Analysis and Study on Residue of Florasulam in Wheat by Liquid Chromatography–Tandem Mass Spectrometry

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ABSTRACT: *To more accurately assess the risk of residues a quick accurate approach for finding florasulam pesticide residue in wheat was used. Herbicide residues in wheat samples were identified using the GC/MS-MS and QuEChERS sample preparation techniques. Gas chromatography-tandem mass spectrometry (GC-MS/MS) was used to identify the presence of florasulam residue in wheat. Pesticide in wheat was extracted, purified and concentrated. The linearity of the calibration curves was satisfied in matrix-matched standard solution with R2 = 0.99. Acceptable recoveries are in the range of 70-120%, with repeatability RSD ≤ 20%. The LOQ is at the lowest spike level of validation that meets this method performance acceptance criterion. The method has been successfully applied in pesticide analysis in wheat. It shows that spraying the pesticide florasulam on wheat is safe.*

KEYWORDS *- Herbicide, Wheat, Liquid Chromatography-Mass Spectrometry, Florasulam*

I. INTRODUCTION

Weed management appears to be as old as agriculture itself because weeds have been a challenge for man ever since he cultivated plants [1]. Weed control strategies are evolving in tandem with agricultural modernization. Chemical weed control has introduced a number of herbicides, and more target-specific compounds are being introduced with time. Weeds are causing massive output losses in the wheat harvest [2].

One of the most significant food crops worldwide is wheat. Weeds are responsible for yield losses in many of the major crops farmed worldwide. These yield losses are greater than the losses brought on by other agricultural pests [3]. A wide range of pests, illnesses, and weeds harm crops. Without adequate crop protection, losses for wheat can reach 50%, and they can be significantly greater for other crops [4].

A triazolopyrimidinesulfonanilide is florasulam. The molecular structure of florasulam is shown in figure 1.

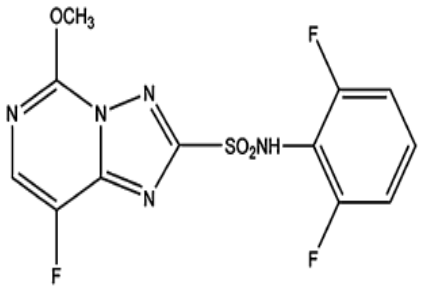

Figure1: Molecular structure of florasulam

Herbicide used to manage broadleaf weeds after they have emerged. The plant enzyme acetolactate synthase is inhibited by florasulam. It is absorbed by the roots and shoots of plants and transported through the xylem and phloem. For the detection of florasulam in plant, fruit, vegetables, and grains, researchers created the liquid chromatography-tandem quadrupole mass spectrometry (LC-MS/MS) multi-residue approach [5], [6].

As a result the usage of herbicides for wheat is required. Florasulam is a herbicide containing triazolopyrimidinesulfonanilide. It is a pesticide employed in cereals to combat broadleaf weeds [7], [8], [9].

After the use of pesticides, pesticide residues are an inevitable phenomenon. Pesticide residues may become hazardous and cause harmful consequences on people, animals, and the ecosystem if the maximum residual limit (MRL) is surpassed. MRLs of 0.01 mg/kg for florasulam were also set for wheat.

Herbicide application and the lingering effects of its residues in the environment have increased concerns about environmental pollution, food safety, human health, and soil and water contamination. To accurately analyze the impacts of herbicides on the environment and to ensure the safe use of these chemicals for the following rotation of delicate crops, it is crucial to comprehend the persistence and degradation of herbicides in soil [10].

Studies on residue screening and food intake assessment involving herbicides in wheat and other cereal crops needs to be carried out in order to ensure food safety. There have been some published multiresidue analytical techniques for pesticides, notably florasulam. By using UHPLC-MS and solid-phase extraction (SPE) with C18, [11] established a method to identify triazolopyrimidine herbicides in soil, water, and wheat (plant and grain). A technique was developed to locate monosulfuron-ester residues in wheat grains, straw, green plants, and soil using LC-MS/MS [12] and modified QuEChERS with C18.

Name and formulas of fluorosulam compound were listed in table 1.

Table 1: The name and formulas of florasulam

Compound	Molecular formula	Molecular weight	IUPAC
Florasulam	$C_{12}H_8F_3N_5O_3S$	359.29	N-(2,6-Difluorophenyl)-8-fluoro-5-methoxy-[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

Widespread use and high toxicity of florasulam have necessitated the use of a selective, sensitive, and quick approach for this herbicide's detection [13]. Numerous pesticides, including florasulam and pyroxsulam, were examined in surface water samples by [14], [15] using LC-Orbitrap-MS. In theirexamined the residues of seven triazolopyrimidinesulphonamide herbicides, including florasulam and pyroxsulam, in samples of rice, wheat, corn, soybeans, and soil.

