

## Spectrophotometric Method for Quantification of Fenoxaprop-*p*-ethyl Herbicide in Commercial Formulations and Agricultural Samples

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**Summary:** A simple spectrophotometric method was developed for determination of fenoxaprop-*p*-ethyl herbicide in agricultural samples. The method is based on the acid hydrolysis of fenoxaprop-*p*-ethyl to form 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The 6-chloro-2,3-dihydrobenzoxazol-2-one was used as a coupling reagent in diazotization reaction. The absorbance of the azo dye was measured at 430 nm. The reaction conditions were optimized for hydrolysis as well as for the diazotization reaction. The Beer's law was obeyed over the concentration range of 0.2-25  $\mu\text{g mL}^{-1}$  with molar absorptivity of  $1.4 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.029 and 0.098  $\mu\text{g mL}^{-1}$ , respectively. The method has been successfully applied to the determination of fenoxaprop-*p*-ethyl in commercial formulations and various agricultural samples. The % recoveries were found in the range of 95-97%.

### Introduction

Fenoxaprop-*p*-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionates} is one of the aryloxyphenoxy propionic acid which is used for control of perennial and annual grass weeds in many crops [1]. Fenoxaprop-*p*-ethyl is grass specific herbicide that inhibits the synthesis of enzyme required for lipid synthesis. Its mechanism of action is to inhibit fatty acid biosynthesis. The shoot and rhizome growth ceases, and shoot meristems and rhizome buds begins to die [2, 3]. The more frequently used formulations are amines or alkaline salts, alkyl esters or free carboxylic acids. The esters, emulsified in oil, are commonly used because of their higher herbicide activity, penetration power and low vapour pressure [1, 4]. Due to their persistence, polar nature and water solubility, the phenoxy acids are dispersed in the environment, and their residues and transformation products are present in several matrices like water, soil, cereals and other vegetable products [5].

Most of the methods reported in the literature for determination of fenoxaprop-*p*-ethyl herbicide are mainly chromatographic like HPLC [4, 5-10], GC [11], HPTLC [1] and LC-MS [12, 13]. Chromatographic methods need expensive instrumentation and cleanup of sample which is a tedious procedure. The aim of the present study is to develop simple and rapid spectrophotometric method for the determination of fenoxaprop-*p*-ethyl in

environmental matrices and food samples. Fenoxaprop-*p*-ethyl is hydrolyzed at different conditions to check the toxicity of fenoxaprop-*p*-ethyl herbicide [4]. The products after acid hydrolysis reported are also 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The reaction is based on coupling of hydrolyzed of 6-chloro-2,3-dihydrobenzoxazol-2-one with diazotized aniline for azo dye formation. The absorbance of the colored azo dye was measured at 430 nm.

### Results and Discussion

Fenoxaprop-*p*-ethyl was hydrolyzed in the presence of acid to form 6-chloro-2,3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The hydrolyzed product 6-chloro-2, 3-dihydrobenzoxazol-2-one was coupled with diazonium ion. The resultant colored azo dye absorption was measured at 430 nm. The proposed reaction mechanism is given in Fig. 1.

#### *Spectral characteristics*

The azo dye formed in the proposed reaction shows maximum absorption at 430 nm. All spectral measurements were carried out against reagent blank. The Beer's law was obeyed in the concentration range of 0.2-25  $\mu\text{g mL}^{-1}$  of fenoxaprop-*p*-ethyl. The

