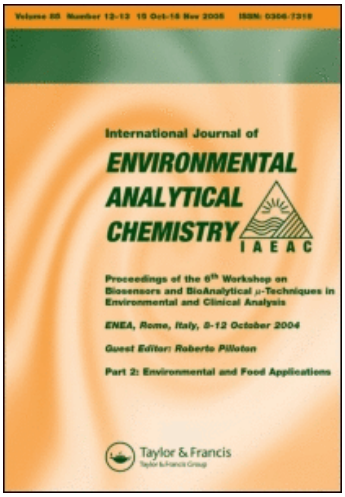

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Spectrophotometric method for determination of atrazine and its application to commercial formulations and real samples

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Spectrophotometric method for determination of atrazine and its application to commercial formulations and real samples

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A simple sensitive spectrophotometric method has been developed for the determination of atrazine in herbicide formulations and real samples. The method was based on the reaction of atrazine with pyridine to form a quaternary halide which in the presence of alkali forms a carbinol base. The heterocyclic ring of the carbinol base breaks and forms the glutaconic dialdehyde. The glutaconic dialdehyde group was coupled with sulfanilic acid to form a yellow coloured product having λ_{max} 450 nm or coupled with aniline to form a orange red coloured product having λ_{max} 480 nm. The Beer's law was obeyed over the range from 0.1 to 25 $\mu g\,mL^{-1}$ and molar absorptivity $1.5 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ for sulfanilic acid, and from 0.08 to 12 $\mu g\,mL^{-1}$ and molar absorptivity $1.3 \times 10^4\,L\,mol^{-1}\,cm^{-1}$ for aniline were observed. The reaction conditions and other analytical parameters were optimised. The proposed method has been successfully applied for the analysis of commercial formulations and real samples.

Keywords: atrazine; carbinol base; glutaconic dialdehyde; sulfanilic acid; aniline

1. Introduction

Atrazine (2-chloro-4-ethylamine-6-isopropylamine-*S*-triazine) has a low acute toxicity as compared to other herbicides and is used to control broadleaf weed and some grassy weeds on many crops like sugar cane, corn, wheat, sorghum, etc. [1,2].

Due to its wide use and toxicity, a large number of instrumental methods such as GC-MS [3], HPLC [4], TLC [5], capillary LC [6], potentiometery [7] and simultaneous determination by spectrophotometric method [8] are reported for its determination. Very few reagents are reported for spectrophotometric determination of atrazine like ethylcyanoacetate [9], barbituric acid [10] and *p*-aminoacetophenone [11].

In the present work, a sensitive spectrophotometric method using sulfanilic acid and aniline as a coupling reagents has been developed for the determination of atrazine in various environmental samples. The method is based on reaction of atrazine with pyridine in the presence of hydrochloric acid on heating followed by addition of alkali. The yellow coloured product was formed after coupling with sulfanilic acid and the orange red coloured product was formed with aniline.

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2. Experimental

2.1 *Apparatus*

A UV–Visible spectrophotometer (Unico 2100 United Products Instrument Inc., Dayton, NJ, USA) with 1 cm cells was used.

2.2 *Reagents*

All chemicals used were of analytical reagent grade or similar. Pyridine (79.1 g mol^{-1}), HCl (37%), methanol, NaOH and sulfanilic acid (Merck, Darmstadt, Germany) were used throughout the work without further purification. Reference standard of atrazine was purchased from Dr Ehrenstofer GmbH (Germany). Commercial formulations were purchased from the local market.

Sulfanilic acid solution was prepared by dissolving 1.0 g in 36 mL of concentrated hydrochloric acid and diluted upto 100 mL with distilled water. Aniline solution was prepared by mixing 6 mL of aniline in methanol and diluted up to 100 mL with methanol. Atrazine stock solution (1000 μg mL^{-1}) was prepared by dissolving 0.1 g of standard in 40 mL methanol and diluted with distilled water up to 100 mL. Working standard of 100 μg mL^{-1} and 10 μg mL^{-1} solutions were prepared from a stock solution by dilution.

