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RESEARCH ARTICLE

Base Hydrolysis Using Aniline for the Method Development of Spectrophotometric Determination Of Isoproturon And Its Applications To Food Samples

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Abstract

A simple spectrophotometric method has been investigated for the determination of isoproturon herbicide. Total 7 parameters were optimized for the base hydrolysis using aniline for the method development of spectrophotometric determination of isoproturon. Isoproturon was hydrolyzed in alkaline media on heating. The hydrolyzed product, *p*-isopropylaniline was diazotized with nitrite in acidic media and coupled with aniline on heating, to form an azodye, 4-((4-isopropylphenyl)diazenyl)benzenamine. The absorbance of the resulting azodye was measured at 490 nm. Conditions for the complete hydrolysis of isoproturon and quantitative determinations were optimized. A linear relationship between absorbance and concentration was observed in the range of 2-12 ppm. Molar absorptivity was found to be $4.12x10^3$ $L.mol^{-1}.cm^{-1}$ with relative standard deviation (RSD) of 3.58%. The developed method was successfully applied for the residue determination of isoproturon in food samples. The residue level found in wheat grain and flour were 41.33±3.06 $\mu g.g^{-1}$ and 20±3.16 $\mu g.g^{-1}$, respectively. Good percent recoveries have shown the potentiality of the method for the determination of isoproturon in food samples.

Introduction

Isoproturon, [3-(4-isopropylphenyl)-1,1-dimethylurea)], is a type of the phenylurea herbicides, and is widely used in Europe to protect crops from broad-leaf weeds [1]. It is a widely used urea herbicide for pre- and post-emergence control of black grass, silky bent grass, wild oats, annual meadow grass, ray grass, many broadleaf weeds in spring and winter wheat, spring and winter barley, winter rye and winter cefoods [2-5]. Due to the slight solubility in water isoproturon can easily migrate through the soil to crops and thus enter the human food chain. Depending on the conditions of a particular rainfall and properties of soil, it can also reach the groundwater. Because of the absence of microbial activity, its degradation processes are very slow and thus, with accumulation, endured concentrations may be achieved in drinking water [6]. Different analytical methods such as titrimetric [7], gas chromatography (GC) [8] and high performance liquid chromatography (HPLC) [9] have been investigated for the determination of isoproturon. The success of these techniques largely depends on proper sample preconcentration because the concentration of isoproturon in food samples generally is below the detection limits of these techniques [2]. Solid-phase extraction (SPE) has become the method of choice for sample preconcentration in many analytical applications, especially in herbicides analysis, due to its easy operation and high preconcentration ability [10]. But the SPE may not fully distinguish analyte and interfering substances during preconcentration, which might prevent accurate quantitative determination or, occasionally, qualitative identification of an analyte. Another limitation of SPE is that, along with application of more polar pesticides to environment [11], quantitatively trapping them from aqueous sample cannot be achieved with reverse-phase sorbent-based SPE, which is the main category of SPE used in food environmental sample analysis.

Isoproturon had been determined by sol–gel immunosensor in flow injection format [12]. However, the reported limit of detection was 2µg.L^{-1} which is far beyond MAC (maximum admissible concentration) of European Union (0.1 maximum admissible concentration µg.L^{-1}).
Isoproturon was also determined selectively from environmental materials (water samples) using an immunosorbent column containing anti-isoproturon antibodies encapsulated in a silica matrix by a sol-gel process [13]. RIANA (River analysis) immunosensor was used for the analysis of isoproturon in water samples. A solid-phase fluoroimmunoassay combined with an optical transducer to get excitation and collection of fluorescence from fluorophore labeled antibodies locally bound at the planar interface was developed [14]. Spectrophotometric methods using p-dimethylaminobenzaldehyde [15], ethyl acetoacetate [16], and *p*-aminoacetophenone [17] as reagents are also available in the literature for the determination of isoproturon but these are less sensitive and use methanolic sodium hydroxide for the hydrolysis that can affect the colour development of the solution [18].
Here is a simple, sensitive and selective method using a new reagent, *p*-isopropylaniline, reported for the determination of isoproturon in various samples. The proposed method is based on the alkaline hydrolysis of isoproturon, followed by diazotization and coupling with *p*-isopropylaniline in alkaline medium. The method has been satisfactorily applied for the determination of isoproturon in food samples.

1. Materials

1.1. Apparatus and Reagents

Spectronic 20D (Milton Roy USA, Spectrophotometer) and pH meter (Inolab pH 720) were used. All reagents used were of analytical reagent grade purity. Sodium hydroxide, Sodium nitrite, Ethanol, conc. HCl and Aniline (Merck) were used. Commercial samples containing isoproturon, grain and wheat were purchased from the local market.

