Residual Determination of (Pyroxsulam 4.5%OD) in Iraqi Wheat and Field Soil Using QuEChERS / HPLC

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Abstract: ***Simple, accurate and sensitive method for determination the herbicide residues (Pyroxsulam 4.5% OD) in wheat cultivar (Iba 99) and cultivar (Abu Ghraib 3) and a field soil, a field trial was conducted during winter seasons of 2016-2017 at Abu Ghraib Research Station, the Agricultural Research Department, Ministry of Agriculture. A simple method QuEChERS includes extraction by acetonitrile for Pyroxsulam from (wheat, soil) after that clean up by dispersive solid phase extraction -with PSA, GCB and MgSO4. Using High performance liquid chromatography having UV/visible detector for the detection of herbicide residues. The two cultivars were similar in dissipation of pyroxsulam in wheat plant, where as the concentration rises to a higher value in fifth day with respect to cultivar (Abu Ghraib 3) and in fourth day with respect to cultivar (Iba 99), the average recovery percentage of Pyroxsulam for wheat plant, was 100.4% and 99.8% for field soil. The average of relative standard deviation percentage of Pyroxsulam for wheat plant was 0.02% and 0.04% for field soil. Residue of pyroxsulam was not detected in harvest samples of wheat grain and field soil. Pyroxsulam could be consider as a good herbicide of low –toxic and no residual effect on wheat and soil when used in the recommended dose and in optimal conditions in Iraq.***

Keywords: wheat, pyroxsulam, residue, QuChERS, HPLC

1. Introduction

Wheat is one of the most abundant sources of carbohydrates and protein for human being and animals, to achieve food security it is necessary to increase production [1]. Weeds are one of the most major reasons for the lower yields of crops in the country [2]. Weeds are strong competitors and concealed enemies of crops because of their abundant growth under different environmental conditions. Herbicides used to control weeds in different parts of agriculture, has many of benefits for example increase amount of food crops [3]. Several herbicides are used in Iraq to control weeds of the wheat such as Topic, Harmony, 2.4.D., Lintur, Chevalier, Granstar, Illoxan, Atlantis and Pallas. The herbicide used in this study is Pallas 45% OD the selective herbicide used to control of weeds in spring and winter wheat by ground or aerial application register in 2008 by Dow Agro Sciences LLC, its standard material **Pyroxsulam**, which is absorbed by the leaves and inhibits the formation of the enzyme Acetolactate Synthase Enzyme (ALS) thus preventing manufacturing amino acids and then stopped cell division and growth and then the death of the weeds.

The wrong use of herbicide leads to accumulation of residues in food crops and soil [4], the method of accumulation monitoring is important when herbicide go by different dissipation pathways in plants and in soil [5]. For this reason, this study was determinate herbicide residues in wheat plant and soil by using QuEChERS[6] with high performance liquid chromatography (HPLC). Using Simple, accurate and sensitive method for extraction Pyroxsulam from the wheat cultivars (Abu Ghraib 3) (Iba 99) and field soil, after that clean up by dispersive solid-phase extraction[7] with a different sorbent of (Primary Secondary Amine, anhydrous Magnesium Sulfate and Graphitized Carbon Black) for wheat plant, used C_{18} rather than GCB for clean up the soil.So as to better balance between benefits of pesticides in agriculture and risks to human being, environment and food, in this study determination of herbicide residues (Pyroxsulam 4.5%OD) in Iraqi wheat cultivar (Aba 99) and cultivar (Abu Ghraib 3) and a field soil using QuEChERS to extraction and clean up the pyroxsulam from wheat and soil, detection by HPLC.