2.3 *Procedure*

Atrazine solutions of 0.1–25 μg mL^{-1} were taken in reaction flasks followed by the addition of 0.35 mL of pyridine (79.1 g mol^{-1}) solution and 0.1 mL of concentrated hydrochloric acid solution (37%) to get 0.86 M pyridine and 0.15 M HCl solutions after dilution. These solutions were heated on boiling water bath for 25 min. After cooling, 4 mL of 1.75 M NaOH were added followed by the addition of 6 mL of 1% sulfanilic acid. The solutions were diluted to 100 mL with distilled water. The same procedure was applied for aniline as a coupling reagent. The absorbances of the coloured products were measured at 450 nm for sulfanilic acid and 480 nm for aniline against reagent blank.

2.4 *Procedure for determination of atrazine in sugar cane juice and corn samples*

Samples were collected from an agriculture field, where atrazine was sprayed and were first analysed for the residue determination. Known amounts (60 mL) of sugar cane juice and corn (15 g in 60 mL of distilled water) were taken separately and stirred on magnetic plate for 30 min. The homogeneous solution of each sample was then divided into three equal parts. Atrazine was extracted from the sample by step wise addition of 2×10 mL of chloroform. The chloroform extract was evaporated to dryness and the residue was dissolved in 5 mL methanol. The methanolic solution was then analysed by the proposed method.

2.5 *Standard addition method for real samples*

The validity of the method was confirmed by applying the standard addition technique. In this method several different concentrations of the standard solution of atrazine (6, 7.8, 9.7 μg mL^{-1} for sugar cane juice and 6, 7.4, 9.8 μg mL^{-1} for corn) were added to the

sample and the above-mentioned procedure was used for extraction in triplicate. The colour was developed by the recommended procedure and the % recoveries were calculated.

2.6 *Commercial formulations of herbicide A (bestrazine) and B (mixture of atrazine and ametryn)*

The standard addition method was also applied to the commercial formulations to check the recovery of these herbicides in the presence of adulterants. A known amount (0.25 g in 20 mL of distilled water) of herbicide A (bestrazine) and herbicide B (mixture of atrazine and ametryn) were taken separately and stirred on magnetic plate for 30 min. The homogeneous solution of each sample was then diluted with water up to 100 mL. Atrazine was extracted from the sample by step wise addition of 2×10 mL of chloroform. The chloroform extract was evaporated to dryness and the residue was dissolved in 50 mL methanol and diluted with distilled water in a 100 mL volumetric flask. Known volume of this sample solution was then analysed by the proposed method in triplicate. For the standard addition method the same procedure was applied as mentioned above to check the recovery.

3. Results and discussion

The proposed reaction mechanism for atrazine reaction is given in Figure 1. Atrazine was reacted with pyridine in the presence of alkali. An electrophilic attack occurs between atrazine through its strongly electron attracting chlorine and pyridine at the unshared electron pair of nitrogen forming the quaternary pyridinium salt. This undergoes addition of a hydroxyl group resulting in carbinol base. Glutaconic dialdehyde was formed from carbinol base due to breaking of heterocyclic ring of the carbinol base in the presence of alkali. Glutaconic dialdehyde is an unstable yellow coloured product and can become stable by reacting with sulfanilic acid and resulted in the formation of a new yellow coloured product with maximum absorbance at 450 nm or with aniline and an orange red coloured product formed with maximum absorbance at 480 nm (Figure 2). The corresponding reagent blanks have negligible absorbance at these wavelengths. The effect of various experimental parameters on the absorbance of the final coloured product was studied.

3.1 *Effect of the reagent concentration*

The effect of various reagent concentrations on the reaction of atrazine into yellow coloured product formation was studied. Pyridine solution concentration was investigated in the range of 0.22–2.0 M. The absorbance increased from 0.22 to 0.86 M (Figure 3). Beyond this decrease in absorbance was observed with increase in concentration of pyridine solution.