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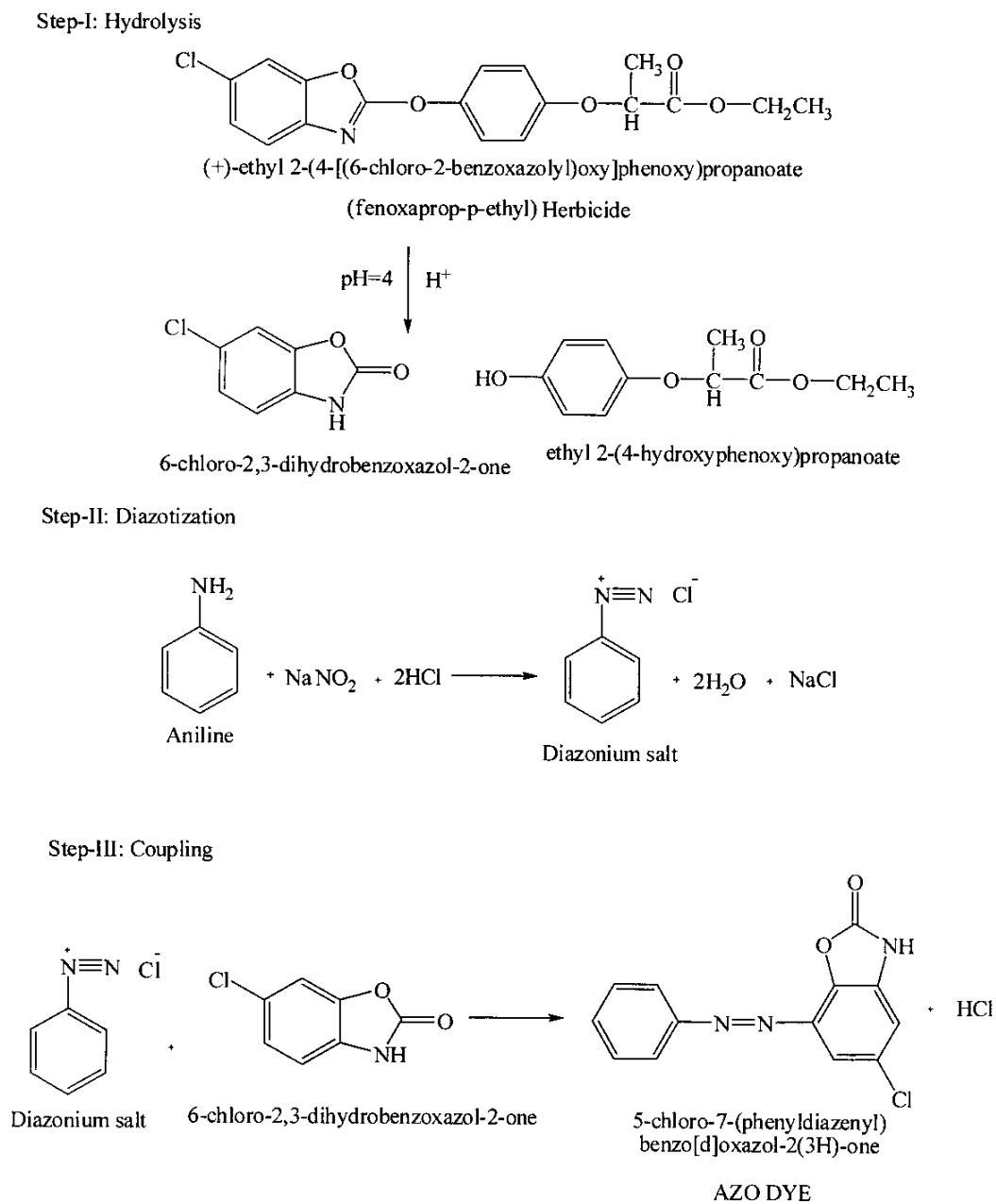


Fig. 1: Proposed reaction mechanism for spectrophotometric determination of fenoxaprop-*p*-ethyl herbicide.

molar absorptivity was found to be  $1.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ . The limit of detection (3s/b) and quantification (10s/b) were  $0.029 \mu\text{g mL}^{-1}$  and  $0.098 \mu\text{g mL}^{-1}$ , respectively (Table-1).

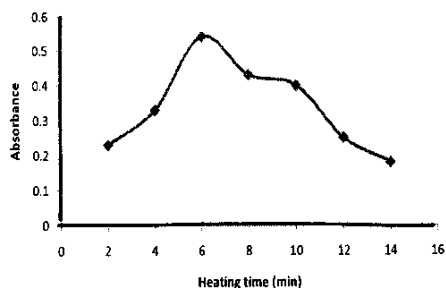
#### Conditions Optimization

Effect of acidity, temperature, and heating time on hydrolysis of fenoxaprop-*p*-ethyl to 4-(5-