2. Materials and Methods

Chemicals and apparatus 1-

All Chemicals and solvents were used in this study are HPLC- grad were of the highest purity and obtained from National Center for Pesticide Control (NCPC) /Ministry of Agriculture (Baghdad). which were described in following: pyroxsulam standard purity (96.5%) were obtained from DowAgro Sciences (USA); Acetonitrile purity (99.9%) from Labscan Ltd. (Ireland); Deionize water super purity solvent from Romil-SpS (Cambridge);Acetic acid glacial purity 99.5% from Scharlab S.L. (Spain); Anhydrous magnesium sulfate $MgSO_4$ from Sigma Chemical (U.S.A); Sodium chloride NaCl from Avantor (U.S.A). Primary secondary amine (PSA) (40-60 µm), Graphitized Carbon Black (GCB) (200-400 mesh) and C18 (50 µm, 60Aº) were from Restek Q-sep (U.S.A).

2-HPLCsystems

A Shimadzu LC-20AD (UFLC) Ultra Fast Liquid Chromatography, High performance liquid chromatography with UV/visible detector was used for determination of herbicide. Separation was performed on reversed phase Kromasil C_{18} column (4.0mm ×250 mm) with a 5µm particle size (Touzart & Matignon, France)The column was thermostated at 40°C. Mobile phase consisted of acetonitrile: water (90:10 v/v) as isocratic elution was pumped at flow rate of 0.5 mL min-1, injection volume was 5 µL, the wavelength of the UV/visible detector was fixed at 280 nm and the run time was 10 min per sample.

3-Field experiments

A field trial was conducted during winter seasons of 2016-2017 at Abu Ghraib Research Station, the Agricultural Research Department, Ministry of Agriculture. The experiment was laid out in Randomized Complet Block Desgin (RCBD) in three replicates, each experimental plot was 12 m^2 (3m×4m) for each plot and was separated by irrigation channels, soil was examined before the cultivation process in the laboratories of the soil department of Agricultural Research as the results were as follows: organic matter, 0.86%; pH 8; EC 4.27 $ds.m^{1-}$; texture, Clay Soil; sand 16%;silt 39%;clay 45%; and NPK (avail.) Nitrogen70.0 ppm; Potassium 592ppm; Phosphorus 24.16ppm. Manually planted the wheat seeds cultivar (Iba 99) and cultivar (Abu Ghraib 3) on the lines of distance between line and another 20 cm at a seed rate (120 $Kg.ha^{1-}$) in 2016/11/15. When the wheat at 2-3 leaf to jointing stage, were sprayed with Pallas herbicide at the applied dose was 125 ml/donum (Iraqi donum) in 2017/1/19. wheat plant and soil samples were randomly collected at about 2h, 1, 2, 3, 4, 5, 6, 7, 14, 30, 45, 60, and 90 d after spraying.

4-Standard preparation

For preparation of stock solution, were weighted 0.02 gm of pyroxsulam standard and dissolved in acetonitrile and was transferred to volumetric flask of 100 ml diluted to mark by using acetonitrile solvent to prepare 200 $\mu g.ml^{1-}$ stock standard, the stock solutions were then diluted with acetonitrile in order to obtained the standard solutions for spiking and calibration curve. The standard solutions were freshly prepared, filtered through 0.45 µm membrane filters, and kept at 4 C° in the dark.

5-Extraction and D-SPE cleaned up of Pyroxsulam residue in wheat

Homogenized samples of (5g of wheat plant, 2g of straw and5g of grain) were taken separately in 50 mL centrifuge tube, 10 mL acetonitrile containing 1% (v/v) of acetic acid were then added to the sample, subjected to vortex (REAX top, Heidolph–Germany) for 1 min. After added anhydrous MgSO4 (2 g) and NaCl (1 g) and vortexed for (1 min). Following that centrifugation (4K 15, Sigma –Germany) at 4000 rpm for (5 min), after that which provides a perfect physical separation of phases. For cleaned up using dispersive solid phase extraction (D-SPE), 1 mL of the supernatant was transferred into a 1.5 mL microcentrifuge vial containing 50 mg PSA sorbent;150 mg anhydrous MgSO4and 50 mg GCB, vortexed vigorously for 1 min, centrifuged (Micro ceterifuges, Hettich zentrifugen –Germany) for (3 min) at 6000 rpm, the acetonitrile layer was filtered through a 0.45 µm membrane filter (Cellulose acetate membrane filter, Chm-Spain) for injected to HPLC. Flow diagram of the QuEChERS method for wheat plant was summarized in below Figure (1). Added 1 mL of water to (straw, grain) samples in the extraction step, because this technique was used for samples with 75% wetness, and to be modified to dry samples such as wheat grains, for products with a water content lower than 25% like straw, spices and dried fruits, added water so as to make sample pores more easily to the extraction solvent [13-11].