Pyridine reacted with atrazine in acidic solution to form quarternary pyridinium halide. Because the reaction is acid dependent, the effect of hydrochloric acid concentration was studied. The maximum reaction product was formed when the hydrochloric acid solution concentration reached up to 0.15 M in the final solution (Figure 4).

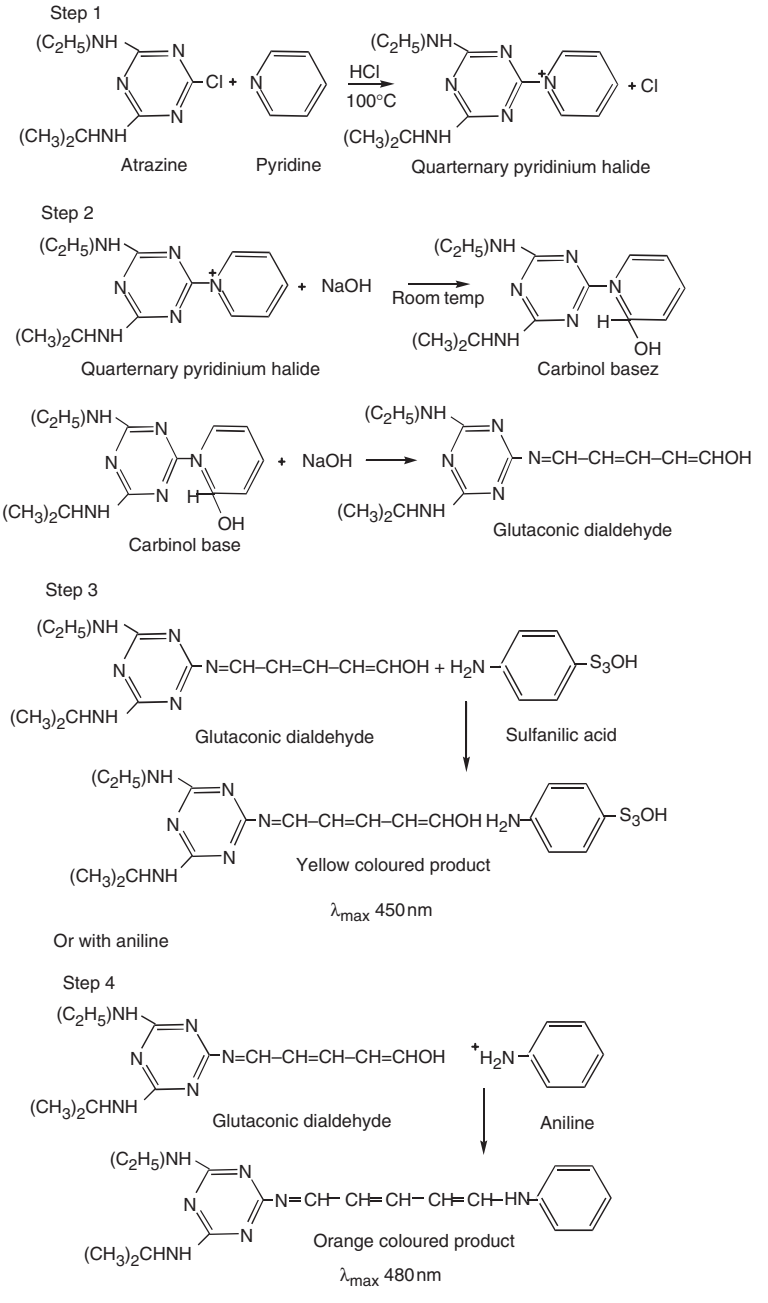

Figure 1. Proposed reaction mechanism for spectrophotometric determination of atrazine.

Formation of carbinol base from quarternary pyridinium halide took place in the presence of alkali. The concentration of sodium hydroxide was studied in the range of 0.75–2.0 M. Maximum absorbance was measured at 1.75 M (Figure 5). More than 1.75 M sodium hydroxide results in hydrolysing the carbinol base and destroying the aromaticity of the pyridine ring yielding glutaconic dialdehyde.

