Pak. J. Weed Sci. Res., 19(2): 167-177, 2013

QUANTIFICATION OF PINOXADEN HERBICIDE IN WHEAT GRAINS AND VEGETABLE SAMPLES BY ULTRASONICATION-ASSISTED EXTRACTION AND HIGH- PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A method for high-performance liquid chromatographic (HPLC) for the residue determination of Pinoxaden in food samples was developed and validated. The chromatographic analysis was carried out by HPLC, on a C_{18} packed capillary column with temperature gradient, 20µl injection volume and ultraviolet detector at 280 nm. Wheat potato and cabbage samples were collected from fields near agricultural forms in Peshawar where the herbicide pinoxaden was not sprayed. Samples were spiked with amount between 5-and 15µg g^{-1} of herbicide and were isolated from samples by applying ultrasonication extraction at ambient temperature. Percent recoveries were improved by optimizing solvent types, solvent volume, and time. Calibration curve range determined by HPLC was 0.2-40µgmL^{-1}. Application of this procedure to the analysis of herbicide in ester and acid form showed the effectiveness of the methodology proposed.

Key words: Barley, HPLC, pinoxaden, ultrasonication extraction, vegetable samples.

INTRODUCTION

Weed interference is one of the most important but less noticed factors, contributing towards lowering the yields of wheat. Weeds not only reduce the crop yield, deteriorate the quality of farm produce but also trim down the market value of crop (Hussain *et al*., 2012). For several decades herbicides have been widely used for agricultural purposes both in the developed and developing countries in order to control pest damage to crop yields. However the trend in the use of herbicides is increasing day by day to control different kinds of weed as part of the modern agriculture practices. This intensive use has resulted in to a serious contamination of the environment (Pacanoski, 2007).

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Pinoxaden is a recently introduced post-emergence, selective herbicide discovered by Syngenta (Crop Protection AG). It is a member of the chemical class phenylpyrazolines and is marketed worldwide under the trade name AXIAL (Porter *et al.*, 2005). To ensure the permissiveness of primal crops, wheat and barley, a patented safener Cloquintocet-mexyl is added to the formulation. In addition, an efficient adjuvant is included into the formulation to assure the most advantageous spread of the spray on the surface of grass leaves and to have speedy uptake of solution by the leaf tissues (Muelebach *et al*., 1998). Pinoxaden is used for a variety of annual grass weeds such as black grass, silky bent grass, wild oats, and little seed canary grass, found in wheat and barley crops. And adjuent added in formulation enhances the performance of pinoxaden herbicide (Mousavinki *et al*., 2009).

Although the reported toxicity and ecotoxicity of pinoxaden is minimum and below the trigger values, it is harmful if inhaled, irritates eyes and respiratory system, is harmful to aquatic flora and fauna, and may cause long-term adverse effects in the aquatic environment (Shah *et al*., 2011).

Concern about the potential adverse effects of herbicides on the environment and human health have growing steadily because herbicide residues have been detected widely in the air, water ,soil and agricultural generate (Hurley *et al*.,1998).

Due to herbicides toxicity on human health, monitoring residue of herbicides in food samples is a priority objective in order to get knowledge of food quality avoiding possible risk on our health (Kim *et al*., 2005). The methods reported for the residue determination are (Navarroa *et al*., 2009) presently available proposed analytical methods for the determination of aryloxyphenoxy herbicides (Ascenzo *et al*., 1998; Carabias-Martynez *et al*., 2006; Conrado, 2002) involved acid digestion of the homogenized samples. The extracts are cleaned up on SPE cartridges and concentration of free and conjugated dihydroxy metabolites are determined using a two column HPLC system with UV detection at 223nm (Public Release Summary 2006) and Flow injection (FI) analysis (Shah *et al*., 2011).

For quantification of pinoxaden, various samples like cereal grains, soil and water were analyzed by LC/MS/MS after a series of extraction steps on Nexus Absolute SPE columns with LOQ range of 0.0005-1 mg Kg^{-1} (Report Released UK, 2005).

Based on toxicology studies indicating fetal anomie-lies, maternal morality and liver toxicity field measurements studies are required. This needs to develop easy analytical methods for quantities exposure assessments. The aim of the present study was to develop a simple and quick HPLC method with fast ultrasonication extraction

procedure for the determination of pinoxaden in formulation and food samples.