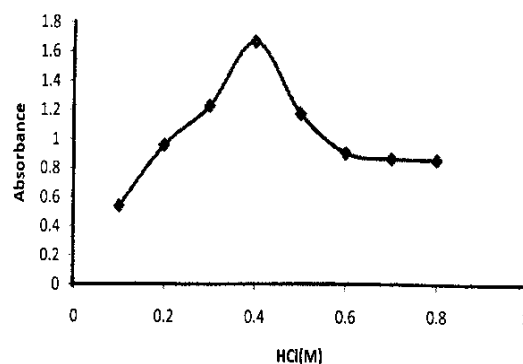
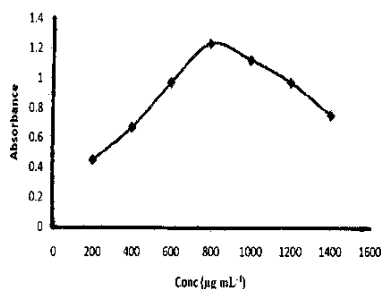
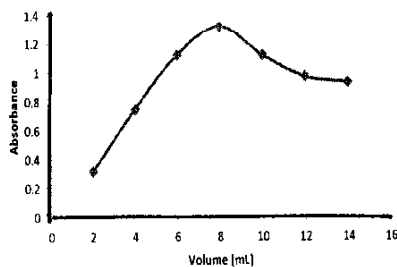
Table-1: Optical characteristics for spectrophotometric determination of fenoxaprop-*p*-ethyl.

Analysis	Characteristics
Calibration curve range ( $\mu\text{g mL}^{-1}$ )	2-25
Limit of detection ( $\mu\text{g mL}^{-1}$ )	0.029
Limit of quantification ( $\mu\text{g mL}^{-1}$ )	0.098
Relative standard deviation	3.5
Slope	0.086
Correlation coefficient	0.996

chlorobenzo[*d*]oxazol-2-yl-*o*-xyloxy phenol was studied. Maximum hydrolysis was observed with 0.4 M hydrochloric acid for 6 min heating on boiling water bath (100 °C) (Figs. 2 and 3). Nitrite concentration for diazotization was optimized in the range of 100  $\mu\text{g mL}^{-1}$  to 1200  $\mu\text{g mL}^{-1}$  (Fig. 4). It was found that 10 mL of 800  $\mu\text{g mL}^{-1}$  nitrite concentrations was sufficient as it gave good stability and quantitative results. The effect of acidity on the diazotization reaction was studied and it was observed that constant absorbance values were obtained at the concentration range of 0.1 to 0.8 M hydrochloric acid solution. Therefore, in further analyses the concentration of hydrochloric acid in solutions was adjusted at 0.4 M. Aniline solution was used as a diazotizing reagent with nitrite. The diazonium ion concentration was increased with increase in concentration of aniline from 0.02 to 0.08 % solution and remained constant up to 0.12% concentration (Fig. 5). Therefore 0.08% solution of aniline was used for diazonium ion formation which was further coupled with hydrolyzed fenoxaprop-*p*-ethyl.

Fig. 2: Effect of heating time on hydrolysis of fenoxaprop-*p*-ethyl herbicide at 100 °C.

Precision of the method was checked by the replicate analysis of solution containing 1.0  $\mu\text{g mL}^{-1}$  fenoxaprop-*p*-ethyl solution. The standard deviation and relative standard deviation were found to be  $\pm 0.0098$  and 3.5%, respectively.

Fig. 3: Effect of acidity on the hydrolysis of fenoxaprop-*p*-ethyl.Fig. 4: Effect of nitrite concentration on diazotization of hydrolyzed fenoxaprop-*p*-ethyl herbicide.Fig. 5: Effect of aniline solution (2%) volume on azo dye formation of diazotized fenoxaprop-*p*-ethyl herbicide.

#### Application

Food samples (barley and wheat) were collected from agriculture fields, where fenoxaprop-*p*-ethyl had been sprayed. The method was applied

for residue determination in food samples. The values for fenoxaprop-*p*-ethyl found in barley and wheat were found to be  $1.35 \pm 0.13 \mu\text{g g}^{-1}$  and  $1.05 \pm 0.20 \mu\text{g g}^{-1}$ , respectively, (Table-2). A recovery test was performed on the same sample after residue determination and fortified with known concentration of fenoxaprop-*p*-ethyl solution. Six replicates of the fortified samples were analyzed using the proposed method. The results are given in Table-3. The average recoveries were found in the range of 96-98% in all cases. The proposed method was also applied to check the purity of commercially available fenoxaprop-*p*-ethyl herbicide. The results are given in Table-4 and can be seen that the results are in good agreement with the label value.

Table-2: Residue level of fenoxaprop-*p*-ethyl in real samples.

Sample	Residue ( $\mu\text{g g}^{-1}$ )
Barley grains	$2.3 \pm 0.32$
Wheat grains	$1.2 \pm 0.22$
Barley flour	$0.15 \pm 0.32$

Table-3: Percent recovery of herbicide from real samples.