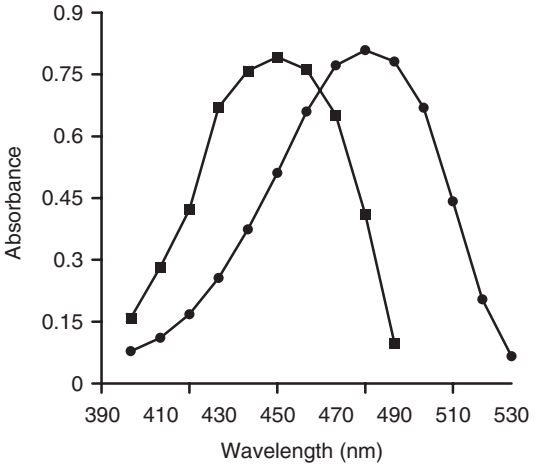

Figure 2. Absorption spectra of atrazine coupled to sulfanilic acid $20\,\mu g\,mL^{-1}$ (■) and aniline $15\,\mu g\,mL^{-1}$ (●).

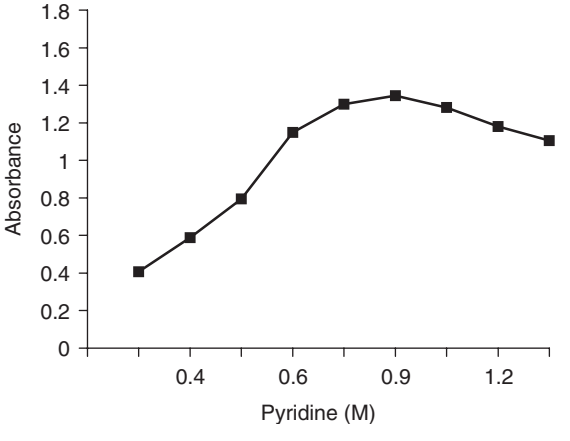

Figure 3. Effect of pyridine concentration on reaction with atrazine.

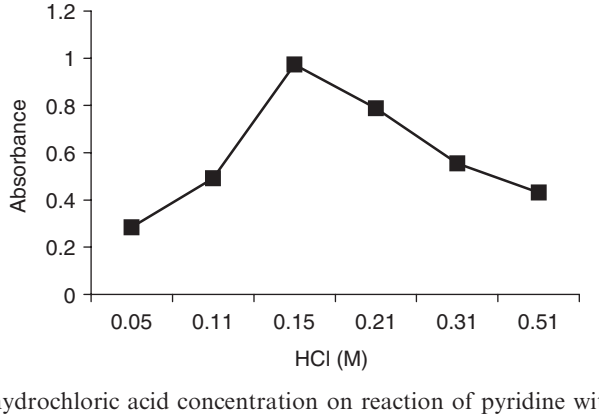

Figure 4. Effect of hydrochloric acid concentration on reaction of pyridine with atrazine.

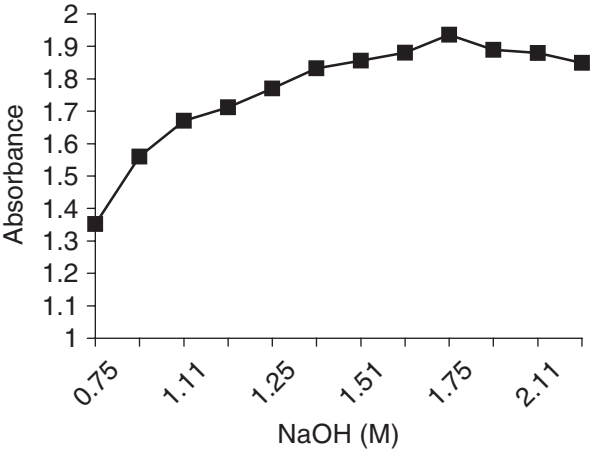

Figure 5. Effect of sodium hydroxide concentration on reaction of atrazine.