MATERIALS AND METHODS

Instruments

Herbicide was analyzed by HPLC. A Perkin Elmer chromatograph, from (Perkin Elmer, Series 200 Norwalk, CA, USA) equipped with HP series 1100 LC system Germany, gradient pump (Model 600), an auto sampler (Model 717 plus) with 20 µL injection loop with Perkin Elmer vacuumed degasser, and UV/Vis detector set at 280 nm was used. Pinoxaden was separated on Zorbax-C_8 (80 A^0, 4.6 mm x150 mm, 5mm particle size) with a C_{18} reversed phase packing (4x4 mm) with guard cartridge.

An ultrasonic bath (ks 300 KUM SUNG, Korea) was used to carry out the extraction step. International clinical centrifuge (U.S.A) was used for centrifugation.

HPLC conditions

The mobile phase consisted of acetonitrile and methanol. The gradient elution was carried out according to the program, Acetonitrile: Methanol (30:70, 50:50, and 70:30) for 8, 5, 7 min. The flow rate was 0.7 ml min^{-1}. The amount injected was 20 µl. Wavelength 280 nm as working wave length for HPLC analysis of pinoxaden.

Standard solution and sample preparation

Stock solution of pinoxaden was prepared by dissolving 10mg of pinoxaden in 10 ml of acetonitrile and diluted with acetonitrile and methanol. Working standards were prepared from this stock solution in range of 2-40 ppm by dilution with acetonitrile. All solutions were kept in aluminum foil wrappings to avoid photo degradation.

Extraction procedures

Grains samples

To analyze pinoxaden in barley and wheat, 5g aliquots of the samples were directly weighed in three different flasks and were spiked at three levels (5, 15, 20 µg g^{-1}) by adding standard solution of herbicide to each samples mixing it thoroughly and were kept for 6 hr. Then 20 ml of acetone was added to each sample and sonicated for 30 min at ambient temperature. After sonication the samples were filtered on paper. The filtrate was collected in flask and was evaporated up to dryness and then redissolved in 10 ml of acetonitrile. The solution were centrifuged and filtered through 0.45 µm micro pore before HPLC analysis.

Vegetables samples

A chopped and homogenized cabbage samples (10 g) were fortified with 5,10 and 20µg mL^{-1} standard solution of pinoxaden

herbicide .Each sample was centrifuged and was kept to equilibrate for 6.0 h at room temperature before extraction. Methanol and acetone (10:10) were added as extractant to ultrasonic extraction vessel. The vessels were placed for 30 min at room temperature in ultrasonic bath. The extract was separated from fibers by filtration, and transferred in to 250 mL separatory funnel and was diluted with methanol and then evaporated near to dryness, and redissolved in 5 mL of acetonitrile and diluted up to 10 mL . The filtrate was centrifuged and filtered through 0.45 µm membrane for HPLC analysis.

RESULTS AND DISCUSSION

HPLC analysis

The starting mobile phase composition was 100% methanol and then 30:80, 50:50, 70:20 CH_3CN: methanol with flow rate 1 ml min^{-1}. Calibration curve was prepared by plotting peak heights versus concentrations (Fig. 1).

Optimization of extraction conditions

The effects of various experimental parameters of ultrasonication extraction step were studied. Parameter optimized were solvent type, ratio of mixtures of solvents, ultrasonication time, and temperature.

Optimization of solvent

Selection of solvent for extraction is important because it effects on % recoveries of herbicides. Pinoxadenl is partially soluble in water and maximum soluble in acetone, methanol and ethanol. These solvents were used for extraction of Pinoxaden from vegetables and barley. The maximum extraction is achieved by acetone then for HPLC the dried extract was dissolved in acetonitrile. The % recoveries were in range of 70-78 without ultrasonication and improved to 92-100% by ultrasonication extraction methods in different samples (Fig. 2).

