SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FENOXAPROP-P-ETHYL HERBICIDE IN WHEAT AND BARLEY GRAINS USING CHARGE TRANSFER COMPLEX

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ABSTRACT

A simple and sensitive spectrophotometric method was developed for determination of fenoxaprop-p-ethyl herbicide in food samples. The method is based on acid hydrolysis of fenoxaprop-p-ethyl it gives the product 6-chloro-2, 3- dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP).The 6-chloro-2,3- dihydrobenzoxazol-2-one (CDHB) was reacted with Fe (II) to form yellow color complex. Its λ_{max} was found to be 430nm. The influences of various experimental parameters on the absorbance of the charge transfer complex of fenoxaprop-p-ethyl with Fe (II) are studied. The absorbance of complex was measured at 430 nm. The charge transfer complex of herbicide with Fe (II) shows molar absortivity $4.2x10^4$ L. $mol^{-1.}$ cm^{-1}. Analytical parameters were optimized and successfully applied to the determination of fenoxaprop-p-ethyl in various samples. The method shows a linear range from 2-24 µg mL^{-1}. The percent recovery for determination of fenoxaprop-p-ethyl in commercial formulations was found to be 84 ± 0.067 –98 ± 0.060 %. The limit of detection and quantification was found to be 0.20 µg mL^{-1} and 0.67µg mL^{-1}respectively.

Key Words: **Fenoxaprop-p-ethyl, spectrophotometric, charge transfer complex**

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INTRODUCTION

The increasing world population demands a continually growing supply of food and food products. Because of relative lack of new areas suitable for agriculture, the performance of the existing areas is enhanced by using herbicides Cherhati *et al.* (2004). Herbicides are one of the crucial factors in a worldwide increase in agricultural production. Herbicides contribute effectively and profitably to weed control and benefit society as a whole. But, use of herbicides has created considerable concern for human health and environment risk associated with herbicides use Giuseppe *et al.* (1998). Despite the benefit, the use of these kinds of chemicals must be controlled because an important fraction of these pesticides are released in to the environment presenting a potential hazard risk Saladana *et al.* (2005).

Fenoxaprop-p-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionates} is a member of aryloxy phenoxy-propionate class of herbicides and is used only for the control of perennial and annual grass weeds in many crops. Fenoxaprop-p-ethyl (FPE) is used for post emergence control of grasses weeds in potatoes, beans barely and cotton Paconosi (2007). It is grass specific herbicides that inhibit the synthesis of enzyme required for lipid synthesis Porprom *et al.* (2006). The corresponding acid, fenoxaprop is the first degradation product in the metabolic sequence in soil. 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 vapor pressure Song *et al.* (2005). 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 and cereals (Celi *et al* (2006). FPE is a selective herbicide with contact and systemic action, absorb principally by leaves. It translocates both acropetally and basipetally to the roots or by leaves. It is used for post emergence control of grasses weeds in potatoes, beans barely and cotton (Lucini *et al.* (2010).

High potential toxicity of fenoxaprop-p-ethyl was shown in developmental toxicity studies entering the risk assessment process (Ma *et al.* 2004) Toxicity studies have been conducted using fenoxaprop-p-ethyl involving 4 species of rat, mouse, and monkey. The study both for dietary and inhalation studies were identified for liver toxicity includes increased liver weight, a fall in body weight was reported (Giuseppe.*et al.* (1998). Nevertheless, the risk assessment related to pesticide exposure through diet is a main concern and must therefore be considered.

Most of the methods reported for determination of fenoxaprop-p-ethyl are chromatographic like GC (Diez. *et al.* (2008) and Funtus *et al.* (2006), HPLC (Giuseppe *et al*. Celli *et al.* (2006) and Scnchis *et al*. (1998) and HPTLC (Song *et al.* 2005) Flow injection spectrophotometric (Jasmin *et al.* 2010) method need derivitization step and heavy instruments with proper care.

The aim of present work is to develop simple method for the determination of FPE residues in food samples. The developed spectrophotometric method is simple rapid and could be used for analysis of fenoxaprop-p-ethyl in environmental and food samples.

MATERIALS AND METHOD

Instrument

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

Reagents and Chemicals

All chemicals used were of Analytical Reagent grade purity. Ferrous sulphate, ethanol, acetic acid, sodium acetate, acetone and ethanol (Merck), were used during this work. Reference standard fenoxaprop-p-ethyl was purchased from Dr. Ehrenstofer GmbH, Germany.Commercial formulations containing fenoxaprop-p-ethyl purchased commercially from the local market.