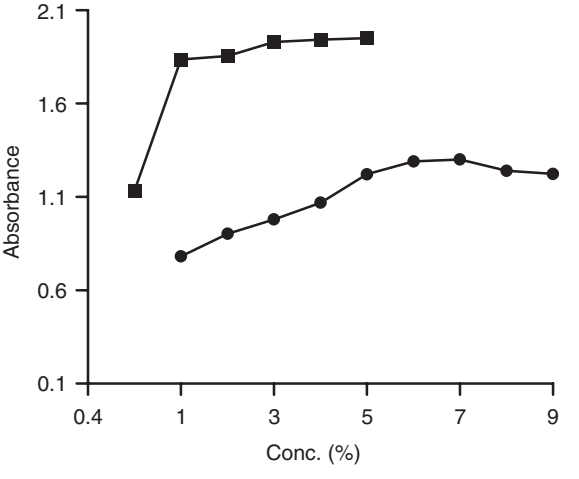

Figure 6. Effect of coupling reagents concentration on the coloured product formation (sulfanilic acid (■), aniline (●)).

Sulfanilic acid and aniline were used as coupling reagents to stabilise the unstable glutaconic dialdehyde. The effect of sulfanilic acid concentration was investigated in the range of 0.5–5.0% and aniline was studied in the range of 1.0–9.0% (Figure 6). The absorbance increased from 0.5 to 1.0% sulfanilic acid and no effect was observed with further increase in sulfanilic acid concentration, while with aniline maximum absorbance was found at 6.0% aniline solution.

3.2 *Effect of temperature, pH and time*

Maximum absorbance of yellow coloured product was observed when the solution containing glutaconic dialdehyde with sulfanilic acid was heated in a boiling water bath for 25 min. The effect of pH on the yellow coloured product formation was studied in the

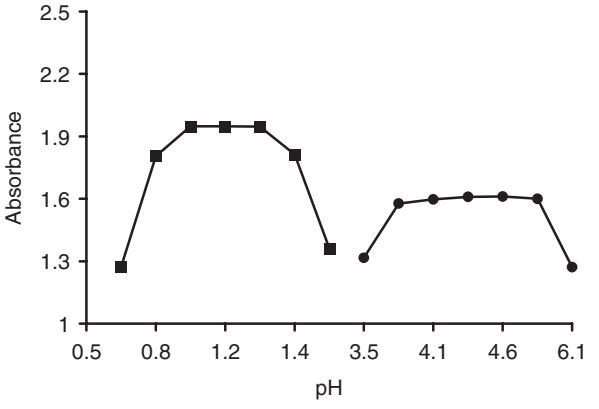

Figure 7. Effect of pH on the coloured product formation (sulfanilic acid (■), aniline(●)).

range of 1.0–2.5 and it was found that the final product formed only in strongly acidic medium with sulfanilic acid at pH 1.0–1.2 and aniline formed orange red coloured stable product with glutaconic dialdehyde at pH 4.0–5.0 (Figure 7).

The effect of time on the stability of yellow dye was investigated up to 24 h. Coupling of sulfanilic acid and aniline was found to be stable for 24 h at room temperature.

3.3 *Figures of merit*

The absorbance concentration curve was found linear over the concentration range of 0.1–25 $\mu g\,mL^{-1}$ and 0.08–12 $\mu g\,mL^{-1}$ with sulfanilic acid and aniline as a coupling reagent, respectively. The absorbance with aniline used as a coupling reagent is high but found linear over a smaller concentration range, whereas in the case of sulfanilic acid as a coupling reagent the absorbance value is bit linear over a wider concentration range. The linearity of the calibration graph was proved by the high value of the correlation coefficient (r^2).

The limit of detection was determined by establishing the minimum level at which atrazine can be detected reliably (3s) using six replicate determinations and the limit of quantification was calculated by establishing the lowest concentration of atrazine that can be measured with acceptable precision and accuracy (10s) also with six replicate determinations for each coupling reagent. The validity of the method was evaluated by statistical evaluation of the regression lines. The precision of the method was tested by the standard deviation (SD) and relative standard deviation (RSD). The small values of the SD and RSD show low scattering of the point on the calibration curve. The results are summarised in Table 1.