1.2. Solution preparation

a) **Aniline solution**: an aniline solution (2%) was prepared by dissolving 2 mL of aniline in 70 mL of absolute ethanol and diluted upto 100 mL with distilled water.

b) **Nitrite solution**: a nitrite solution (0.15%) was prepared by dissolving 0.15g of $NaNO_2$ in small amount of distilled water and then diluted upto 100 mL with distilled water.

c) **Isoproturon standard solution**: a standard isoproturon solution (100 ppm) was prepared by dissolving 0.01 g of isoproturon in 100 mL of absolute ethanol in 100 mL volumetric flask. Then from this solution, 10 mL was taken and hydrolyzed in 0.4 M HCl. The resulting was 100 ppm in the hydrolyzed product. Through stepwise dilution, 10 and 1 ppm isoproturon solutions were prepared with distilled water. Similarly, working solutions in the range of 2-20 ppm were prepared by using dilution method.

1.3. Optimization of various parameters for the method development:

1.3.1. Optimization of suitable wavelength for Spectrophotometric determination of Isoproturon

10 mL isoproturon solution was taken in a 100 mL beaker from 100 ppm stock solution. Added to it 5 mL of NaOH solution (5 g.dm^{-3}) and heated on a boiling water bath for 30 min to hydrolyze the pesticide. The hydrolyzed solution was cooled and followed by the addition of 12 mL of nitrite solution (1000 ppm), 5 mL concentrated hydrochloric acid and 10 mL 2 % aniline solution. Again heated on a boiling water bath for 10 min to complete the diazotization and coupling reactions. The resulting azodye was cooled and diluted upto the mark with distilled water. Blank solution was prepared by the same procedure without addition of pesticide. The absorbance of the azodye was measured at different wavelengths from 360-570 nm using Spectronic 20D. The absorbance was measured with increasing wavelength and each wavelength was calibrated with blank solution.

1.3.2. Optimization of heating time for hydrolysis of Isoproturon

10 mL isoproturon solution was taken from 100 ppm stock solution in different beakers to which 5 mL of 5 M NaOH solution was added. The solutions were heated on a boiling water bath and the heating time was varied from 10-40 min. The hydrolyzed solutions were then cooled and 12 mL $NaNO_2$ (1000 ppm), 5 mL conc. HCl, 10 mL Aniline (2%) were added to each in a sequence and again heated at 100°C for 10 min. The resulting solutions were cooled and diluted upto the mark with distilled water. The absorbance of the resulting solutions was measured at 490 nm using blank solutions.

1.3.3. Optimization of Sodium hydroxide concentration (M) for hydrolysis of Isoproturon

10 mL isoproturon solution was taken from 100 ppm stock solution in different beakers. Different volumes of sodium hydroxide solution (5 M) were added to adjust the molarity of sodium hydroxide solution in the range of 0.6 M. The solutions were heated on a boiling water bath for 20 min to complete the hydrolysis of isoproturon. The hydrolyzed solutions were cooled and diazotizing and coupling reagents were added in sequence. The solutions were

heated on a boiling water bath (100 ºC) for azodye formation for 10 min. After completion of reaction, the resulting solutions were cooled and diluted upto the mark with distilled water. The absorbance was measured at 490 nm against a blank solution.

1.3.4. Optimization of nitrite solution (0.1 %) volume for diazotization of Isoproturon

Isoproturon standard solution 10 mL from 100 ppm stock solution was transferred to six separate beakers followed by the addition of 10 mL of 5 M sodium hydroxide solution to adjust the concentration of 0.5 M. All the solutions were heated on a boiling water bath for 20 min to hydrolyze isoproturon. After cooling, varied volumes of nitrite solution from 0.1 % in the range of 2-12 mL was added. To each solution, 5mL concentrated hydrochloric acid and 10 mL of 2 % aniline solution were added. The solutions were heated again for 10 min. on a boiling water bath for azodye formation. An orange colored azodye was formed in each solution and diluted upto 100 mL with distilled water. Separate blank solutions were prepared by the same procedure without the addition of isoproturon.

1.3.5. Effect of acidity on diazotization of Isoproturon

Isoproturon standard solution 10 mL from 100 ppm stock solution was transferred to four separate beakers. To each beaker 10 mL of NaOH (5 M) solution was added and heated on a boiling water bath for 20 min to hydrolyze isoproturon. After cooling, 8 mL of 0.1 % nitrite solution, different volumes of concentrated hydrochloric acid were added to adjust the acid molarity in the range of 0.24-0.96 M and 10 mL of 2 % aniline solution were added. The solutions were again heated on a boiling water bath for 10 min to complete the reaction. After cooling, the resulting solutions were cooled and diluted upto 100 mL with distilled water. Separate blank solutions were prepared by the same procedure without the addition of isoproturon. The absorbance of the resulting azodye was measured at 490 nm.