Solution

i. Standard fenoxaprop-p-ethyl solution (1000 ugmL$^{-1)}$) was prepared by dissolving 1.0 g of fenoxaprop-p-ethyl in ethanol and diluting up to 100 mL in volumetric flask.

ii. Ferrous sulphate solution (1000ug mL^{-1}): 1.0g of ferrous sulphate was dissolved in 30 ml of water and diluting up to 100 mL in volumetric flask.

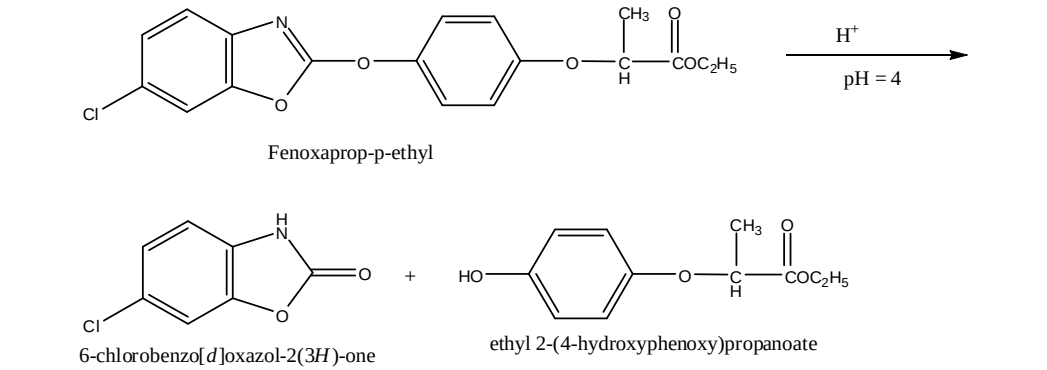
iii. Acetate buffer (pH4) was prepared by mixing acetic acid (0.2M) solution and (0.2M) sodium acetate solution.

Procedure

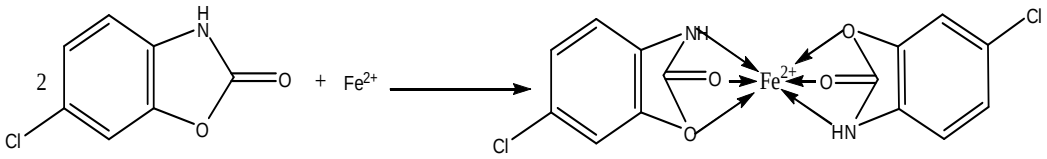
The method for spectrophotometric determination is based on the acid hydrolysis of fenoxaprop-p-ethyl to form 6-chloro-2, 3-dihydrobenzoxazol-2-one and ethyl2-(4-hydroxyphenoxy) propanate. For analysis from 100 ugmL^{-1} stock solution of fenoxaprop-p-ethyl 10 mL was taken in a 100 mL volumetric flask. To this solution 10 mL of $FeSO_4$ (1000 ugmL^{-1}) and 10 mL of acetate buffer (pH-10) was added. The mixture was boiled on water bath for 10 minute after hydrolysis, the proposed hydrolyzed product 6-chloro-2, 3-dihydrobenzoxazol-2-one is used as complexing reagent with metals. For investigation of charge transfer complex of fenoxaprop-p-ethyl with iron (II), initially ferrous sulphate solution was added drop wise to the hydrolyzed product on boiling water bath. This product forms a yellow colored charge transfer complex with iron (II). The resulted yellow color charge transfer complex was extracted in 10 mL ethanol. The reaction mixture containing the resulting yellow colored complex was transferred to50 mL volumetric flask and the volume was made up with ethanol. The absorbance was measured from 350 to 460 nm using UNICO UV-2100 spectrophotometer, for finding out the optimum wavelength. Each wavelength was calibrated with blank solution. The optimization of the complex was measured at 430 nm (optimized wave length).

RESULTS AND DISCUSSION

Fenoxaprop-p-ethyl on acid hydrolysis at different condition gave the product 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP). The reaction is based on complexing of fenoxaprop-p-ethyl with Fe (II). Its λ_{max} was found to be 430 nm. The proposed reaction path way is shown in scheme (Fig. 1).