6-Extraction and D-SPE cleaned up of pyroxsulam residue in Soil

Homogenized soil (5 g) was weighed into 50 mL centrifuge tubes. Samples were ultrasonically extracted (Ultra sonic bath, FS300, Decon-England) in a mixture of 10 mL acetonitrile, 2 mL water and 100 mL acetic acid, for 15 min. Anhydrous MgSO4 (2 g), NaCl (1 g) were added and vortexed for (1 min). After that centrifugation at 4000 rpm for (3 min). For clean up, 1mL of the supernatant was transferred into a 1.5 mL microcentrifuge tube (D-SPE tube) already containing, 50 mg PSA sorbent, 150 mg anhydrous $MgSO_4$ and 50 mg C18, vortexed vigorously for (1 min), following centrifuged at 6000 rpm for (3 min), the acetonitrile layer was filtered through a 0.45 µm membrane filter for analyzed by High Performance Liquid Chromatography (HPLC). Flow diagram of the QuEChERS method for soil was summarized in below Figure (2).

Extracting steps

Clean up steps

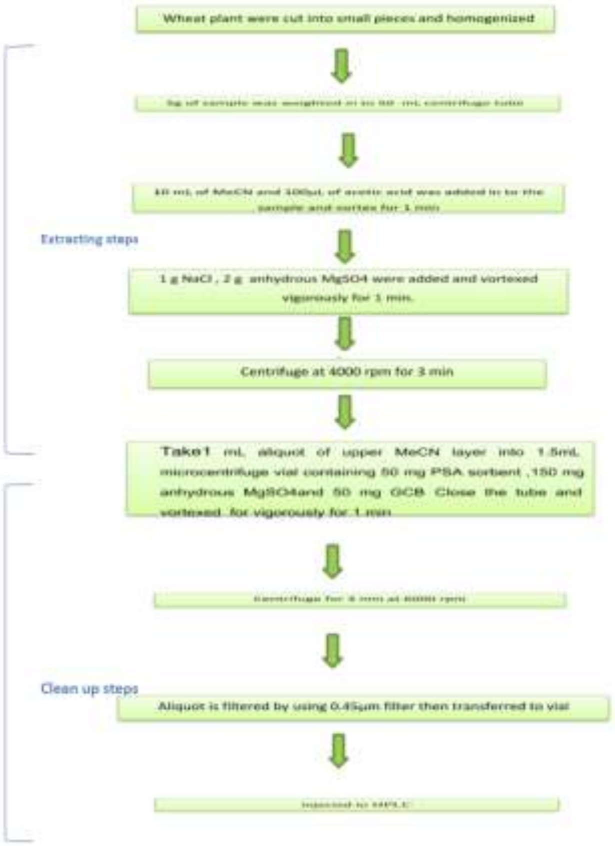


Figure 1: Flow-chart of QuEChERS method for wheat plant

Extraction steps

Clean up steps

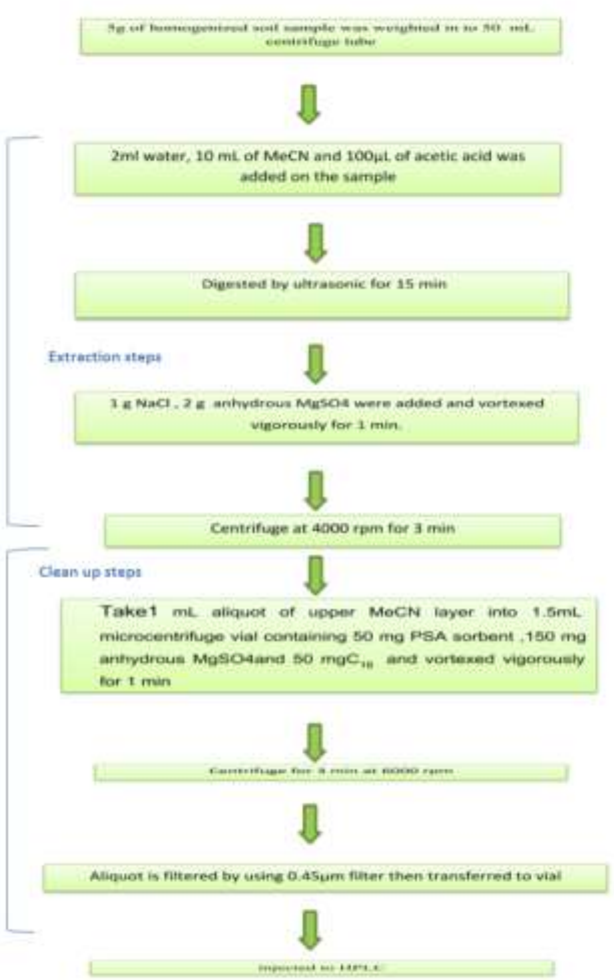


Figure 2: Flow-chart of QuEChERS method for soil sample

3. Results and Discussion

1-Linearity, detection limits and recovery:

In this study a calibration curves were prepared by taking the area corresponding to different concentrations of matrix match calibration standard of pyroxsulam (Fig. 3). A linearity check study was done with the help of matrix match calibration standard (Fig.1, 3), to know the interference of substrate.

Good linearity was observed in the studied range was from 0.03 µg.ml[1] to 200 µg.ml[1]with the determination coefficient R2 = 0.9995, limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were determined as 0.007 µg.ml^{-1} and 0.02 µg.ml^{-1} for pyroxsulam respectively.

Recovery studies were carried out to establish the validity of the analytical method and to know the efficiency of extraction and clean up steps employed in this study, by fortifying the wheat plant and field soil samples with different levels of the analytical standard solution. The standard solutions of Pyroxsulam was added to the untreated wheat plant levels of (20, 1, 0.02)µg.ml^{-1} and soil samples at levels of (25, 1, 0.08) µg.ml^{-1}. The fortified samples were analyzed using the procedure described with five repetitions, (Fig.4) shown the chromatograms obtained from (a) blank real sample, (b) real sample spiked with 1 µg.ml^{-1} (c) pyroxsulam standard solution. The results suggested that the average recoveries of Pyroxsulam in wheat plant was 100.4%, in soil was 99.8%, with the relative standard deviation (RSD) 0.024% and 0.043% respectively were listed in Table 1.