1.3.6. Effect of volume of 2 % aniline solution as a coupling reagent for azodye formation of Isoproturon

Isoproturon standard solution 10 mL from 100 ppm stock solution was transferred to five separate beakers. 10 mL sodium hydroxide solution was added to each beaker and heated on a boiling water bath for 20 min to hydrolyze isoproturon. After cooling, 8 mL of 0.1 % nitrite solution, 4 mL of conc. HCl and varied volume of 2 % aniline solution were added in the range of 2-10 mL followed by heating on a boiling water bath for 10 min for azodye formation. After cooling, the resulting orange colored solutions were diluted with distilled water upto 100 mL. The absorbance of each solution was measured at 490 nm against a blank solution.

1.3.7. Optimization of heating time for coupling reagent

Isoproturon standard solution 10 mL from 100 ppm stock solution was transferred to four separate beakers followed by the addition of 10 mL of 5 M sodium hydroxide solution and heated on a boiling water bath for 20 min for hydrolysis. After cooling 8 mL of 0.1 % nitrite solution, 4 mL of concentrated hydrochloric acid (0.48 M) and 6 mL 2 % aniline solution were added in sequence to each hydrolyzed isoproturon solution. The solutions were heated on a boiling water bath for different time in the range of 5-20 min for azo dye formation. The resulting solutions were diluted with distilled water upto 100 mL .The absorbance of each solution was measured at 490 nm using blank solution.

1.4. Investigation of limit of detection and limit of quantification for spectrophotometric determination of isoproturon

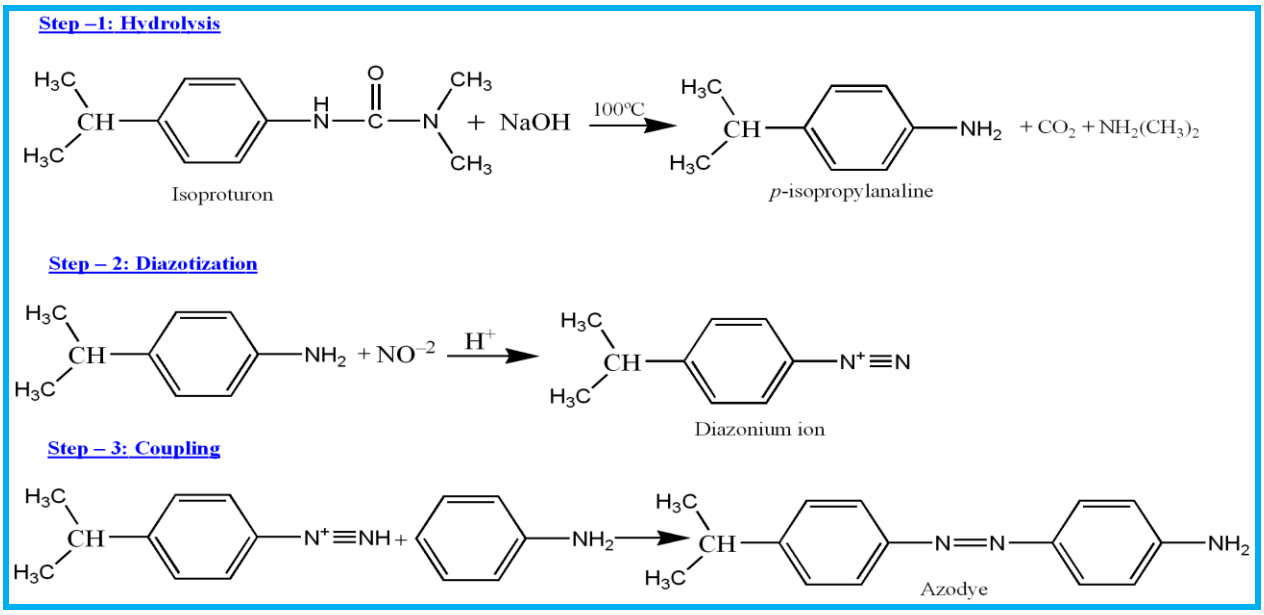
From standard solutions of isoproturon solution (100 ppm) different volume from 2-20 ppm were taken in separate beakers. To each beaker 10 mL of sodium hydroxide solution (5 M) was added and heated on boiling water bath for 20 min, for hydrolysis. All the solutions were cooled and added 8 mL of nitrite solution (0.1 %), 4 mL of concentrated hydrochloric acid and 6 mL of aniline solution (2 %) in a sequence to each solution. All the solutions were again heated for 10 min on a boiling water bath to complete the azodye reaction. After cooling, each solution was diluted up to 100 mL with distilled water. The absorbance was measured at 490 nm using blank solution.

