Quantification of Fenoxaprop-p-ethyl herbicide in Soil and Vegetable Samples by Microwave-Assisted Solvent Extraction and HPLC Method

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Summary: A simple HPLC procedure for the determination of fenoxaprop-p-ethyl herbicide in environmental samples is described. The chromatographic analysis was carried out by HPLC, on a C_{18} packed capillary column (4x4 mm,4.6 X 150 mm, 5mm particle size) with 20 µl injection volume and UV detector at 280 nm. HPLC-grade acetonitrile and methanol were used as mobile phase with flow rate of 1mL min^{-1} . Samples were spiked with amount between 5 - 20µg g^{-1} of herbicide and were isolated from samples by applying microwave assisted extraction (MASE) at ambient temperature. Percent recoveries were improved by optimizing solvent types, solvent volume, extraction temperature and time. Calibration curve range determined by HPLC was 0.5-16µg mL^{-1} .The interaction of different variables for maximum % recovery response was checked by applying factorial design and was found to be in range of 91.22±0.01—99.32±0.01 with good precision (< 5%). Application of this procedure to the analysis of herbicide in ester and acid form showed the effectiveness of the proposed approach.

Key words: Fenoxaprop-p-ethyl, MASE, Soil and Vegetable samples, HPLC.

Introduction

The ubiquitous presence of herbicides as environmental contaminants has created concern about their fates and transport in natural water, agricultural samples and soil. The increasingly intensive and widespread use of aryloxyphenoxy herbicides has resulted in significant contamination of surface and ground water. These herbicides are usually non biodegradable and quite persistent in the environment [1]. Fenoxaprop-p-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionates} is one of the aryloxyphenoxy propionic acid which is used only for control of perennial and annual grass weeds in many crops. Its mode of action is to inhibit fatty acid biosynthesis [2]. Fenoxaprop-p-ethyl 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. Fenoxaprop-p-ethyl is used for post emergence control of grasses weeds in potatoes, beans, cabbage, barely and cotton [3].

The more frequently used formulations are amines or alkaline salts ,alkyl esters or free carboxylicacids.The esters, emulsified in oil, are commonly used because of their higher herbicide activity ,Penetration power and low vapor pressure . Due to their persistence, polar nature and low 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 [4].

Fenoxaprop-p-ethyl control weeds due to inhibition of acetyl-CoA carboxylase found both in mammalian liver and plant chloroplast. Therefore, the toxicity of fenoxaprop-p-ethyl makes it impossible to underestimate the toxicity risk to human beings [5].

Toxicity studies have been conducted using fenoxaprop-p-ethyl on three species of rat, mouse and monkey and were identified for liver toxicity, increased liver weight and fall in body weigh [6].

Most of the methods reported for analysis of herbicides in food and environmental samples are biological methods [7, 8] gas chromatography [9, 10]. Chromatographic techniques are the most widely used one to determine phenoxy acid herbicides HPLC is preferred one on G.C methods because it allows direct analysis of phenoxy acid and their esters without derivitization [11, 12].

In the proposed method we described a method based on microwave assisted solvent extraction (MASE) HPLC approach to determine herbicide fenoxaprop-p-ethyl in environmental samples soil and water. MASE IS effective at room temperature preventing transformation compared to

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traditional extraction techniques by decreased extraction time and reduced solvent consumption.

Results and Discussion

Optimization of HPLC Conditions

HPLC method was optimized for determination of fenoxaprop-p-ethyl using reverse phase Zorbax SB-C_8 column(4.6 X 150 mm, 5mm particle size) (attached with a sample loop of 20 µL capacities. The mobile phase consisted of acetonitrile and methanol. Gradient elution was carried out according to the programme; as 100 % acetonitrile for 5 min, 30 % methanol and 70 % acetonitrile for 12 min and 100 % methanol for 8 min. The flow rate was kept at 0.7 mL min^{-1} using ultraviolet detector at 280 nm for absorption measurement. Standard curve was constructed to encompass anticipated range of fenoxaprop-p-ethyl concentration in range of 5-20 µg mL^{-1} in different samples.