There are many methods for determining florasulam in the literature, including LC-MS/MS, spectrophotometry, HPLC, chromatography, and GC-MS/MS. Most of these methods are either weakened by the instability of the reagent and the instrumental system or require extensive sample pretreatment. When compared to these approaches, LC-MS/MS technologies have a number of benefits, including the analytical processes' simplification and time and money savings [16].

Additionally, many methods for the residue analysis of florasulam field experiments have been reported. Using the QuEChERS method in conjunction with HPLC-MS/MS,investigated the residue and dissipation of florasulam in wheat (plant, grain, and straw) and soil under field settings. In a related work, [17] used rapid resolution liquid chromatography (RRLC) and MS/MS to examine the dissipation kinetics and residual of florasulam (together with tribenuron-methyl) in wheat matrices (plant, grain, and straw) and soil. The residue and dissipation of florasulam in wheat and soil were determined by high performance liquid chromatography–tandem mass spectrometry.

The amount of florasulam residues in wheat grain, wheat straw, and soil was less than the detection limit, which is 0.01 mg/kg, the maximum amount of florasulam residue. These findings would be valuable in establishing florasulam MRL guidelines for wheat [18].

The QuEChERS (rapid, easy, cheap, efficient, rugged and safe) approach is described in this publication for identifying florasulam residues in a wheat field sample. In order to give scientific support for tracking florasulam residue content in environmental, crop, and food samples, the used approach has been put to use on actual samples. In order to simultaneously determine the presence of florasulam in wheat a simple and efficient approach based on QuEChERS in conjunction with LC-MS/MS was adopted in this investigation.

II. MATERIALS AND METHODS

Florasulam standard was purchased from Sigma- Aldrich. QuEChERS extraction salts (6 g MgSO4, 1.5 g NaOAc, 50 ml) and

QuEChERSdSPE (1200 mg MgSO4, 400 mg PSA, 400 mg C18, 15 ml) and formic acid, methanol, ammonium formate were obtained from Sigma- Aldrich. The extraction solvents, acetonitrile (ACN) and acetic acid were of an LC gradient and purchased from Sigma- Aldrich. Pesticide stock standard solutions were prepared at 1,000 µg/ml in ACN with 1% acetic acid and used after dilution to the desired concentration. Wheat samples were obtained from fields grown under control.All standard solutions were stored at -20°C before use. Thermo-TSQ Altis LC-MS instrument was used for all the analyses. AccucoreaQ Dim.(mm)100*2.1 (2.0 µm) with 20 µL of injection volume was used for the separation at 30°C. Ammonium formate-water with 0.1% formic acid (v/v) and ammonium formate-methanolwith 0.1% formic acid (v/v) made up the mobile phase, and the flow rate was 0.5 mL/min. The triple quadrupole MS/MS detection was performed using the Positive Electrospray Ionization (ESI +) mode and Multiple Reaction Monitoring (MRM). The ion source temperature was set at 300°C, and the drying gas flow rate was 10.0 L/min. The MRM parameters for florasulam on LC MS/MS are listed in Table 2.

Table2: MRM parameters of florasulam on LC-MS/MS

Analyte	Florasulam
Electrospray ionisation	ES(+)
Q_1 (m/z)	360.06
Q_3 (m/z)	109.1 129.1
Collision energy (V)	59 29

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method the extracts are analyzed by mass spectrometry (MS) techniques after a chromatographic analytical separation.

In brief, a well-ground wheat sample along with 1 mL of 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous MgSO4/NaOAc (4/1, w/w) per g sample are added to a centrifuge tube or bottle, which is shaken and centrifuged. A portion of the MeCN extract (upper layer) is added to anhydrous $MgSO_4$/PSA sorbent (3/1, w/w; 200 mg per 1 mL extract), mixed, and centrifuged. This final extract is transferred to autosampler vials for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) to identify and determine a wide range of pesticide residues.

Table3: Compositionandproportions of mobile phase

Retention time (min)	Flow(ml/ min)	A %	B %	Cur ve
0	0.5	80	20	5
0.1	0.5	80	20	5
1.3	0.5	30	70	5
5.5	0.5	5	95	5
7	0.5	5	95	5
7.01	0.5	80	20	5
9	0.5	80	20	5

Mobil A: 5 mMAmmoniumformate0.1% formicacid in water, Mobil B: 5 mMAmmoniumformate 0.1% formicacid in methanol

Calibration curve was plotted for concentration of the injected area and the curve was found to be linear with good correlation coefficient (R2 = 0.99). Precision values expressed as relative standard deviation (RSD) were $\leq$ 20% irrespective of sample type.The calibration chart of standard concentrations for Florasulam is shown in figure 2.