Optimization for extraction time and temperature

To study the effect of extraction time on extraction efficiency extractions were carried out at 5 10, 20, 30, and40 min using mixture of acetone and methanol (1:1) as the extraction solvent. The extraction time at room temperature at which herbicide shows maximum recovery is 30 min and % recovery is improved by 20% at ambient temperature. The recoveries are shown in Fig. 3 and Fig. 4, the recoveries increased clearly as the extraction time by ultrasonication is increased up to 100 % but it decreases at longer time. Degradation starts and variation in retention time and peak broadening is observed on heating. The samples for extraction were stored in brown bottles or bottles covered with aluminum foil to prevent photo degradation.

Calibration and linearity

Standard curve was constructed to encompass anticipated range of concentration in range 0f 4-50 µg/mL^{-1} in different samples. The peak height was noted for each concentration. Accuracy and precision of method were determined on three replicates .Based on IUPAC recommendation, points were considered to be in the linear range if their values did not differ by more than 5% from the slop. The percentages of recoveries were calculated by comparing the average chromatographic peak heights of the standards, fortified samples and unfortified samples. The HPLC chromatogram, recorded at 280 nm of standard solution of pinoxaden prepared in acetonitrile. The retention time for pinoxaden standard is 6.37 min (Fig. 5). Similar chromatograms were developed for wheat and vegetable samples for percent recoveries (Fig. 6 and 7). Gradient elution with the acetonitrile and methanol system enabled an efficient separation and wand development of chromatogram peaks of all Pinoxaden samples.

Statistical analysis

Statistical analysis of Wheat, Barley and vegetable samples followed equilibration and injections of the standards and blanks preceded injections of the sample extracts. The concentration of pinoxaden in final extract was calculated using the following formula.

$$\text{Concentration } (\mu g\ g^{-1}) = \frac{\mu g\ mLl^{-1}.\text{peak hight.dilution.total volume of the extract}}{\text{peak highs for standard.sample weight}}$$

Where: µg m Ll^{-1} = concentration of the standard pinoxaden solution.
Peak hight = peak hight of the analyte in the sample
Dilution = Dilution factor, if the sample was diluted prior to analysis.
Peak hight of standard = peak hight of the standard in the sample
Sample weight = weight of the sample extracted

$$\%\ \text{Recovery} = \frac{\mu g\ g^{-1}.\text{Concentration of standard.100}}{\text{Sample weight}}$$

The limits of detection (LOD) and limits of quantification (LOQ) were calculated from minimum concentration for which a proper peak was observed using the following formula.

$$LOD = 3s/b \text{ and } LOQ = 10s/b$$

Where b is slope of the curve.

The results for percent recoveries are given in Table-1. Fig. 5 shows the chromatogram for standard pinoxaden and Fig. 6, 7, and 8 shows the chromatograms for pinoxaden of 5, 10, and 15 µg mL^{-1} spiked pinoxaden wheat, Barley and vegetable samples.

CONCLUSION

HPLC method was developed for determination of pinoxaden herbicide residues in food samples. Reverse phase Zorbax SB-C_8

column 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 program; 5 min a Reversed-phase HPLC, with UV detection, is a good alternative for herbicide determination because derivitization step is not required. A series of preliminary experiments were conducted in selecting conditions for the ultrasonication-assisted extraction step. The method validation studies for spiked samples indicated that the present method provides good recoveries and reasonable precision for aryloxyphenoxy of two levels (10 µg g^{-1} and 5 µgg^{-1}). The recoveries of pinoxaden herbicides are 90–97%. The results obtained for recoveries, precision, and accuracy show that the proposed extraction method (UE) followed by HPLC method is efficient, sensitive, reproducible methods for identification, determination and quantification of herbicides in environmental samples.

Table-1. Recoveries of pinoxaden herbicide from real samples

Real samples	Added amount (ug mL^{-1})	% Recovery
Wheat grain	5	97.00 ± 0.02
	10	94.00 ± 0.11
Barley grain	15	95.00 ± 0.01
Cabbage	5	95.00 ± 0.02
	10	98.00 ± 0.30
	15	98.00 ± 0.30

