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DETERMINATION OF ARYLOXYPHENOXY PROPIONATE GROUP OF HERBICIDES USING HPLC IN FOOD SAMPLES

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ABSTRACT

A method for the separation and determination of herbicide residue, aryloxyphenoxy propionateherbicides, (fenoxaprop-p-ethyl, cyhalofop-butyl pinoxaden) in food samples with high-performance liquid chromatography detection is described. The developed method is based on microwave-assisted solvent extraction (MAE) of wheat flour and maize grains samples for 3 minutes at 550W irradiation energy. The separation was performed by using mixture of solvents acetonitrile/methanol at room temperature. The factors influencing the determination and separation, such as volume, solvents for extraction, temperature and time were optimized. Calibration curve was established using HPLC for fenoxaprop-p-ethyl and pinoxaden are 5-50 µgmL^{-1} and 2.0 - 50 µgmL^{-1}. Recoveries in the range of 86-90% were obtained with ethanol as extraction solvent. It was observed that pinoxaden is 86% recovered in methanol and acetonitrile, respectively.

Key words: Aryloxyphenoxy,HPLC, food samples.

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INTRODUCTION

The environmental circumstances of Pakistan are favorable to grow a broad variety of crops and economy of Pakistan is mainly based on agriculture sector. On the other hand, production of most of the crops in Pakistan is lower as compared to the world's average because of weeds invasion. Weeds are one of the most major reasons for the lower yields of crops in the country (Amir *et al*., 2012). Weeds are strong competitors and concealed enemies of crops because of their abundant growth under different environmental conditions. Selection of suitable herbicides and the treatment timingandat the correct leaf growth stages is important (Rehman *et al.*, 2014). Herbicides are chemicals used to control weeds in several areas of agriculture. The use of herbicides in agriculture has advantages such as increase in

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quality and quantity of food crops. Nevertheless herbicides are toxic substances and to protect consumer health, maximum residue limits have been established at national and international level (Leo and Nollet, 2004).

Peryear losses to wheat crop due to weed invasion are reported to be in billions.These massive losses warrant a well-organized control of weeds. Therefore, now weed management technology has entered a scientific stage and even though chemical weed control is very significant (Khan *et al*., 2012).

The use of chemicals is generally simple, time saving, very effective but excessive approach to weed control. Taking into account the global utilization of herbicides per year, it is not amazing that many of these compounds have been detected in normal waters and therefore, have raised significant concern both from individual health and from ecological point of View. In spite of the benefit, the use of these kinds of chemicals must be controlled because most of degraded part of these herbicides is released into the environment which is hazardous for human health. The ubiquitous presence of herbicides as ecological contaminants has formed distress about their fates and transport in natural water, agricultural samples and soil. The increasingly intensive and extensive use of aryloxy-phenoxy herbicides has resulted in major contamination of surface and ground water. These herbicides are usually non-biodegradable and quit persistent in the environment. (Sanchis-Mallois *et al.*, 1998) Aryloxyphenoxy propionic acid herbicides as Fenoxaprop-p-ethyl isused for the control of perennial and annual grass weeds in many crops. These herbicides inhibit fatty acid biosynthesis (Zhang *et al.*, 2008).

Phenylpyrazoline herbicides like Pinoxaden is a member of the aryloxyphenoxy group supplied worldwide under the trade name Axial, and is sprayed against a variety of annual grass weeds such as grass found in wheat and barley crops (Peter *et al*., 2005). Pinoxaden suppresses the function of acetyl co-A carboxylase enzyme, by this means prevents the fatty acids production and accordingly the formation of bio membranes in weeds (Jasmin *et al*., 2011).

A large amount of methods reported for determination of fenoxaprop-p-ethyl like FIA (Jasmin et al. 2010), HPLC (Santilioet al. 2009), and GC-ECD (Zhanget al. 2008). FIA Method for determination of pinoxaden investigated (Jasmin et al. 2011) need derivatization step before analysis.