Table4: Data of florasulamfromwheatgrainsamples.

Standard solution concentrations (µg/L)	Amount found (µg/kg)	Amount found %
5	4.537	90.74
10	10.504	105.04
25	26.721	106.884
50	49.974	99.948
100	96.462	96.462
200	201.994	100.997

When Table 4 was examined, a decrease was seen in the herbicide values in wheat depending on the standard solution concentrations.

Table5: Residueandothervalues of florasulamobtainedfromwheatgrainsamples

Amounts found for florasulam (mg/kg)	Retention time (min)	Qualitative ion (m/z)	Response
4.537	2.20	129.146	350233
	2.15	109.152	391720
10.504	2.24	129.146	137028
	2.24	109.152	137941

26.721	2.23	129.146	355103
	2.24	109.152	329813
49.974	2.21	129.146	655496
	2.23	109.152	630277
96.462	2.23	129.146	121255
	2.21	109.152	118955
201.994	2.23	129.146	226205
	2.23	109.152	213623

When Table 5 was examined, the relationship between amounts found for florasulam, retention time, qualitative ion, response values was shown.

III. RESULTS AND DISCUSSIONS

The QuEChERS has been used in residue analysis all over the world.In this study, QuEChERS, a fast and simple residue extraction method, was used for the determination of florasulam [19]. The detection of herbicide residues in food products is now often done using GC/LCMS and GC/LCMS-MS[20].The extraction techniques for wheat grain satisfied the recovery and RSD standards (70%–120% with RSD 20%).

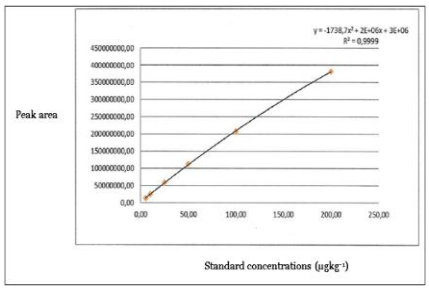

Figure2: Calibration curves of standard concentrations for florasulam

For the cleanup of florasulam in wheat grain (5 g),discovered that 50 mg of PSA with 150 mg of anhydrous MgSO4 was effective, with recovery. In this investigation, 50 mg of PSA was applied to wheat grain, and good florasulam recoveries were attained.

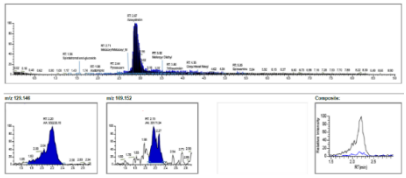

Figure 3: General chromatogram formed in LC-MS/MS device in wheat grain sample residue analysis

The florasulam study was carried out in MRM mode, and one of the targets showed equivalent ionization in both positive and negative modes. The ESI in positive mode was used in this investigation because the results showed that it elicited stronger reactions than ESI in negative mode.

The relationship between the peak area of the quantification ion and the concentration was evaluated for the range of the analyte from 5 to 200 µg/L. At six different concentration levels (5, 10, 25, 50, 100 and 200 µg/L), final extracts of all the blank matrices, including wheat grains were used to create matrix-matched calibration standards. Acetonitrile was used to create neat calibration standards [21]. Good linearity connections between peak area and florasulam concentrations were demonstrated by the results in Figure 1. the correlation coefficient (R2) was all greater than 0.9966 in wheat grain in the range of 0.005-0.2 mg/L.This investigation shown that florasulam rapidly decomposed in wheat, resulting residues.

Triazolopyrimidinesulfonanilide herbicide florasulam is used to control broadleaf weeds in cereal, but it's possible that it will eventually be approved for usage in other cereal crops as well. The validated method was used to find the florasulam residues. Wheat grain samples from the market were not obtained for the method verification because with this method, it was detected in real wheat grain samples obtained from field trials. Results revealed that all analyte residue levels were below matching LOQ and MRL indicating[22].

IV. CONCLUSION

Herbicides have been identified as an indispensable part of the crop production programme. While using herbicides, all the prevention and management aspects should be kept in mind for huge harvest as well as for quality food production without deteriorating the environment. Hence, integrating the mechanical and cultural management practices with herbicides for managing weeds is a viable option.

Florasulam in wheat grain was quantified using QuEChERS and LC-MS/MS using a sensitive and very straightforward residue analysis approach. The method for determining the pesticide in wheat grain was suc-cessfully applied, and average recoveries were 70-120%with RSD ≤ 20%. For the purpose of suppressing weeds in wheat fields, it may be regarded safe to apply a formulation of 8% oil suspension (1.42% florasulam) at the recommended dosage.

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