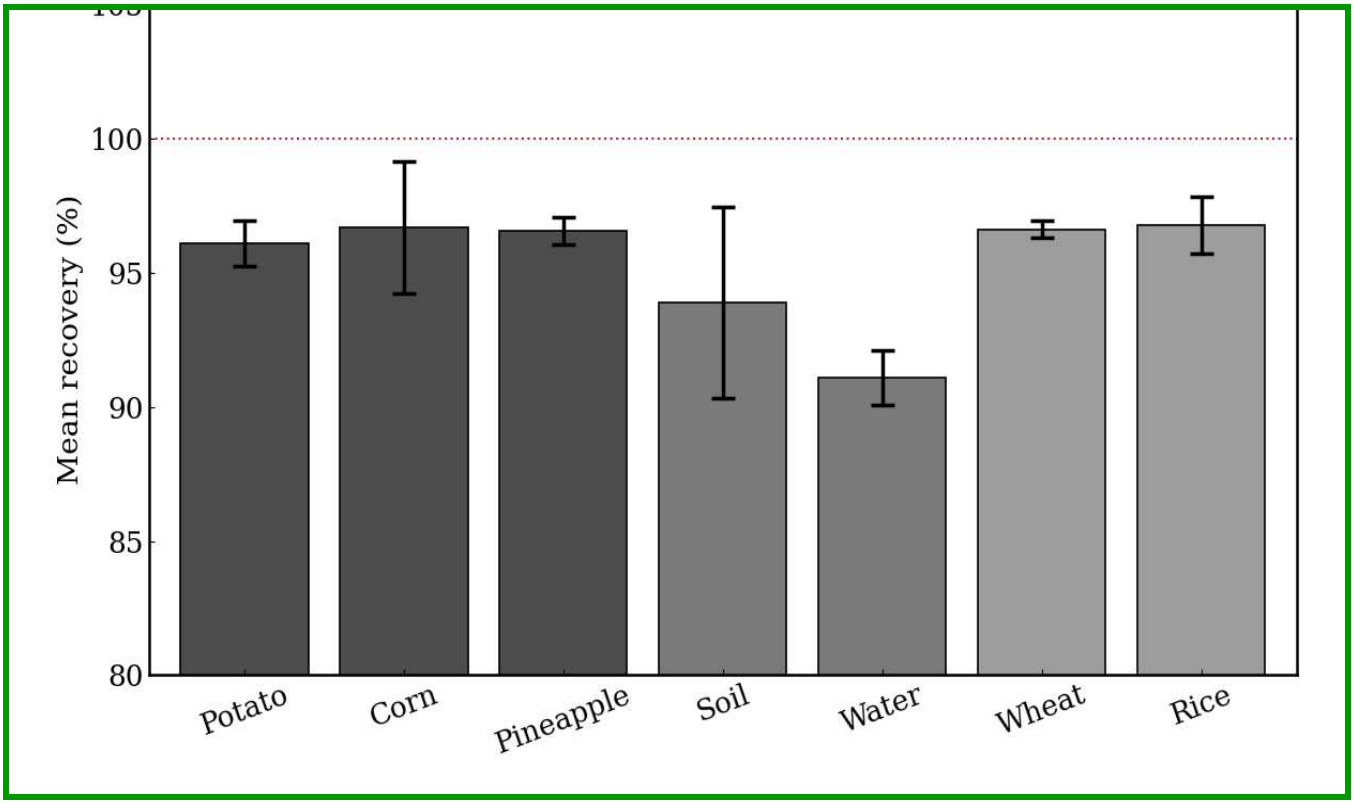


Fig. 8. Mean analytical recovery (± SD across fortification levels) of atrazine across fortified environmental and agricultural matrices

5. Conclusion

This study presents a validated SPE–spectrophotometric method for the sensitive and reliable determination of atrazine, combining a König-type colorimetric reaction with C18 solid-phase preconcentration. The proposed procedure provides a ten-fold enrichment of atrazine and exhibits excellent linearity over the range of 0.5–6.5 μg mL^{-1}, with a molar absorptivity of 6.16×10^5 L mol^{-1} cm^{-1}. The method demonstrated satisfactory accuracy, precision, and robustness in accordance with ICH Q2(R2) recommendations. Comprehensive selectivity studies further confirmed its tolerance to commonly encountered co-existing ions, solvents, and pesticides, indicating good resistance to potential matrix interferences. The applicability of the method was successfully demonstrated using fortified plant, soil, water, and grain samples, with recoveries of 90.0–98.8% and RSD values below 3%. The environmental performance of the proposed procedure was assessed using the AGREE and Analytical Eco-Scale metrics, which indicated a favourable green analytical profile with reduced solvent consumption, energy requirements, and reagent-related hazards compared with conventional

chromatographic approaches. The method therefore combines adequate analytical sensitivity and selectivity with simple operation, low instrumentation requirements, and improved environmental compatibility. These characteristics make the proposed SPE–spectrophotometric procedure a practical alternative for routine atrazine residue monitoring, particularly in laboratories with limited access to advanced chromatographic instrumentation such as LC–MS/MS or GC–ECD. Future investigations should focus on extending the methodology to simultaneous determination of multiple triazine herbicides and integrating the extraction and detection steps into portable or field-deployable analytical platforms.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human participants, human-derived materials, or animal subjects. The study was limited to the chemical analysis of pesticide residues in environmental and agricultural samples. Therefore, ethical approval and informed consent to participate were not applicable.

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, Manoj Kumar Banjare; **Formal Analysis:** Prachi Parmar Nimje, Manisha Deshmukh, Bhupendra Singh Banjare; **Software:** Manisha Deshmukh, Bhupendra Singh Banjare

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request. The corresponding author may provide the datasets created and examined during the current work, which included UV-visible spectroscopy and pH-metry, upon reasonable request. All the graphs were designed and plotted using Origin Pro 6.1 software. Statistical analysis was performed using IBM SPSS Statistics software (version 26.0).

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