This method was developed with the aim of finding out easy and simple high-performance liquid chromatography (HPLC) procedure for partition of the herbicides for detection and quantification of

selected aryloxyphenoxy herbicides in food and environmental samples using microwave-assisted extraction with suitable extraction solvent and cleanup process.

MATERIALS AND METHODS

A CA Series 200 Liquid Chromatographic system Perkin Elmer (USA) equipped with HP series 1100 outfitted with gradient pump, Model 600, an auto sampler Model 717 plus and UVdetector at 240 nm was used. The determination and separation of fenoxaprop-p-ethyl and pinoxaden was performed using Zorbax SB-C8 (80 A0, 5mm particle size, 4.6 X 150 mm) with a Zorbax C18 (4x4 mm) guard cartridge and. 20 µL injection loop. Microwave (KENWOOD) with temperature control system (Model KEN SS25/ST) was used for microwave extraction.

Reagents

All chemicals wereHPLC-grade. Acetonitrile and ethanol were obtained from Rathburn, Walkerburn, UK. LC grade water was used by purifying distilled water with a Milli-Q water purification system by Millipore Co, USA. All other chemicals used were of analytical reagent grade purity (Merck) Standard fenoxaprop-p-ethyl [-ethyl-2-[4[(6-chloro-2-benzoxazolyl) oxy] phenoxy] propionate] 99 % and pinoxaden 98% was purchased from Dr.Ehrenstofer GmbH, Germany.

Fenoxaprop-p-ethyl and Pinoxaden standard stock solutions (400 µg mL^{-1})

For preparation of standard, the stock solution of pinoxaden and fenoxaprop-p-ethyl, 10 mg of each standard was dissolved in 10 mL of acetonitrile and diluted with acetonitrile up to 25mL. Working standards were prepared from this stock solution in the range of 5-40 µg mL^{-1} by dilution with acetonitrile in 10 mL volumetric flask.

Mixture solution

Stock solutions containing each analyte at 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10 µg mL^{-1} were also prepared in methanol by mixing both analytes. These were used for separation, optimization and constructionof calibration curves.

Sample preparation (Wheat flour and vegetables samples)

Recovery studies were carried out using three control samples (10g) of wheat flour and maize grains fortified with (5 and 10 µg g^{-1}) pinoxaden and fenoxaprop-p-ethyl mixture in 1:1 ratio. Two parts of spiked wheat flour and maize grainssamples (10 µg g^{-1} and 5 µg g^{-1}) were prepared by adding asuitable volume of herbicides solution of both fenoxaprop-p-ethyl and pinoxadento the wheat sample, then stirring carefully to homogenize it. The other part was used as a blank.

All of these samples were kept in the dark. The sample flasks were wrappedwith aluminum foil. There were no detectable levels of the target analyte in the wheat and vegetables before spiking. The samples were kept for four hours for sorption and were shaken occasionally for uniform mixing. A mixture of 20 mL methanol and acetone was added to each sample and kept in microwave for 4 min. On cooling the samples and the filtrate was evaporated to dryness. Then the residue was redissolved in acetonitrile, filtered, centrifuged and analyzed using HPLC at optimized conditions. The results are given in Table-1.

RESULTS AND DISCUSSION

Separation of fenoxaprop-p-ethyl and pinoxaden was done by liquid column chromatographyafter preliminary analysis by TLC. Different solvents separately and mixture were screened for the best separation of pinoxaden and fenoxaprop-p-ethyl herbicides.

Optimization of HPLC Conditions

Instrumental parameters of HPLC were optimized for the separation of fenoxaprop-p-ethyl and pinoxaden like mobile phase solvents, mobile phase flow rate, ratio of solvents and gradient elution. Flow rate of mobile phase was varied in the range of 0.5-1.0 mL min^{-1} using acetonitrile and methanol in the ratio 20:80, 50:50 and 80:20 for the partition of both herbicides. The complete declaration of mixture was achieved using 0.7 mL min^{-1} flow rate and mobile phase proportion 50:50 in time range of 15 min and gradient elution for 5, 10, and 5 min was suitable for clear partition.

