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Development and Validation of a QuEChERS-LC-MS/MS Multiresidue Method for 35 Herbicides in Wheat and Flour: Implications for Food Safety Monitoring

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ABSTRACT

The foundation of each nation's economy and social structure, agriculture is essential to its entire development. The role of agricultural products has recently become key due to wars, persistent population growth, and significant food deficits in some essential crops. There is a global food crisis right now, with over 60% of its strategic food requirements expected to go unmet. Grown worldwide, cereals such as wheat, corn, and rice are staple food crops rich in fiber, vitamins, and minerals. Therefore, controlling weeds can help increase agricultural production and help satisfy the growing demand for food. One type of pesticide used to control weeds and increase crop yields is called an herbicide. Detecting pesticide residues on food, especially cereals, is therefore essential. To detect herbicides in some cereals at lower concentrations by direct injection into LC-MS/MS without the need for additional, more expensive analytical processes, this work examines, optimizes, and validates the QuEChERS approach. With relative standard deviations of less than 13% and recovery rates ranging from 75% to 116% for the majority of the compounds tested using the LC-MS/MS approach by ESI$^+$, with a total runtime of 16 minutes.

Keywords: QuEChERS, LC-MS/MS, agriculture, herbicides, wheat.

INTRODUCTION

Due to the ongoing global population growth, the use of pesticides in modern agriculture has become essential for supplying society's food needs. Millions of tons of pesticides are applied globally each year in this manner Rani (2012) Pesticides are among the most hazardous substances that harm people, animals, and surface water in particular, despite their utility (Mcknight et al., 2015). Pesticides have the potential to negatively impact the environment and human health when used in excessive amounts (Poulier et al., 2014; Mohdly et al., 2025; Sharawi et al., 2025). Any undesired plants that proliferate in a field and endanger the health of crops, animals, or people are considered weeds. Herbicides are a group of pesticides that eliminate weeds that threaten plants and increase agricultural productivity (European Commission, 2024). In agriculture, herbicides are widely used. Almost 70% of all agricultural pesticides used globally are used on farms (Van den Brink and Mann, 2011). In the world, the top ten countries, which utilize pesticides, are China, the US, Argentina, Thailand, Brazil, Italy, France,

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Canada, Japan, and India (Worldatlas, 2018). Worldwide, pesticide use is estimated to be 2 million tons, of which 47.5% are herbicides (Carvalho, 2017). Fifty-nine percentage of people in Africa make their living from agriculture, which makes up a large portion of the continent's economy (Abate et al., 2000). Additionally, the African continent has the lowest rate of pesticide usage worldwide and contributes between 2% and 4% of the global pesticide market share (Abate et al., 2000). The growing population was predicted to lead to a sharp increase in food demand over the next three decades, as well as a rise in the need for fungicides, herbicides, and pesticides (Snyder et al., 2015). Herbicide analysis is most effectively done using Multi-residue methods, both in terms of cost and turnaround time. Most techniques involve several steps and employ a wide variety of chemicals and solvents. The QuEChERS method, in conjunction with liquid chromatography-tandem mass spectrometry (LC-MS/MS), was found to be the best combination for identifying herbicides in specific foods due to its strong recovery, rapid analysis time, low cost, and safety. The increased ionization of herbicides has led to the widespread use of LC-MS/MS (Wang et al., 2011; Yang et al., 2013; Raina-Fulton, 2014; Santilio et al., 2014). For the safety of consumers, it is crucial to monitor and establish maximum residue limits (MRLs) to regulate herbicide residues in food products. In compliance with food residue levels that are safe for consumers, the Codex Alimentarius Commission (CAC) and the European Commission (Wang et al., 2011) set MRLs. The MRL of 0.01 mg/kg is the lowest limit of analytical quantitation (LOQ) in EU legislation, as it is considered safe for consumers (FAO, Codex, 2019) if the MRL is obtained after multiple experiments. Bi et al. 2020 (EU Pesticides report, 2018) developed and confirmed a QuEChERS method implementing liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine florasulam and pyroxsulam residues in wheat grain and straw. The process was tried with oat, millet, corn, and rice cereals. RSDs varied from 2 to 15%, while average recoveries ranged from 76 to 113%. Guo et al. (2019) developed a reliable technique for figuring out the different phenoxy acid pesticide residues in cereals. Using a QuEChERS technique in conjunction with HPLC-MS/MS, the research of phenoxy acid herbicides in rice, corn, and wheat was optimized. Wheat is one of the world’s most important staple crops, cultivated widely due to its high-quality, versatile flour, adaptability to temperate climates with short growing seasons, and high yield per unit area (World Population Review, 2024). It ranks second after maize among cereal crops and is the most dominant (FAO, 2023), international trade volumes. Beyond its central role in producing bread, pasta, cereals, and pastries, wheat is strategically significant because it suffers from major diseases-particularly rusts (yellow, stem, and leaf rust) and insect pests that threaten productivity (Abou_zeid et al., 2025, Mohamed et al., 2026). In Egypt, wheat represents the cornerstone of national food security, with around 3.5 million feddans cultivated annually, yielding 18-20 million tons in total (about 2.7-3 tons per feddan) (USDA, 2024). As the primary ingredient in traditional Egyptian bread, wheat holds unparalleled social and economic value, making the sustainable production and protection of wheat from diseases and pests a national priority.