Optimization of MAE Condition

The effects of various experimental parameters on microwave-assisted extraction step were studied. Parameters optimized were solvent type, ratio of mixtures of solvents, time and temperature.

Selection of Solvent

Selection of solvent for extraction is important because it affects the % recoveries of herbicides. Fenoxaprop-p-ethyl is highly soluble in acetone and ethanol. These solvents were used for extraction of fenoxaprop-p-ethyl from soil and vegetables samples. Maximum extraction was achieved using acetone. Recoveries in range of 70-78 % without microwave and improved to 92-100 % by microwave assisted extraction methods in different samples. (Fig. 1)

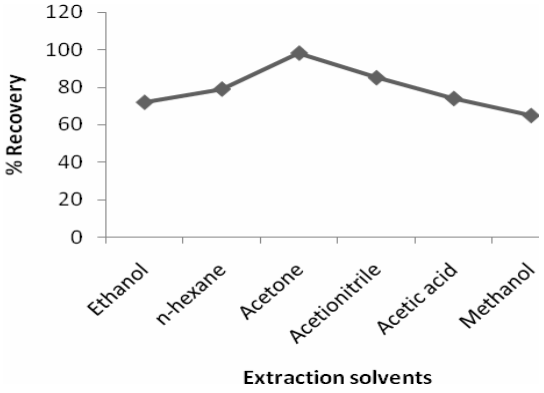

Fig. 1**:** Investigation for suitable solvent for extraction of fenoxaprop-*p*-ethyl.

Effect of Extractent Solvent Volume

Volume of solvent for MASE was optimized to select the minimum volume of solvent required for maximum % recovery. Optimum volume for microwave assisted extraction 40-50 mL is (Fig. 2).

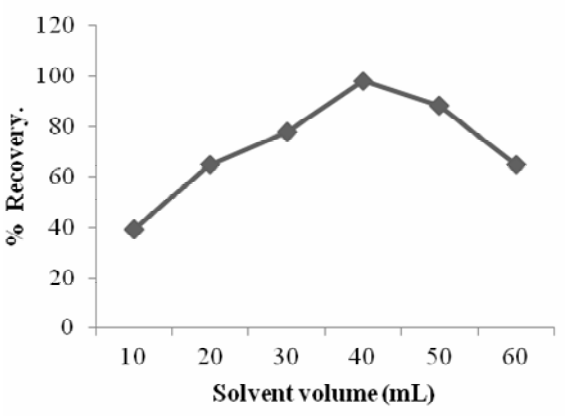

Fig. 2**:** Investigation of suitable volume of solvent for extraction of fenoxaprop-*p*-ethyl.

Effect of Time on Extraction

Extraction time optimizations were carried out for 1-6 min. The extraction time at room temperature at which herbicide shows maximum recovery is 4 hrs and % recovery is improved by 6 min microwave heating at 60 ^{0}C.(Fig. 3)

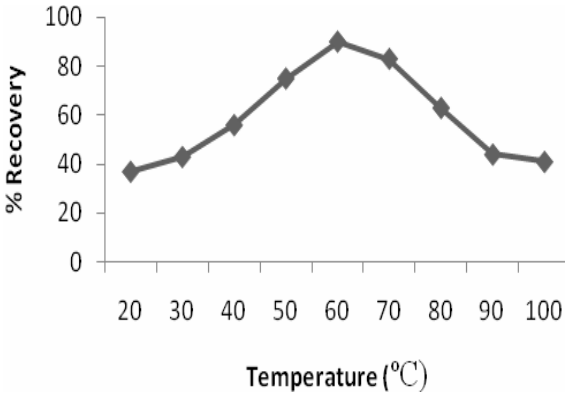

Fig. 3: Effect of microwave temperature on % recovery of fenoxaprop-p-ethyl**.**

At high temperature degradation starts and peak broadening is observed. Therefore, 6 min irradiation time was selected for MASE (Fig. 4)