The conditions such as solvent type, volume, extraction time and temperaturefor microwave–assisted extraction were also optimized.

Optimization of Solvent

A study was carried outfor extraction of both herbicides separately to see the effect of different solvents on extraction. Methanol, acetonitrile acetone, chloroform, dichloromethane, n-hexane, and ethanol were used at 850 W of microwave for 3 minutes and average recoveries found are given in figure1. Fenoxaprop-p-ethyl was found to be well extracted in acetone with average recoveries of 98%.

Effect of temperature on extraction

Effect of temperature on the extraction efficiency was investigated and extractions were carried out at 30-80 ºC. The recoveries for extraction are shown in Fig. 2. For both herbicides the recoveries increases as the extraction temperature changes from 30-

60°C and the % recoveries decreases with further increase in temperature from 60 - 80 °C.

Time optimization for maximum extraction

Extraction time was optimized for 1-6 min at 850 W. Solvents used for extraction were acetone/methanol. Under the condition of changing the extraction time, the results of the MAE recoveries of fenoxaprop-p-ethyl and pinoxaden from wheat grains are shown in figure 3. The recoveries of both herbicides increased, when the extraction time was increased from 1 to 3 minbecause at high temperature degradation of herbicide affects the recovery. The optimum extraction time was selected to be 3 minute.

Optimization of volume of extracting solvent

Huge amounts of volume of solvent may make the process complicated andHowever if solvent volume is not enough the marked extraction would not be complete. Therefore different volumes of extract were studied. The effect of the extractant and its volume on the extraction efficiency for MAE is shown in figure 4.The recoveries of fenoxaprop-p-ethyl and pinoxaden increasedwhen the solvent volume was increased from 10 mL to 20 mL.

Figure 5 and 6 show the chromatograms of fenoxaprop-p-ethyl and pinoxaden standards solution 20 μgmL^{-1} and Figures 7 and 8 show chromatograms for separation and determination of fenoxaprop-p-ethyl and pinoxaden in a mixture using optimized reverse phase HPLC method.

Table-1: Recoveries of fenoxaprop-p-ethyl and pinoxaden from real samples

Real samples	Added (ug mL^{-1})	Found (ug mL^{-1})	% Recovery
Wheat flour	5	4.82	97.00±0.017
	10	9.57	94.00±0.11
Maize grains	5	9.52	95.00±0.021
	10	9.73	98.00±0.30

Table-2. Residue level of fenoxaprop-p-ethyl and pinoxaden in real samples

S. No.	Sample	Residue (µg g^{-1}) Fenoxaprop-p-ethyl	Residue (µg g^{-1}) Pinoxaden
1	Maize grains	2.6 ±0.29	1.13±0.20
2	Wheat flour	1.7±0.25	1.15±0.22

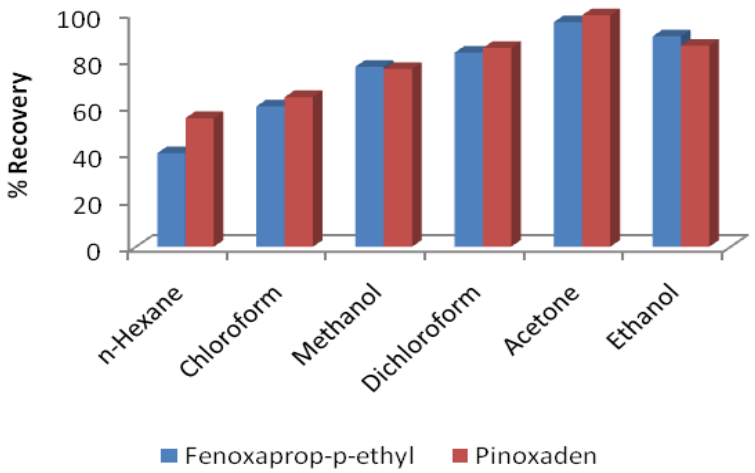

Fig. 1. Optimization of solvent for extraction of fenoxaprop-p-ethyl and pinoxaden