The present study applies QuEChERS technology to extract a mixture of herbicides and subsequently evaluate that mixture across different matrices. Specifically, a QuEChERS procedure that removes the need for cleanup or acid treatment was assessed, optimized, and validated to enable the determination of 35 herbicides in wheat and flour at lower concentrations through direct injection into LC-MS/MS. Identification of these compounds was carried out using electrospray positive ionization (ESI+) with an Exion HPLC coupled to a SciexQtrap API 6500+ LC-MS/MS System.

MATERIAL AND METHODS

Analysis and instrumentation

(a) LC-MS/MS analysis was performed using an ExionLC AC system coupled to a

Qtrap API 6500+ MS/MS detector (AB Sciex, USA).

(b) Chromatographic separation was achieved on an InfinityLab Poroshell 120 EC-C18 column (3.0 × 50 mm, 2.7 µm; Agilent, USA).

The column temperature was maintained at 40°C, and a 2 µL aliquot was injected for each run. The 35 herbicides were separated using a gradient elution program with 10% 50 mM ammonium formate in deionized water as the LC mobile phase. Eluent A consisted of 0.1% formic acid, while methanol served as eluent B, delivered at a flow rate of 300 µL/min. The gradient began with the A bottle at 60% for 1 min, followed by a continuous change until 11.5 min; it then shifted progressively to 12 min, reached 0% for 2 min, and subsequently returned to 60% A at min 14 for an additional 2 min, resulting in a total run time of 16 min. MS/MS detection was conducted in multiple reaction monitoring (MRM) mode using electrospray ionization in positive ion mode.

The LC mobile phase stock solution was prepared by combining a 50 mM ammonium formate solution in methanol/water (1:9), 1.73 mL formic acid added to 900 mL of water, 33% ammonia solution to adjust the pH to 3.78 ± 0.02, and 100 mL of methanol. Following dilution of 200 mL of this stock solution with 800 mL of methanol/water (1:9), the pH was required to be 4 ± 0.1. The final LC mobile phase consisted of a 10 mM ammonium formate solution in methanol/water (1:9).

Reagents and materials

HPLC-grade methanol (99.9%) was obtained from J.T. Baker (PA, USA), and HPLC-grade acetonitrile (99.9%) was sourced from J.T. Baker (Pennsylvania, USA). Deionized water (<18 M_cm resistivity) was produced using a Millipore (Billerica, MA, USA) MilliQ water purification system. A 33% ammonia solution was purchased from Riedel (Seelze, Germany).