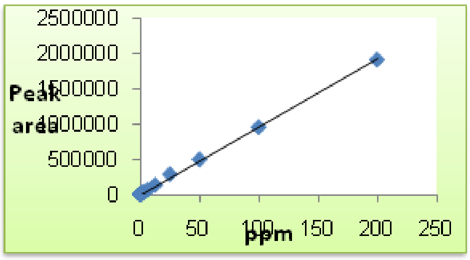
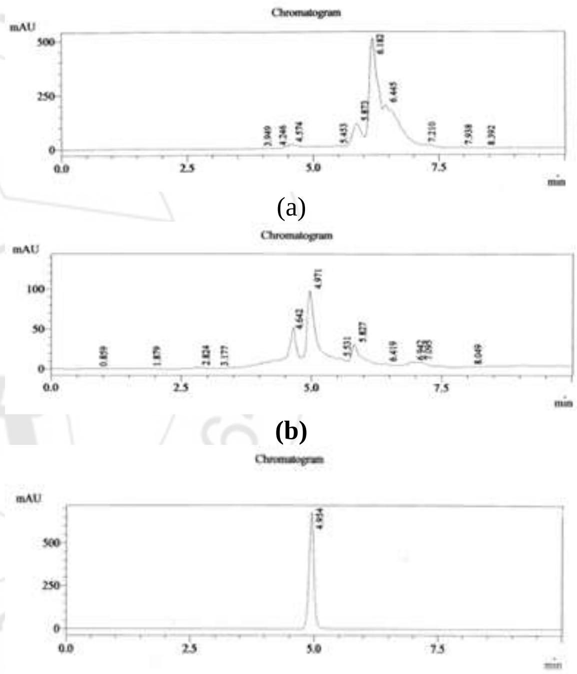


Figure 3: Calibration curve of pyroxulam from (0.03 to 200)



(a) (b) (c)

Figure 4: HPLC chromatogram of (a) blank real sample, (b) real sample spiked with 1 µg.ml^{-1} (c) pyroxsulam standard solution

Table 1: RSD and average recovery of Pyroxulam in samples spiked at different levels

Sample	Spiked levels (µg.ml^{-1})	Found (µg.ml^{-1})	Rec. (%)	RSD (%)
Wheat	20	19	101.8	0.004
	1	0.96	100.1	0.03
	0.02	0.019	99.5	0.004
Average			100.4	0.012
Soil	25	21	103.1	0.07
	1	0.82	96.7	0.04
	0.08	0.65	99.5	0.02
Average			99.8	0.043

2-Pesticide residues in wheat and field soil

The dissipation trends of pyroxsulam in wheat plant cultivar (Abu Ghraib 3) and cultivar (Iba 99) were shown as in figure (5, 6) respectively. The results showed that pyroxsulam dissipated in two cultivars rapidly after application. The initial concentration of pyroxsulam in the cultivar (Abu Ghraib 3) was 52 ppm, while the initial concentration of pyroxulam in the cultivar (Iba 99) was 64 ppm, the two cultivars were similar in dissipation of pyroxsulam in wheat plant. Whereas the concentration rises to a higher value in fifth day with respect to cultivar (Abu Ghraib 3) and in fourth day with respect to cultivar (Iba 99), after that, the pyroxsulam concentration was rapidly decreased to reached 0.01 in fourteenth day in two cultivars, then continued declined regularly to the harvest period, At after harvest intervals not detected pyroxsulam residue in wheat grain. The dissipation of pyroxsulam in field soil as shown in figure (7). The initial concentration of pyroxsulam in the soil at two hours after spraying was reached to the highest concentration, thereafter continued declined regularly to the harvest period, not detected of pyroxsulam residue in field soil after harvest intervals.

Figure 5: dissipation of pyroxsulam in wheat cultivar (Abu Ghraib 3)

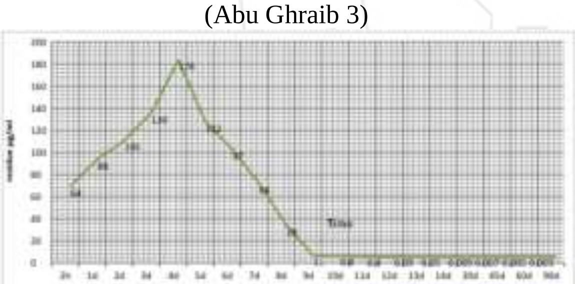


Figure 6: dissipation of pyroxsulam in wheat cultivar (Iba 99)