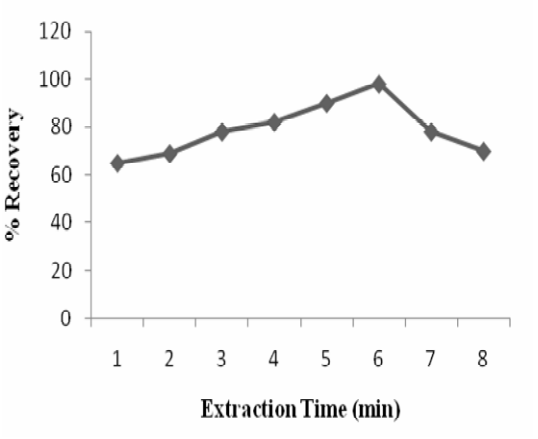

Fig. 4: Effect of microwave extraction time on % recovery of fenoxaprop-*p*-ethyl.

Statistical Optimization

Three factors were defined to evaluate for Analysis of variance (ANOVA) using factorial design and their contribution to herbicide extraction efficiency from water and food samples 'A' temperature, 'B' time of extraction and 'C' solvent volume. The ANOVA test ($p<0.05$) showed that amount of solvent and temperature had significantly positive effect on fenoxaprop-p-ethyl extraction (Table-1). Recoveries higher than 98 % were observed in preliminary studies on partitioning of herbicides between methanol and acetonitrile assisted by microwave.

Table-1: Results of the ANOVA for selected factorial model for HPLC determination of fenoxaprop-*p*-ethyl.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	**1027.00**	**6**	**171.17**	**342.33**	**0.0413**	
A-Temp	**40.50**	**1**	**40.50**	**81.00**	**0.0704**	
B-Time	**40.50**	**1**	**40.50**	**81.00**	**0.073**	
C-Solvvol	**684.50**	**1**	**684.50**	**1369.00**	**0.0172**	**significant**
AB	**40.50**	**1**	**40.50**	**81.00**	**0.0704**	
AC	**40.50**	**1**	**40.50**	**81.00**	**0.0704**	
BC	**180.50**	**1**	**180.50**	**361.00**	**0.0335**	
ABC	**40.50**	**1**	**40.50**	**81.00**	**0.0704**	
Residual	**0.50**	**1**	**0.50**			
Cor Total	**1027.50**	**7**				

A central composite design was applied based on a two level factorial design, supported the low numbers of factors to be optimized. The result of ANOVA for solvent volume, extraction time and temperature appeared to have significant effect on extraction efficiency with 2^3 factorial response surface were obtained and all these responses were well fitted with R^2 (0.9998).Fig. 5 shows response surface plot of the composite desirability function estimated from design.

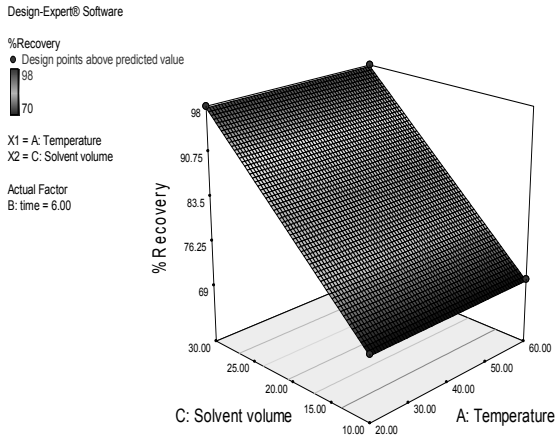

Fig. 5: Response surface plot for % recovery of fenoxaprop-p-ethyl.

Fig. 6 shows HPLC chromatograms, recorded at 280 nm for standard solution of fenoxaprop-p-ethyl prepared in acetonitrile. The retention time for fenoxaprop-p-ethyl standard is 4.37 min. similar chromatograms were obtained for soil and vegetables samples for percent recoveries.

Chromatograms obtained for fortified soil (Fig. 7) and vegetable (fig. 8) were used for identification and quantification.

Experimental

Instrument

The HP series 1100 LC system (Perkin Elmer Series 200 CA, USA) equipped with gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop and ultraviolet detector at 280 nm was used. Zorbax SB- C_8 (80 A^0, 4.6 X 150 mm, 5mm particle size) was used as a separation column with a Zorbax C_{18} (4x4 mm) as a guard cartridge. Microwave extractions were performed with KEN ST/SS25 KENWOOD (China) microwave with temperature control system.