1.5. Application of the investigation method for the determination of Isoproturon in food sample

Wheat samples collected from the field with spray of isoproturon was used. 20 g of homogenized sample (wheat grain and flour) was taken in a beaker and 25 mL ethanol, 25 mL water (1:1 ratio) was added to it and was left for 2 h with constant shaking. It was then filtered and washed with ethanol. The solvent was evaporated to reduce the volume. 10 mL NaOH solution (5 M) was added to the reduced volume of extracted sample and heated on boiling water bath for 20 min. The hydrolyzed solution was cooled and 8 mL $NaNO_2$, 4 mL concentrated HCl, and 6 mL aniline were added and heated again for 5 min. The solution was cooled, diluted up to the mark with distilled water. The absorbance was measured at 490 nm using blank solution. The process was repeated three times.

Results and Discussion

Isoproturon is chemically 3-(4-isopropylphenyl)-1, 1-dimethylurea or 3-(p-isopropylphenyl)-1, 1-dimethylurea. Isoproturon undergoes a rapid hydrolysis in the presence of strong alkalis [15,21] and aqueous sodium hydroxide solution was used here for hydrolysis. On hydrolysis, isoproturon would lead to the hydrolyzed product p-isopropylanaline. The proposed method is based on diazotization of the hydrolyzed product and then coupling with aniline to form azodye, 4-((4-isopropylphenyl)diazenyl)benzenamine. The reaction involves three steps as shown in Scheme 1.



Scheme 1: Proposed reaction mechanism for spectrophotometric determination of Isoproturon

Preliminary studies were conducted to investigate the possible hydrolysis of isoproturon and the use of hydrolyzed product as a diazotizing reagent. Initially isoproturon was hydrolyzed in alkaline media on a boiling water bath and the hydrolyzed product was tried for the formation of diazotization and azodye (In acidic medium the colour of the resulting azodye was negligible). The formation of colored azodye, 4-((4-isopropylphenyl)diazenyl)benzenamine, indicates the possibility of the reaction and its application for spectrophotometric determination of isoproturon by this method. Further studies were focused on optimization of various parameters.

The 1st parameter, absorption maxima of the azodye formed was found to be at 490 nm. The absorption spectrum of isoproturon is shown in **Fig. 1**.

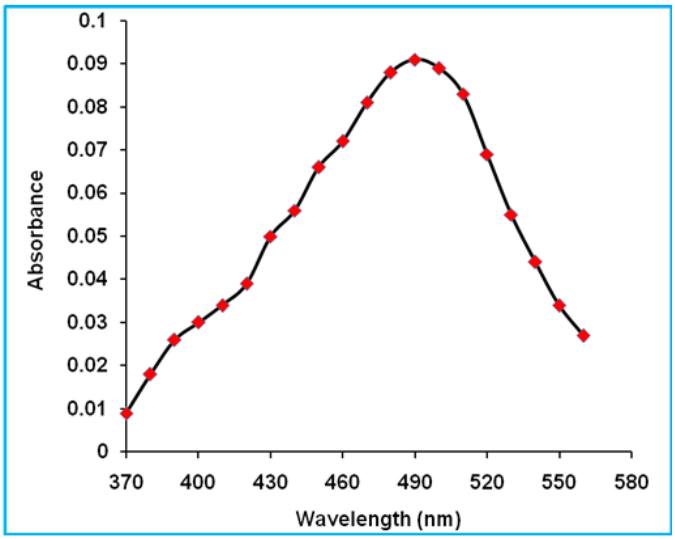


Fig. 1: Wavelength (nm) optimization for spectrophotometric determination of isoproturon

The reaction conditions have been optimized to obtain maximum colour development. Optimum conditions for maximum colour development were obtained by varying each parameter at a time, keeping all other variables constant.

The 2nd parameter is the optimization of heating time. The results are shown in **Fig. 2**. The hydrolysis of isoproturon was found to maximum for 20 min heating time at 100 °C and above this time decrease in absorbance indicates the decomposition of isoproturon. Maximum absorbance observed at 20 min heating time and above this time decrease in absorbance indicates the decomposition of isoproturon. Therefore, further analysis was carried out at 20 min heating time for hydrolysis of isoproturon.