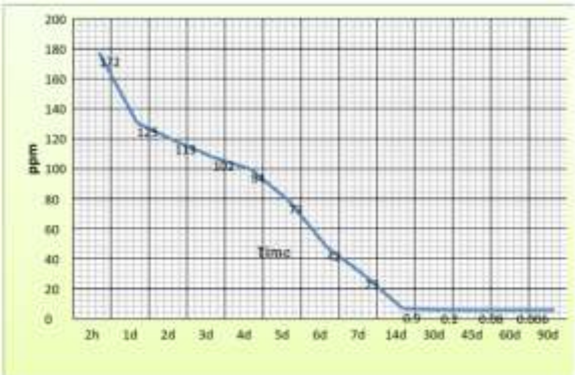


Figure 7: dissipation of pyroxsulam in soil field

4. Conclusions

The effect of herbicide Pallas (active ingredient pyroxsulam) was studied on two cultivars of Iraqi wheat and assessment the residual of herbicide in wheat plant and soil. The dispersion of pyroxsulam in the wheat plant for both cultivars Iba 99 and Abu Ghraib 3 is not very different, as the concentration gradually increases after spraying until it reaches its highest value and then decreases rapidly and fades. The concentration of Pyroxsulam in wheat and soil after harvest time less than detection limit when used at the recommended dose and in optimal conditions. No codex, European Union or United States of America MRLs value for pyroxsulam in wheat or other material were established, but found tolerances for pyroxsulam in wheat plant (0.001ppm) were awaiting in Canada and Australia [12] therefore, this work helps to spread MRLs for pyroxsulam in wheat plant to give information about fade and right use of these herbicides, Pyroxsulam could be consider as a good alternative to high-toxicity herbicides in Iraq.

References

[1] Devi, K.N., Singh M.S., Singh, N.G. and Athokpam, H.S.2011.Effect of integrated nutrient management on growth and yield of wheat (Triticum aestivum L.). J. Crop weed 7:23-27.
[2] Khatam, A., W. Ahmad and M.Z. Khan. 2012. Perception of farmers regarding effect of various weed management practices in onion crop. Pak. J. Weed Sci. Res. 18 (4): 553-559.
[3] Leo, M. and L. Nollet. 2004. Herbicide Residue, Handbook of food analysis 2nd Ed, Madrid, Spain. 1269.
[4] Jayashree R, and Vasudavan N (2007). Persistance and distribution of endosulfan under field condition. Environ. Monit assess. 131: 475-87.
[5] Koeppe, M.K., C.M. Hirata, H.M. Brown, W.H. Kenyon, and D.P. O'Keefe. 2000. "Basis of Selectivity of the Herbicide Rimsulfuron in Maize." Pesticide Biochemistry and Physiology 66 (3): 170_181. doi:10.1006/pest.1999.2470.
[6] M. Anastassiades, S.J. Lehotay, D. ˇ Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[7] K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1040 (2004) 259.
[8] Diez, C., Traag, W. A., Zommer, P., Marinero, P., & Atienza, J. (2006). Comparison of an acetonitrile extraction/partitioning and ''dispersive solid-phase extraction"method with classical multi-residue methods for the extraction of herbicide residues in barley samples. Journal of Chromatography A, 1131, 11–23
[9] Pizzutti, I. R., Kok, A., Zanella, R., Adaime, M. B., Hiemstra, M., Wickert, C., et al.(2007). Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid chromatography–tandem mass spectrometry usingpositive and negative electrospray ionization. Journal of Chromatography A, 1142, 123–136
[10] Walorczyk, S. (2007). Development of a multi-residue screening method for thedetermination of pesticides in cereals and dry animal feed using gaschromatography–triple quadrupole tandem mass spectrometry. Journal ofChromatography A, 1165, 200–212
[11] Walorczyk, S. (2008). Development of a multi-residue method for thedetermination of pesticides in cereals and dry animal feed using gaschromatography–tandem quadrupole mass spectrometry II. Improvement andextension analytes. Journal of Chromatography A, 1208, 202–214
[12] EPA. 2008. Pyroxsulam: Pesticide fact sheet. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances (7501C)