3.4 *Application*

A recovery test was performed on samples fortified with known concentration of atrazine solution. Six replicates of the fortified samples were analysed using the proposed method. The results are given in Table 2. The average recoveries were found in the range of 92–97% from corn as well as sugar cane samples. A recovery test was also applied to the

Table 1. Analytical characteristics of the proposed method.

Characteristics	Value sulfanilic acid	Aniline
λ_{max} (nm)	450	480
Colour	Yellow	Orange red
Linearity range ($\mu g\,mL^{-1}$)	0.1–25	0.08–12
Slope	0.0806	0.064
Correlation coefficient (r^2)	0.999	0.998
SD ($n=6$)	9.8×10^{-3}	4.1×10^{-3}
RSD	6.66	5.10
Limit of detection ($\mu g\,mL^{-1}$)	0.029	0.012
Limit of quantification ($\mu g\,mL^{-1}$)	0.098	0.041
Molar^{-1} absorbtivity ($L\,mol\,cm^{-1}$)	1.5×10^{4}	1.3×10^{4}

Table 2. Estimation of atrazine in fortified samples using sulfanilic acid ($n=6$).

Samples	Residue found ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Total conc. found ($\mu g\,mL^{-1}$)	Recovery (%)
Corn	0.360	6.0	6.0	95.1 ± 0.1
		7.4	7.5	96.0 ± 0.1
		9.8	9.9	97.0 ± 0.2
Sugar cane	0.53	6.0	6.0	92.2 ± 0.2
		7.8	8.1	97.0 ± 0.7
		9.7	9.8	93.0 ± 0.2

Table 3. Estimation of atrazine from fortified commercial formulations using sulfanilic acid as coupling reagent ($n=6$).

Herbicide samples	Taken ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Total conc. found ($\mu g\,mL^{-1}$)	Recovery (%)
A (Bestrazine)	8.1	6.0	14.0	99.3 ± 0.1
		9.0	16.9	98.8 ± 0.2
		10.0	17.8	98.9 ± 0.2
B (Mixture of atrazine and ametryn)	5.8	6.0	11.4	94.3 ± 0.5
		8.2	8.2	94.0 ± 0.6
		9.8	15.2	95.9 ± 0.3

commercial formulations (Tables 3 and 4). The average recoveries found with sulfanilic acid was in the range of 94.0–99.9% while with aniline in the range of 91.1–96.0%.

In order to evaluate the analytical applications of the proposed method, it was applied for atrazine determination in commercial formulations. In one formulation, atrazine was present in a mixture with ametryne that was separated by extraction into chloroform and determined in the extract. The values obtained were comparable with the label values.

Table 4. Estimation of atrazine from commercial formulations using aniline as a coupling reagent.

Herbicide samples	Originally found ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Total conc. found ($\mu g\,mL^{-1}$)	Recovery (%)
A (Bestrazine)	2.0	6.0	7.8	96.0 ± 0.3
		9.0	10.5	94.4 ± 0.1
		10.0	11.5	95.0 ± 0.4
B (Mixture of atrazine and ametryn)	2.0	6.0	7.4	91.1 ± 0.1
		8.0	9.3	91.3 ± 0.6

The method was also applied for residue determination in corn and sugar cane samples. The values for atrazine in corn and sugarcane were found to be $0.15 \pm 0.2\,\mu g\,g^{-1}$ and $1.2 \pm 0.2\,\mu g\,mL^{-1}$, respectively.

4. Conclusion

The colour of the final product after coupling with sulfanilic acid and aniline is stable at room temperature for 24 h. The proposed method is simple and sensitive and can be used for atrazine in low concentrations. Moreover, the method does not involve any cleanup procedures because it is free from interference of a large number of foreign species. The method can be suitably adopted for routine analysis of the purity of atrazine in their formulations and real samples.

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