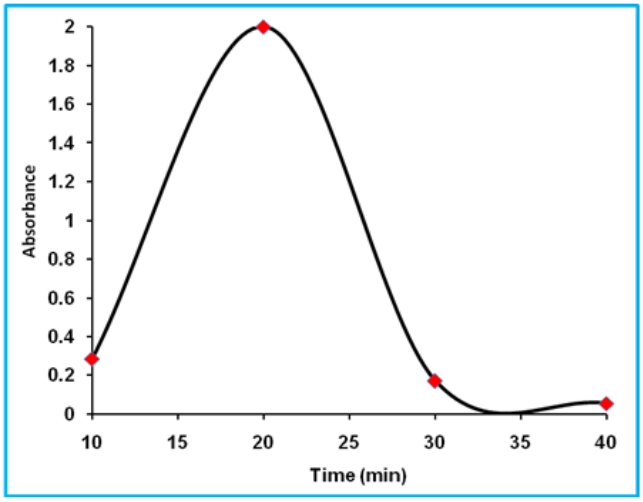


Fig. 2: Optimization of heating time for hydrolysis of isoproturon

After the determination of heating time for the hydrolysis of isoproturon, the 3rd parameter is the conc. of the base (NaOH). The results are shown in **Fig. 3**. When the molar conc. of sodium hydroxide was increased from 0.1-0.5, increase in absorbance was observed. Above 0.5 M conc., precipitates appeared in the solution. Therefore, 0.5 M sodium hydroxide solution was used for further analysis.

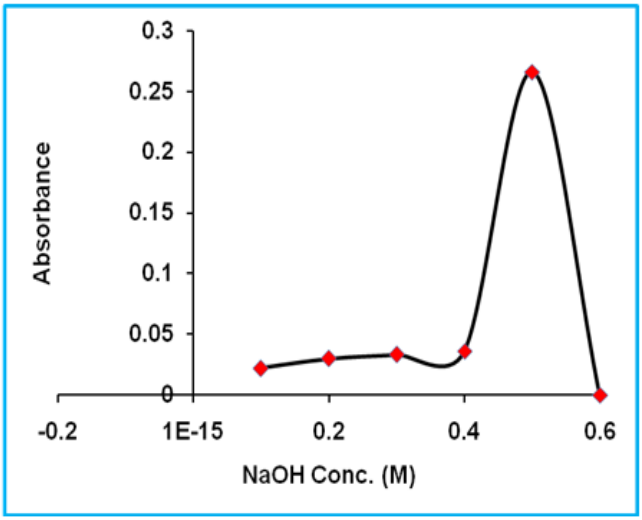


Fig. 3: Optimization of sodium hydroxide concentration (M) for hydrolysis of isoproturon

The 4th important parameter after the optimization of base conc. is the optimization of nitrite conc. because its conc. is important for the formation of diazonium ion. The resulting p-isopropyl aniline, formed during the hydrolysis of isoproturon, reacts with nitrite to form the diazonium ion. The absorbance of the resulting diazonium ion was measured at 490 nm. The results for the optimization of nitrite conc. are shown in **Fig. 4**. The absorbance increased from 2-8 mL of 0.1 % nitrite solution but after 8 mL volume decreased in absorbance was measured. Therefore, 8 mL of 0.1 % nitrite solution was used for further analysis.

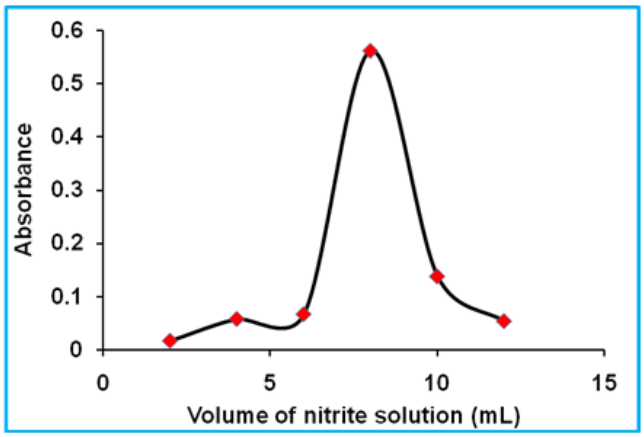


Fig. 4: Effect of volume of nitrite solution (0.1%) on the diazotization of isoproturon

The effect of acid (HCl) conc. on the formation of diazonium ion was also studied as its conc. is important for coupling because coupling takes place in acidic media. The results for the optimization of 5th parameter (effect of acid conc.) are shown in **Fig. 5**. The absorbance increased from 0.24-0.48 M of conc. HCl solution but after 0.48 M decreased in absorbance was measured. Therefore, 0.48 M of conc. HCl solution was used for further analysis.