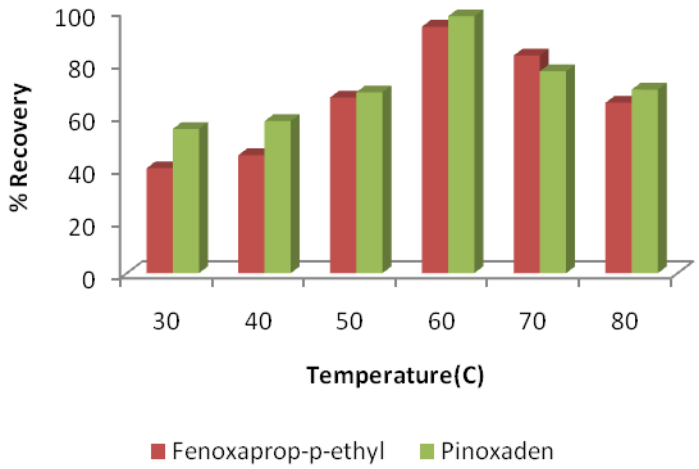

Figure 2. Study of appropriate temperature for extraction and determination of fenoxaprop-p-ethyl and pinoxaden by reverse-phase HPLC method.

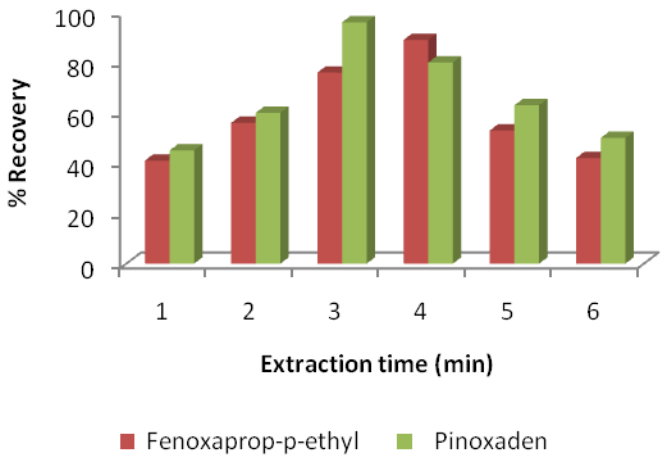

Figure 3. The result of extraction time on the extraction recoveries of fenoxaprop-p-ethyl and pinoxaden

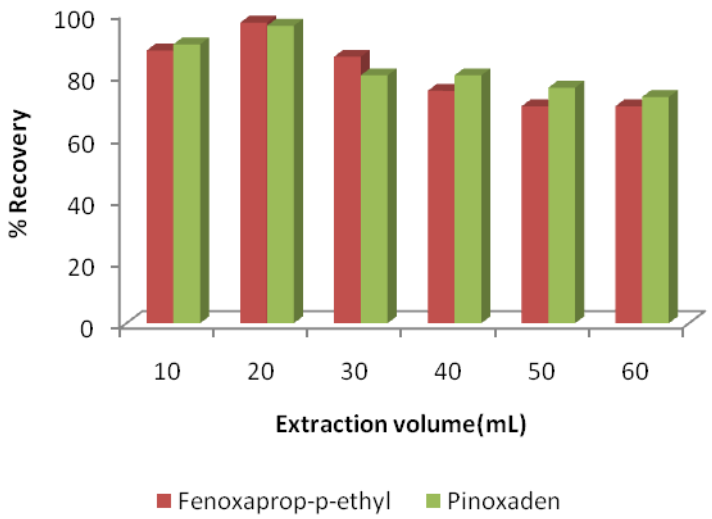

Figure 4. Analysis of suitable quantity of solvent for extraction and determination of fenoxaprop-p-ethyl and pinoxaden by reverse-phase HPLC method