Step-1. Dissociation



Step-2. Complex formation
Yellow coloured complex

Fig.1. Proposed chemical reaction for the Spectrophotometric determination of fenoxaprop-p-ethyl by charge transfer complex formation with iron (II)

The influence of various experimental parameters on the absorbance of the charge transfer complex of fenoxaprop-p-ethyl with Fe (II) was studied. It was observed that the solution acidity has a great effect upon the reaction of Fe (II) with fenoxaprop-p-ethyl. Acetate buffer was selected for maintaining the pH and pH optimization studies were carried out in the range of 3.5 - 5.5 pH. Maximum charge transfer complex formed at pH 4.0. The higher or lower pH value caused a decrease in the absorbance value (Fig.2). The volume of pH 4 acetate buffer was also optimized from 1-12 mL. One mL was found to be the best for completion of the reaction. The effect of concentration of $FeSO_4$ was studied using different volumes of 0.01 M solution in the range of 0.5-3.0 mL . The highest absorbance was using 3 mL (Fig. 3). The charge transfer complex of fenoxaprop-p-ethyl with Fe (II) was formed at room temperature and was stable for 30 minutes. Composition of charge transfer complex was investigated by applying Job's method of continues variations. The plot reached a maximum value at mole fractions of 2.66: 6.67, which indicated the formation of 1:2 complexes (Fig.4). The stability constant (k_f) of the charge transfer complex was calculated from the continuous variation plot and found to be 1.75×10^4 L mol^{-1} $cm^{-1.}$ The spectral characteristics are given in (Table I).

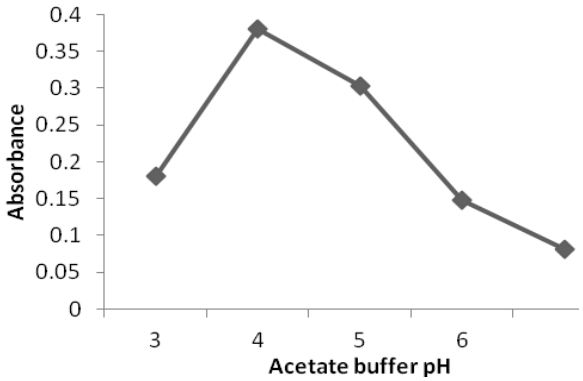


Fig.2. Optimization of acetate buffer pH for the spectrophotometric determination of Fenoxaprop-p-ethyl charge transfer complex

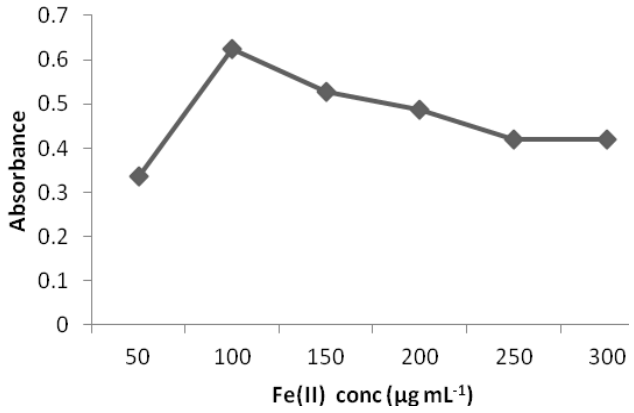


Fig.3. Optimization of Fe (II) concentration for the extractive spectrophotometric determination Fenoxaprop-p-ethyl using charge transfer complex

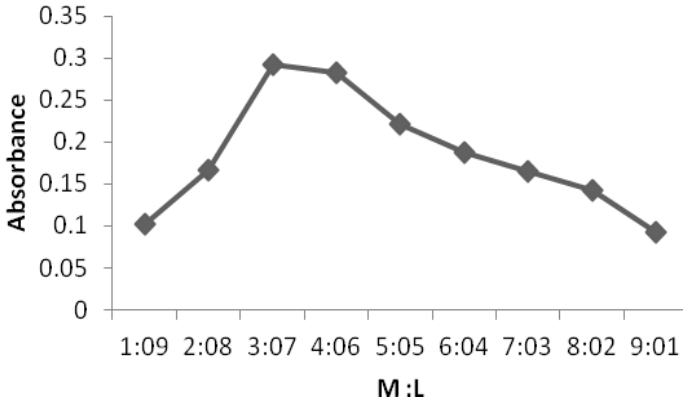


Fig.4. Determination of formula of the complex using job's method

Table I ***Optical characteristics for spectrophotometric determination of fenoxaprop-p-ethyl***