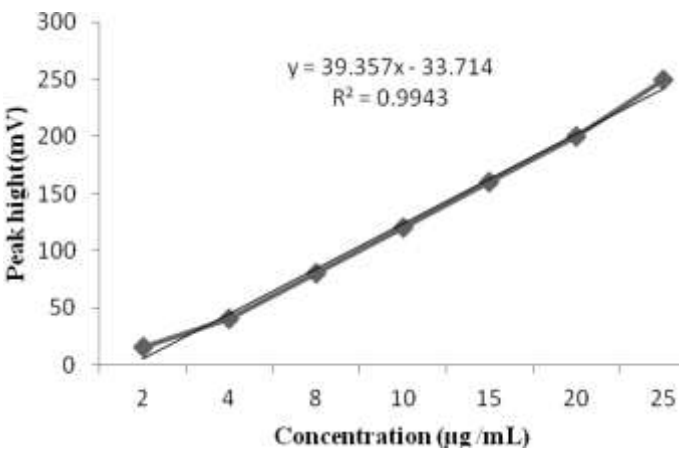

Figure 1. Calibration plot for the determination of pinoxaden herbicide

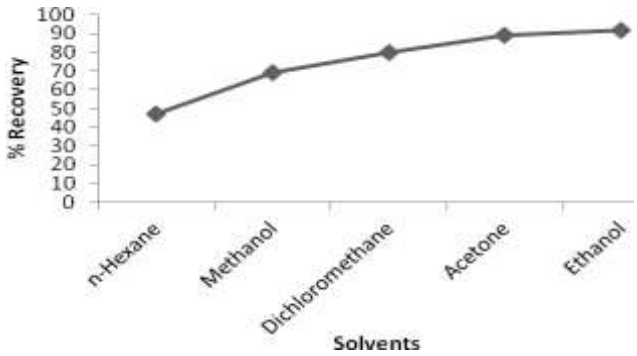

Figure 2. Investigation of suitable solvent system for the determination of pinoxaden herbicide by reverse-phase HPLC method

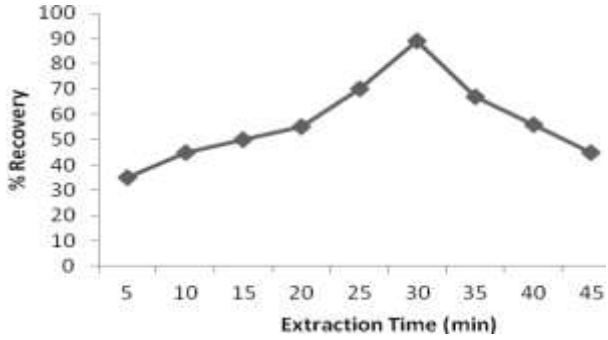

Figure 3. Effect of extraction time on percent recovery of pinoxaden herbicide

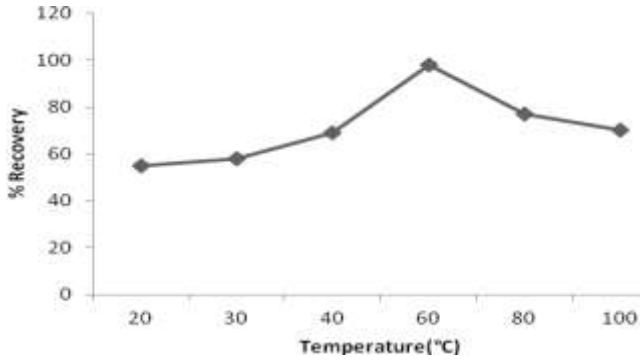

Figure 4. Investigation of suitable temperature for extraction and determination of pinoxaden herbicide by reverse-phase HPLC method