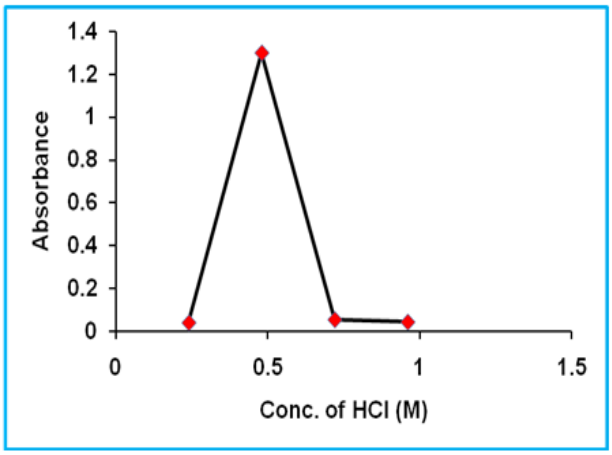


Fig. 5: Effect of Acidity on diazotization of isoproturon

The main step (6th parameter) of this assay is the coupling step in which azo dye formation occurs. For the maximum azo dye formation, the conc. of aniline was determined and the data is shown in **Fig. 6**. As it is evidenced from **Fig. 6** that maximum azodye formation occurs at 6 mL of 2 % aniline solution.

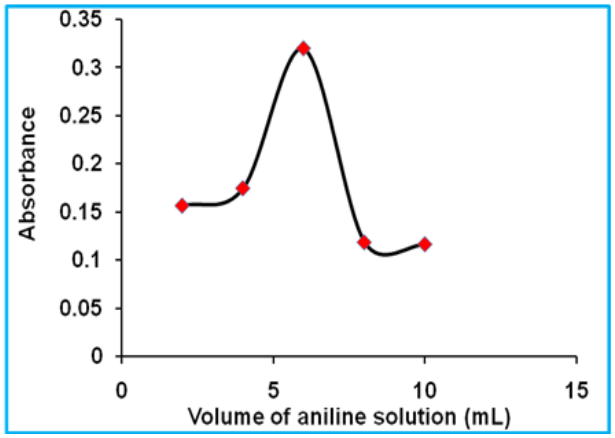


Fig. 6: Effect of volume of 2 % aniline solution as a coupling reagent for azodye formation of isoproturon

The final parameter (7th parameter) of this method is the optimization of heating time for the coupling step. The data is shown in **Fig. 7**. As the azodye formation reaction is slow at room temperature, therefore, 5 min heating on boiling water both is sufficient for azodye formation.

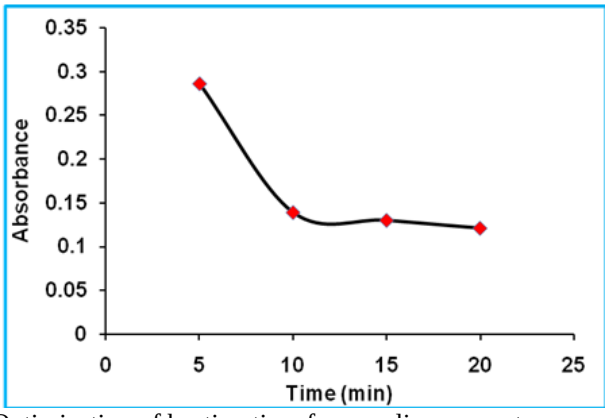


Fig. 7: Optimization of heating time for coupling reagent

The applicability of the proposed method was tested on a technical-grade sample like wheat and formulations of isoproturon. Beer's law was obeyed over the concentration range of 2-12 ppm of isoproturon. The molar absorptivity was found to be 4.12 x 10^3 L mol^{-1} cm^{-1}. The Standard Deviation (SD) and Relative Standard Deviation (RSD) of absorbance values were found to be 7.75 x 10^{-2} ± 1 and 3.58 %, respectively. The low RSD indicates the reproducibility of the method. The results are shown in **Fig. 8**. A linear relationship between absorbance and concentration of isoproturon was observed from 2-12 ppm of isoproturon. At higher concentration deviation from straight line observed. Molar absorptivity was calculated by using the following formula:

$$\text{Molar absorptivity } (\varepsilon) = {}^{A}/_{Cl}$$

Where A = Absorbance, C = Concentration (mol L^{-1}) and l = Pathlength (cm)
For calculation of limit of detection (LoD) and limit of quantification (LoQ) six replicates of 2 ppm isoproturon were prepared by the same procedure and the absorbance readings were measured. The correlation coefficient is also calculated. The overall spectral charactertics of the method investigated for spectrophotometric determination of isoproturon after alkaline hydrolysis is given in **Table 1** while comparison with other methods reported in literaature is given in **Table 2.** The Limit of Detaction (LOD) and Limit of Quantification (LOQ) were found to be 15.50 µg mL^{-1} and 51.67 µg mL^{-1}, respectively. The residue concentration of isoproturon was 41.33 ± 3.06 µg g^{-1} and 20.0 ± 3.16 µg g^{-1} in wheat grain and flour, respectively. The residue found was higher in grain than in flour which may be due to heat loses during the processing [22].