Reagents

Fenoxaprop-p-ethyl {ethyl-2-[4-[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionate} (99 %) was purchased as standard from Dr. Ehrenstofer GmbH, Germany.

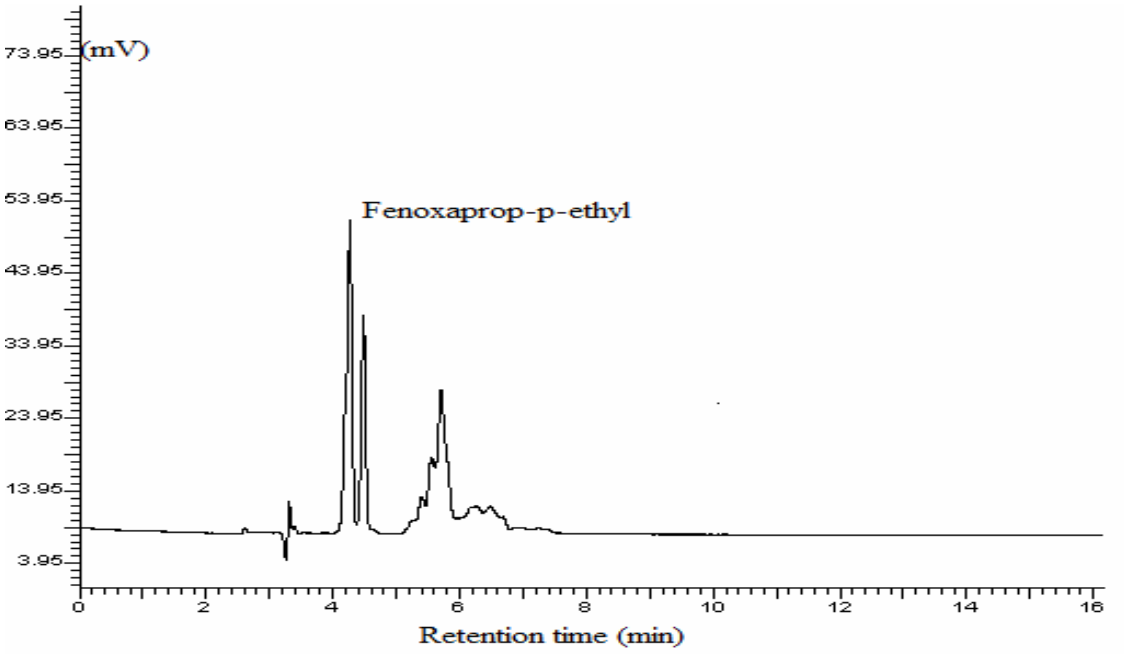

Fig. 6**:** Chromatogram of standard fenoxaprop-*p*-ethyl herbicide using reverse-phase HPLC.