Sample	Recovery (%)
Barley	$98.80 \pm 1.34$
Wheat	$97.95 \pm 2.43$
Barley flour	$98.15 \pm 2.82$

Table-4: Concentration of fenoxaprop-*p*-ethyl in commercial formulation.

Commercial formulation	Label Amount ( $\text{g mL}^{-1}$ )	Found Amount ( $\text{g mL}^{-1}$ )
Active ingredient	$7.5 \pm 0.46$	$8.2 \pm 0.31$

## Experimental

### Instrument

UV-Vis spectrophotometer (UNICO UV-2100 united product and instruments Inc, Dayton, U.S.A) with matched cells was used for all spectral measurements. A WTW pH meter model pH-422 (Wissenschaftlich-Technische Werkstätten, W. Germany) was used for pH measurements.

### Reagents

All chemicals used were of analytical reagent grade or of similar purity. Sodium nitrite, hydrochloric acid, sodium hydroxide (Merck, Darmstadt, Germany) and distilled ethanol were used

during this work. Commercial formulation containing fenoxaprop-*p*-ethyl was purchased from the local market. Reference standard fenoxaprop-*p*-ethyl was purchased from Dr. Ehrenstofer GmbH, Germany.

### Solutions

#### Nitrite Solution ( $2000 \mu\text{g mL}^{-1}$ )

Nitrite solution was prepared by dissolving 0.3 g sodium nitrite in distilled water and diluted up to 100 mL.

#### Aniline Solution (2%)

Aniline solution was prepared by adding 2 mL of aniline in 50 mL of ethanol and diluted with distilled water up to 100 mL.

#### Hydrochloric Acid Solution

1M HCl solution was prepared by dilution method.

#### Standard Fenoxaprop-*p*-ethyl Solution ( $1000 \mu\text{g mL}^{-1}$ )

0.1 g of fenoxaprop-*p*-ethyl was dissolved in methanol and diluted up to 100 mL.

### Procedure

An aliquot of fenoxaprop-*p*-ethyl solution containing  $2-10 \mu\text{g mL}^{-1}$  were taken in a titration flask and added 10 mL of 2M hydrochloric acid solution. The solutions were heated on a boiling water bath for 6 min to hydrolyze fenoxaprop-*p*-ethyl. After cooling the acidity of the solutions were adjusted to 0.4 M in the final solution and added 10 mL of  $800 \mu\text{g mL}^{-1}$  nitrite solution and 8 mL of 2% aniline solution for diazotization. An azo dye was formed and the solutions were diluted up to 50 mL with ethanol to get clear solution and absorbance was measured against reagent blank at 430 nm.

#### Determination of Fenoxaprop-*p*-ethyl in Commercial Formulations

For determination of fenoxaprop-*p*-ethyl in commercial formulation, 0.04 g of commercial sample was dissolved in 10 mL ethanol and hydrolyzed with 10 mL of 2 M HCl at optimum temperature and time. The sample was analyzed by the proposed method.

*Determination of Residue of Fenoxaprop-p-ethyl in Agricultural Samples*

Homogenized food sample (10 g) and 50 mL of ethanol were taken in a flask and shaken on shaking plate for 1 h for extraction of fenoxaprop-*p*-ethyl. After equilibration the sample was filtered and the filtrate was evaporated on rotary evaporator up to volume of 5 mL. The reduced volume of filtrate was centrifuged and analyzed by the proposed method. Each sample was analyzed in triplicates.

*% Recovery*

A recovery test was performed on control samples and fortified with known concentration of fenoxaprop-*p*-ethyl solution. The solvent was evaporated at room temperature for 2 days. For extraction same procedure was used as described for residue determination. Six replicates of the fortified samples were analyzed by the proposed method.

**Conclusion**

Spectrophotometric method is presented for determination of fenoxaprop-*p*-ethyl herbicide in food samples and formulations using diazotization method. The method is sensitive, simple and suitable in routine laboratories for application to agricultural samples and commercial formulations. Compared with chromatographic methods, the present method is cheaper, simpler and does not require as much purification of sample as required for chromatographic analysis. The azo dye of hydrolyzed fenoxaprop-*p*-ethyl is stable and has spectral characteristics suitable for application to the spectrophotometric determination of fenoxaprop-*p*-ethyl herbicide.

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