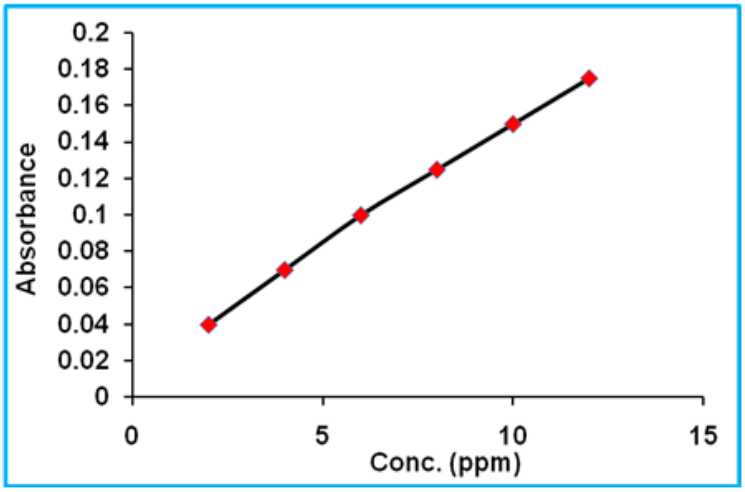


Fig. 8: Effect of isoproturon concentration

Parameter	Value
λmax	490 nm
Molar absorptivity (ε)	4.12×10^3 L mol^{-1} cm^{-1}
Limit of Detaction (LOD)	15.50
Limit of Quantification (LOQ)	51.67
Standard Deviation (SD)	7.75×10^{-2}
Relative Standard Deviation (RSD)	3.58 %
Slope (b)	0.015
Intercept (m)	0.00
Correlation Coefficient (r)	0.954

Table 1: Spectral charactertics of spectrophotometric method for hydrolysis of isoproturon

Parameter	Aniline(proposed method)	*p*-Aminoacetophen-one [7]	Ethyl acetoa-cetate [16]	*p*-dimethylaminobe-nzaldehyde [15]
λmax (nm)	490	525	398	438
Beer's law range (mg mL^{-1})	2-12	0.5-5	1.5-15	1.125-11.25
Molar absorptivity (L mol^{-1} cm^{-1})	4.12×10^3	8.6×10^6	1.37×10^4	0.006

Table 2: Comparison with other spectrophotometric methods

4. Benefits of the Method

The present method does not involve time-consuming distillation, thermal and photo-decomposition as reported earlier [l7-19]. Furthermore, the present method is superior to our earlier methods [16,20] in terms of the simplicity of the reagent and is much more sensitive than the latter method and comparable with the former. Therefore, the present method is simple, sensitive, rapid and useful for routine analysis.

Conclusions

Here a spectrophotometric method for the determination of isoproturon based on its reaction with aniline under acidic conditions is described. The proposed method has been compared with some other spectrophotometric methods and found to more sensitive and selective. The absorption maxima of the coloured compounds formed are observed at 490 nm and the molar absorptivity are 4.12 $x10^3$ L mol^{-1} cm^{-1}. It was concluded that 10 mL of 0.5 M sodium hydroxide, waiting time of 20 min at 100 °C and 8 mL $NaNO_2$ (0.1 %), 4 mL of 0.48 M HCl, 6 mL Aniline (2%) waiting time 5 min at 100 °C temperature are required for complete hydrolysis and diazotization of isoproturon, respectively. The method is simple and convenient and was applied successfully to the determination of isoproturon in technical-grade samples and formulations. The limit of detection (LOD) and limit of quantification (LOQ) with slopes were found to be 15.50 µg g^{-1} and 51.67 µg g^{-1} respectively. The percent recovery for flour and grain samples of wheat was found to 91 % and 100 %, respectively.

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References

[1] Pulido-Tofino, P., Barrero-Moreno J.M., Perez-Conde, M.C. 2000. Flow-through fluoroimmunosensor for isoproturon determination in agricultural foodstuff: Evaluation of antibody immobilization on solid support. Anal. Chim. Acta, 417, 85-94.

[2] Zhang, X., Martens, D., Kramer, P.M., Kettrup A.A., Liang, X. 2006. Development and application of a sol–gel immunosorbent-based method for the determination of isoproturon in surface water. J. Chromatogr. A, 1102, 84-90.