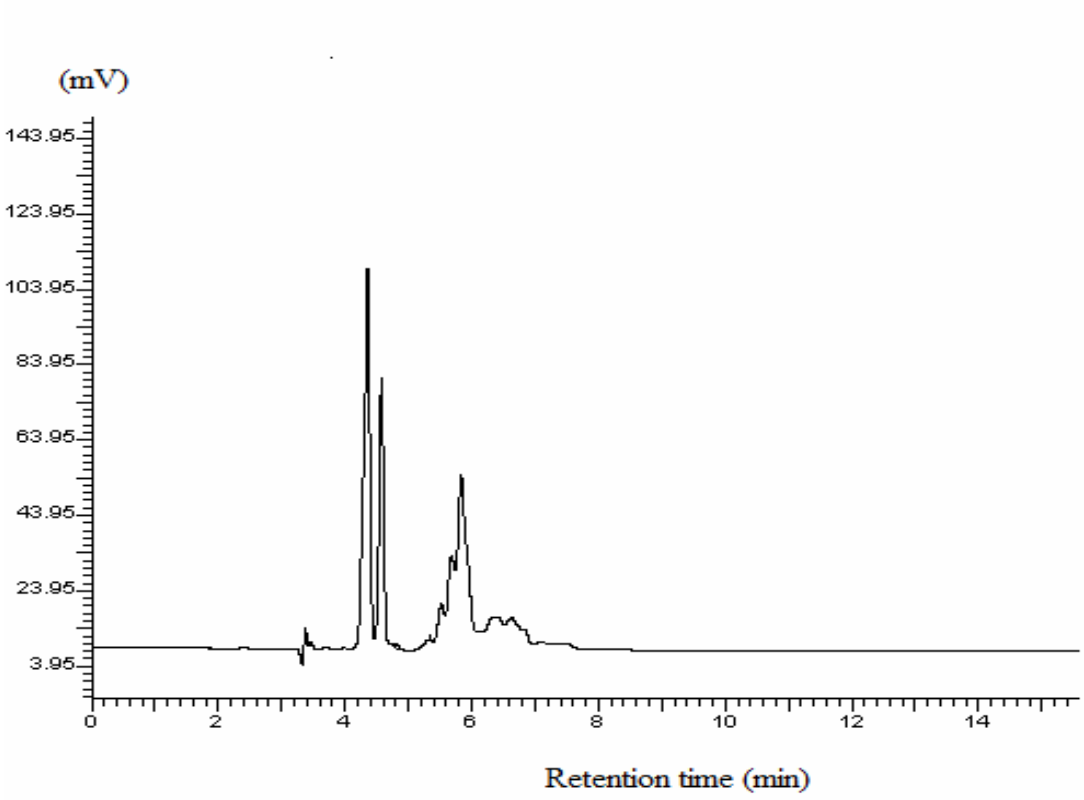

Fig. 7**:** Chromatogram for 10µg g^{-1} fenoxaprop-p-ethyl fortified soil sample using reverse-phase HPLC.