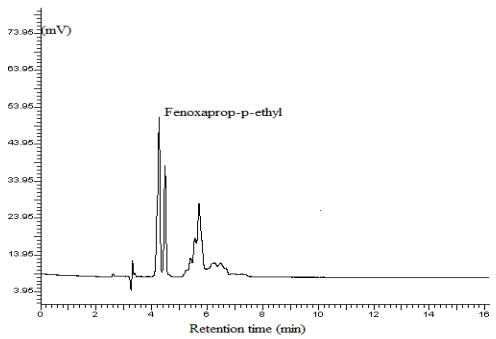

Figure 5. Chromatogram for standard fenoxaprop-p-ethyl herbicide via reverse-phase HPLC

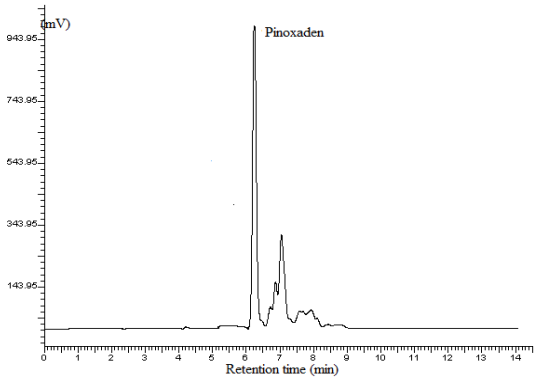

Figure 6. Chromatogram for standard Pinoxaden herbicide via reverse-phase HPLC

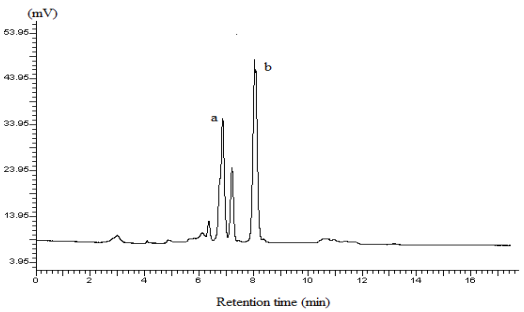

Figure 7. Chromatogram for fenoxaprop-p-ethyl (a) and pinoxaden (b) fortified 5µg g^{-1} wheat flour samples via reverse-phase HPLC

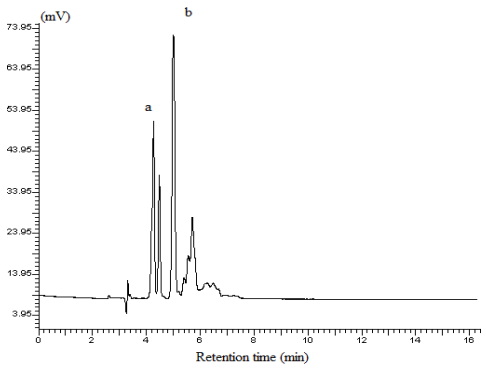

Figure 8. Chromatogram for fenoxaprop-p-ethyl (a) pinoxaden (b) fortified (10µg g^{-1}) wheat flour samples via reverse-phase HPLC

CONCLUSION

The applicability of the method of separation in real samples was checked by analyzing food samples purchased from local market. Figure 8 and 9 shows the chromatograms of mixtures of (5, 10 ug g^{-1}) spiked wheat flour and maize grain sample. The method validationstudies for spiked samples indicated that the present method provides excellent recoveries (90–97%) of fenoxaprop-p-ethyl and pinoxaden. The outcome obtained for, precision and accuracy show that the proposed high performance liquid chromatographic method is an efficient and easy method for identification and quantification of aryloxyphenoxy group of herbicide in food samples.

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