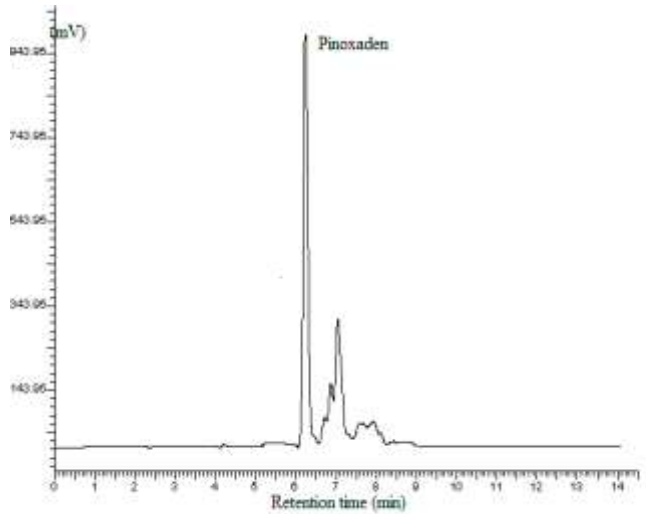

Figure 5. Chromatogram for standard Pinoxaden herbicide using reverse-phase HPLC

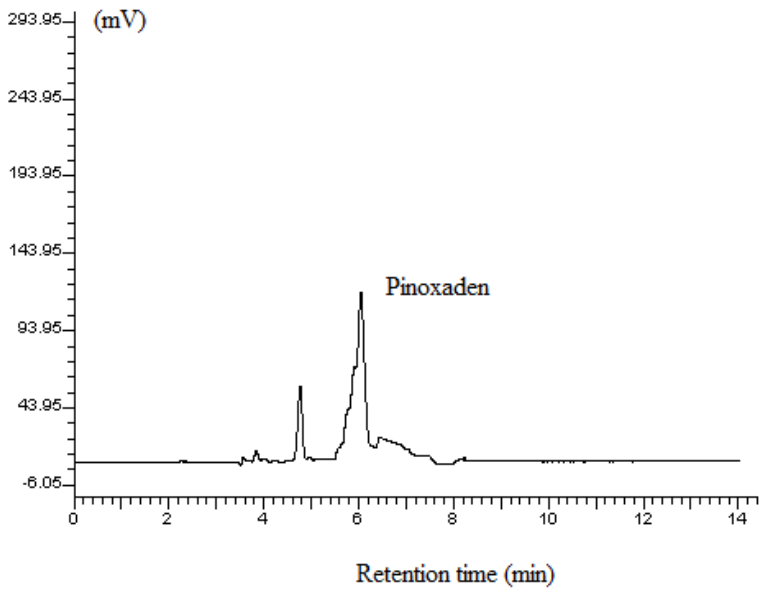

Figure 6. Chromatogram for pinoxaden for (5µg mL^{-1}) using reverse phase HPLC.

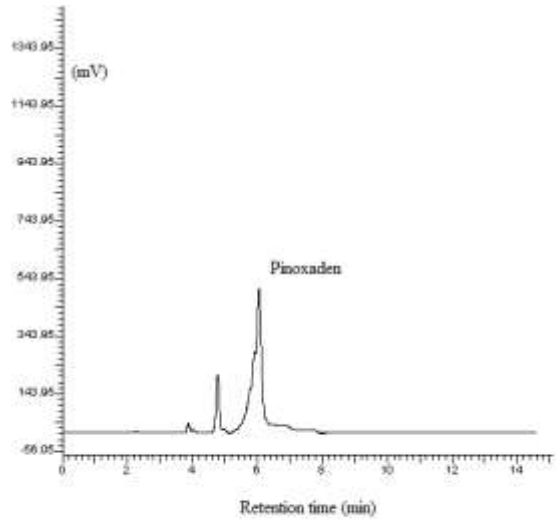

Figure 7. Chromatogram for pinoxaden fortified 15µg g^{-1} wheat samples using reverse-phase HPLC

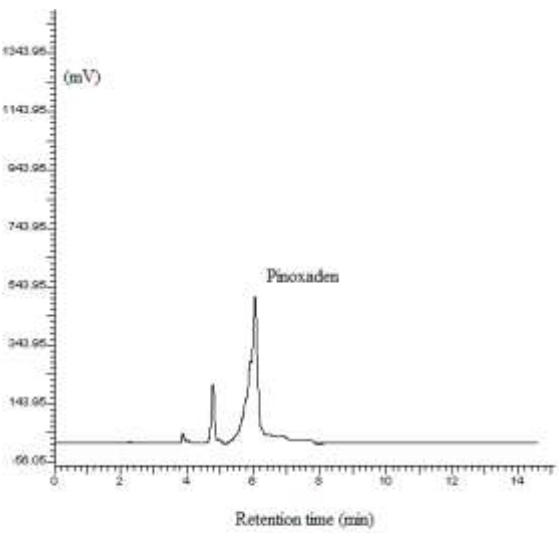

Figure 8. Chromatogram for pinoxaden fortified 15µg g^{-1} Barley samples using reverse-phase HPLC

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