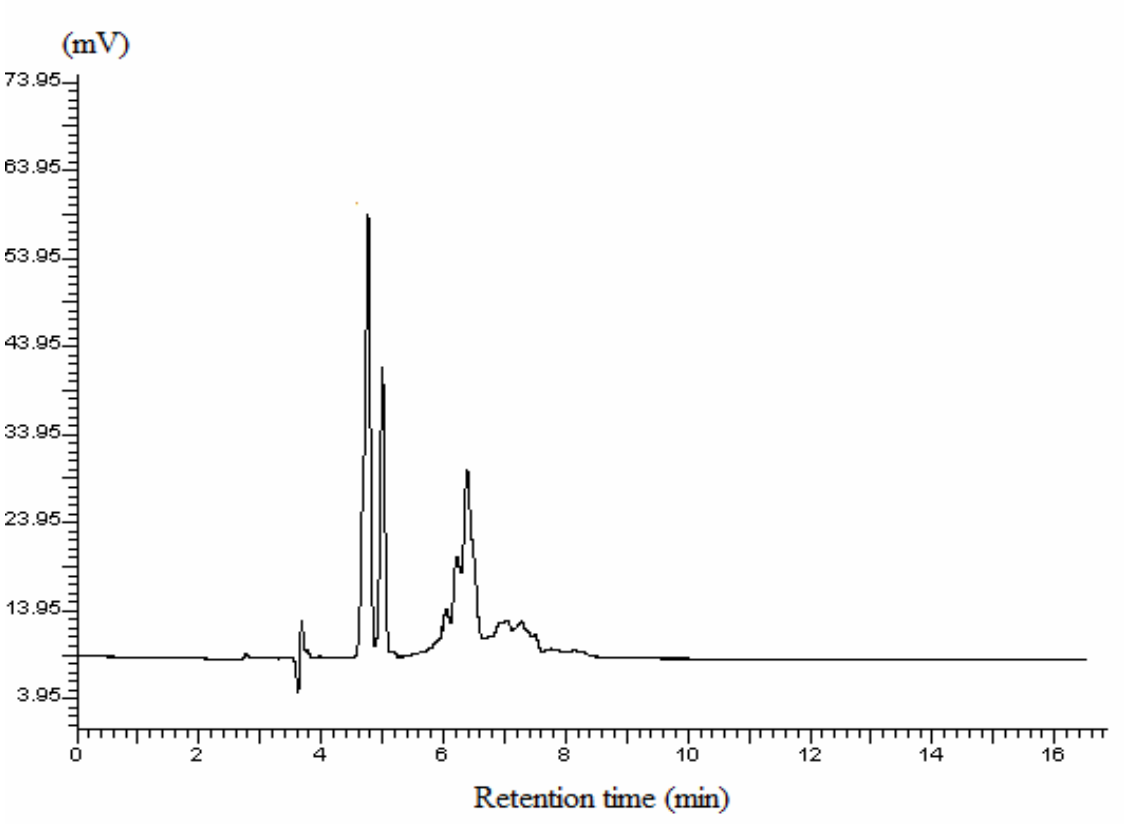

Fig. 8**:** Chromatogram for 5µgg^{-1}fenoxaprop-p-ethyl fortified cabbage sample using reverse phase HPLC.

HPLC-grade acetonitrile and methanol used were products of Rathburn, Walkburn, UK. LC grade water was obtained by purifying distilled water with a Milli-Q water purification system. All other chemicals used were products of analytical reagent grade purity manufactured by Merck (Darmstadt, Germany).

Standard Solution and Sample Preparation

Stock solution of fenoxaprop-p-ethyl (1000 µg mL^{-1}) was prepared by dissolving 0.1 g of fenoxaprop-p-ethyl in 10 mL of acetonitrile and diluted up to 100 mL with acetonitrile. Working standards were prepared from this stock solution in range of 5-80 µg mL^{-1} by dilution with acetonitrile.

Extraction Procedure

Vegetables and soil samples (10 g) were homogenized and fortified with known concentration of fenoxaprop-p-ethyl solution. For mechanical extraction 50 mL of acetone was added to each sample and after mixing the samples were shaken on shaker for 15 min equilibration the samples were filtered and the filtrate was evaporated on rotary evaporator near to dryness, redissolved in 10 mL of acetonitrile passed through 0.45 µm pore, for HPLC analysis.

For microwave assisted extraction optimum volume of acetone was added to each fortified sample in closed-vessels. The starting parameters setting in microwave system were 2.0 min at 350 W and 3.0 min at 500 W. Once the extraction program was completed the vessels were cooled down to room temperature before opening. The extract was evaporated near to dryness and redissolved in 10 mL of acetonitrile passed through 0.45 µm pore, for HPLC analysis.

% Recovery

Control samples soil and vegetable (10 g) of each were separately placed in bottles and known concentration of fenoxaprop-p-ethyl solution was added to adjust the concentration level of 5, 10, 15 µg g^{-1}. The homogenized samples were extracted for 6 min with 10 mL of acetone in microwave. Extraction was repeated three times. The percent recovery was

evaluated by the developed HPLC method. Each recovery was carried out in triplicate. The results for both mechanical and microwave extraction are given in Table-2 and 3..

Table-2: Comparison of different extraction procedure for the extraction of fenoxaprop-*p*-ethyl from real samples

Sample	Added	Found (µg g^{-1})		Average% Recovery± RSD	
	(µg g^{-1})	MASE	ME	MASE	M E
Soil	5	4.8	4.3	96±1.6	83±1.7
	10	9.6	8.3		
	15	14.7	12.0		
Cabbage	5	4.5	3.9	90 ±2.0	77±1.5
	10	8.6	8.0		
	15	14.0	11.		

MASE (Microwave assisted solvent extraction)
ME (Mechanical extraction)

Table-3: Residue level of fenoxaprop-*p*-ethyl in real samples.

S. No	Sample	Residue (µg mL^{-1})
1	Cabbage	1.36 ± 0.16
2	Soil	1.32± 0.22

Conclusion

HPLC method with Microwave-assisted solvent extraction using acetone as extracting solvent has been developed for determination and extraction of fenoxaprop-p-ethyl herbicide from soil and vegetable samples and formulations. MASE followed by HPLC determination provides high recoveries (90-96%) with minimum matrix effects. The results obtained for recovery, precision and accuracy indicated that the MASE followed by HPLC and UV detection is an efficient and simple method for identification, determination and quantification of fenoxaprop-p-ethyl in soil and vegetables samples.

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