[3] Sorensen, S.R., Bending, G.D., Jacobsen, C.S., Walker, A., Aamand, J. 2003. Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. FEMS Microbiology Ecology, 45, 1-11.

[4] Hussain, S., Devers-Lamrani, M., El Azhari, N., Martin-Laurent, F. 2011. Isolation and characterization of an isoproturon mineralizing Sphingomonas sp. strain SH from a French agricultural soil. Biodegradation, 22, 637-650.

[5] Zhang, J., Hong, Q., Li, Q., Li, C., Cao, L., Sun, J.Q., Yan, X., Li, S.P. 2012. Characterization of isoproturon biodegradation pathway in *phingobium* sp. YBL2. International Biodeterioration & Biodegradation, 70, 8-13.

[6] Kramer, P.M., Goodrow, M.H., Kremmer, E. 2004. Enzyme-linked immunosorbent assays based on rabbit polyclonal and rat monoclonal antibodies against isoproturon. J. Agric. Food. Chem. 52, 2462-2471.

[7] Agrawal, O., Das, J. V., Gupta, V.K. 1998. Spectrophotometric determination of isoproturon using< i> p</i>-aminoacetophenone and its application in environmental and biological samples. Talanta, 46, 501-505.

[8] Gerecke, A.C., Tixier, C., Bartels, T., Schwarzenbach, R.P., Muller, S.R. 2001. Determination of phenylurea herbicides in natural waters at concentrations below 1 ng L^{-1} using solid-phase extraction, derivatization, and solid-phase microextraction–gas chromatography–mass spectrometry. J. Chromatogr. A, 930, 9-19.

[9] Wang, J., Guo, R., Chen, J., Zhang, Q., Liang, X. 2005. Phenylurea herbicides-selective polymer prepared by molecular imprinting using< i> N</i>-(4-isopropylphenyl)-< i> N</i>′-butyleneurea as dummy template. Anal. Chim. Acta, 540, 307-315.

[10] Krutz, L.J., Senseman, S.A., Sciumbato, A.S. 2003. Solid-phase microextraction for herbicide determination in environmental samples. J. Chromatogr. A, 999, 103-121.

[11] Hogendoorn, E., Zoonen, P.V. 2000. Recent and future developments of liquid chromatography in pesticide trace analysis. J. Chromatogr. A, 892, 435-453.

[12] Pulido-Tofino, P., Barrero-Moreno, J.M., Perez-Conde, M.C. 2001. Sol–gel glass doped with isoproturon antibody as selective support for the development of a flow-through fluoroimmunosensor. Anal. Chim. Acta, 429, 337-345.

[13] Pulido-Tofino, P., Barrero-Moreno, J.M., Perez-Conde, M.C. 2006. Analysis of isoproturon at trace level by solid phase competitive fluoroimmunosensing after enrichment in a sol–gel immunosorbent. Anal. Chim. Acta, 562, 122-127.

[14] Mallat, E., Barzen, C., Abuknesha, R., Gauglitz, G., Barcelo, D. 2001. Part per trillion level determination of isoproturon in certified and estuarine water samples with a direct optical immunosensor. Anal. Chim. Acta, 426, 209-216.
[15] Raju, K.R., Parthasarthy, T.N., Akella, S.R.K.M. 1990. Spectrophotometric determination of isoproturon or metoxuron using *p*-dimethylaminobenzaldehyde. Analyst, 115, 455-457.
[16] Raju, K.R., Akella, S.R.K.M., Murthy, J.V.S., Balerao, U.T. 1996. Spectrophotometric determination of Isoproturon and Metoxuron using ethylacetoacetate and application to technical and formulation grade samples. Talanta, 43, 577-581.
[17] Giinthar, Z., Analytical Methods for Pesticides and Plant Growth Regulators, Academic Press, New York, 1976. 8, p. 417.
[18] Buser, H., Grolimund, K. 1974. Direct determination of N'-phenyl urea derivatives in herbicide technical products and formulations, using gas-liquid chromatography. JAOAC, 57, 1294-1299.
[19] Indian Standards Institution, Delhi, IS No. 12004, 1987.
[20] Raju, K.R., Parthasarthy, T.N., Akella S.R.K.M., Bhalerao, U.T. 1992. Spectrophotometric assay of Isoproturon and Metoxuron in technical grade and formulation samples using 3-methylbenzothiozolin-2-one hydrazone hydrochloride. Talanta, 39, 1387-1390.
[21] Hartley, D., Kidd, H., "The Agrochemicals Handbook," 2nd Edit. Royal Society of Chemistry, London, 1987.
[22] M.R. Jan, J. Shah, N. Bashir, 2006. Flow injection spectrophotometric determination of bromoxynil herbicide by diazotization method. Anal. Sci. 22, 165.