Parameter	**Batch analysis**
λmax (nm)	430
Molar absorptivity (ε) (L mol-1 cm-1)	1.75×10^4
RSD (%)	3.14
Limit of Detection (µg mL-1)	0.07
Limit of Quantification (µg mL-1)	2.3
Correlation Coefficient	0.997

The effect of absorbance verses concentration for fenoxaprop-p-ethyl was investigated using charge transfer complex and a linear behavior was observed in the range 0.2-12 µg mL^{-1}. Statistical parameters were calculated .The linearity of calibration graphs was proved by the high value of correlation coefficient (r) 0.987. The molar absorpitivity of the resulting colored charge transfer complex was found to be 4.2×10^4 L mol^{-1} cm^{-1}**.**The limit of detection and quantification were calculated and found to be 0.023 µg mL^{-1} and 2.3 µg mL^{-1}respectively. The validity of the proposed method was tested for the determination of fenoxaprop-p-ethyl in real samples. Standard addition technique was applied to test the reliability and recovery of the proposed method in which known concentration of fenoxaprop-p-ethyl was added to the previously analyzed portion of commercial preparations. The results are given in (Table II). The percent recoveries were found to be in the range of 84.9±0.09–.98.0±0.06 %.

Table II ***Percent recoveries of fenoxaprop-p-ethyl from real samples***

Real samples	Taken (g)	Added (mg L^{-1})	Found (mg L^{-1})	% Recovery
Barely grain	10	5	4.71	95.00 ±.017
		10	9.32	93 00±.0.11
		15	14.21	94 ±.0 .009
Wheat grain	5	10	9.62	96.00 ±.021
		15	14.73	98.00 ±0.30
		20	18.87	94.30 ±0 .92

For further confirmation of fenoxaprop-p-ethyl it was determined in commercial formulations (Table III). The proposed method is sensitive; therefore it could be easily applied for routine analysis in pure form and in commercial preparations.

Table III Concentration of fenoxaprop-p-ethyl in commercial formulation

Commercial formulation	Label Amount (g mL^{-1})	Proposed method (g mL^{-1})
Active ingredient	7.8	7.8±0.38

Extraction from Grains Samples

Fortified grain samples were prepared by adding 5, 10 and 15 µg $g^{-1}$of standard fenoxaprop-p-ethyl solution to barley grains and wheat. After fortification the samples were kept at room temperature for 6.0 hours to allow evaporation of the solvent. Then 20 mL acetone was added to each sample and the sonication extraction procedure was used as mechanical for soil samples.

The validity of the proposed method was tested for the determination of fenoxaprop-p-ethyl in real samples. Standard addition technique was applied to test the reliability and recovery of the proposed method in which known concentration of fenoxaprop-p-ethyl was added to the previously analyzed portion of commercial preparations. The percent recoveries were found to be in the range of 84.9±0.09– 98.0±0.30 %. The results are given in (Table II).

For further confirmation of fenoxaprop-p-ethyl it was determined in commercial formulations (Table III). The proposed method is sensitive; therefore it could be easily applied for routine analysis in pure form and in commercial preparations.

For real samples homogenized food sample (10 g) was taken in a flask and 50 mL of acetone was added to it. Each sample was sonicated for 30 min at ambient temperature. After sonication the samples were filtered on filter paper. The filtrate was collected in flask and was evaporated up to dryness and then reconstituted in 10 mL of acetone and diluted up to mark and analyzed by the proposed method. Each sample was analyzed in triplicates. The results are given in (Table IV).

Table IV ***Residue level of fenoxaprop-p-ethyl in real samples***

Sample	Residue (µg g^{-1})
Barley grains	2.7±0.318
Wheat grains	1.5±s0.35

CONCLUSION AND RECOMMENDATIONS

A simple Spectrophotometric method has been developed for determination of fenoxaprop-p-ethyl herbicide based on acid hydrolysis of fenoxaprop-p-ethyl which gives the product 6-chloro-2,3- dihydrobenzoxazol-2-one (CDHB) and ethyl 2-(4-hydroxyphenoxy) propionate (EHPP) followed by complexing of 6-chloro-2, 3-dihydrobenzoxazol-2-one (CDHB) with Fe (II) to form yellow color complex. The spectrophotometric method has low detection limit and high sample throughput. The method is applicable to environmental samples and applied to wheat and barley grains with percent recoveries in the range of 94.0 ±0.92 to 98.0 ± 0.30. Compared to gas chromatographic and liquid chromatographic methods the proposed method is simple, sensitive and can be applied in small scale laboratories for FPE analysis compared to GC and HPLC.

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