Formic acid (98–100%) was obtained from Riedel (Seelze, Germany). QuEChERS extraction kits were purchased from Agilent, with QuEChERS salts and buffers supplied as prepackaged anhydrous formulations containing 1 g trisodium citrate dihydrate, 0.5 g disodium citrate sesquihydrate, 4 g $MgSO_4$, and 1 g NaCl (Santa Clara, CA, USA). Ametryn (99%), Amidosulfuron (98.5%), Azimsulfuron (98.5%), Beflubutamid (99.4%), Butachlor (99%), Carbetamide (96.7%), Chlorbromuron (98.3%), Chlorbufam (98%), Chloridazon (99%), Chloroxuron (99%), Chlorsulfuron (99%), Cinosulfuron (97.5%), Clethodim (96.9%), Clomazone (97.5%), Cyhalofop-butyl (98%), Dimethachlor (98.8%), Dimethenamid (98%), Diuron (98.7%), Florasulam (99.4%), Fluazifop-P-butyl (94.8%), Flufenacet (99%), Fluroxypyr (99%), Foramsulfuron (98.9%), Halosulfuron-methyl (98%), Mefenpyr-diethyl (99%), Metazachlor (98%), Methabenzthiazuron (97.9%), Oxadiargyl (99.2%), Oxadiazon (99%), Oxasulfuron (99.3%), Propachlor (99%), Simetryn (98.5%), Thifensulfuron-methyl (99.4%), Tralkoxydim (99.8%) and Trietazine (99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Standard preparation

To prepare pesticide stock solutions, individual standards were formulated at 1000 µg/mL. Ametryn was dissolved in toluene; Amidosulfuron in acetonitrile; Azimsulfuron in toluene/acetone (7:3 v/v); Beflubutamid in toluene; Butachlor in toluene; Carbetamide in toluene; Chlorbromuron in toluene/acetone (9:1 v/v); Chlorbufam in toluene; Chloridazon in toluene/methanol (8:2 v/v); Chloroxuron in toluene; Chlorsulfuron in toluene; Cinosulfuron in toluene/acetone (8:2 v/v); Clethodim in toluene; Clomazone in toluene/methanol (8:2 v/v); Cyhalofop-butyl in toluene; Dimethachlor in toluene; Dimethenamid in toluene; Diuron in toluene/methanol (9:1 v/v); Florasulam in toluene/acetone (9:1 v/v); Fluazifop-P-butyl in toluene; Flufenacet in toluene; Fluroxypyr in toluene/methanol (7:3 v/v); Foramsulfuron in toluene/methanol (1:1 v/v); Halosulfuron-methyl in toluene; Mefenpyr-diethyl in toluene; Metazachlor in toluene; Methabenzthiazuron in toluene; Oxadiargyl in toluene; Oxadiazon in toluene/acetone (7:3 v/v); Oxasulfuron in toluene/acetone (8:2 v/v); Propachlor in toluene; Simetryn in toluene; Thifensulfuron-methyl in methanol; Tralkoxydim in toluene; and Trietazine in toluene. All stock solutions were prepared and stored at −20 ± 2°C. Working mixtures of the investigated pesticides (5 µg/mL each) and calibration mixtures at 0.001, 0.002, 0.005, 0.01, 0.05, and 0.1 µg/mL were then prepared by diluting appropriate aliquots of the stock solutions with methanol and storing the diluted solutions at 4 ± 2°C.

Spiked Samples preparation

The wheat and flour were bought from the neighborhood market. The samples were homogenized in an electric mill after being finely ground. Wheat and flour samples were spiked with an appropriate volume of working mixture standard solution for recovery trials.

Extraction procedure

Herbicide residues were extracted from wheat and flour using the QuEChERS technique and then analyzed. The first single-phase extraction process involved homogenizing 2 g of material in a 50 mL polytetrafluoroethylene (PFTE) centrifuge tube, adding 10 mL of deionized water, closing the tube, and vigorously shaking it for one minute at 500 rpm using a geno grinder. The second phase involved adding 10 mL of acetonitrile to the same tube, sealing it, and vigorously shaking it for 1 minute at 500 rpm using a Geno Grinder. Next, the tube is filled with a mixture of Agilent QuEChERs salts and buffers. It is then quickly shaken for one minute at 500 rpm using a bio grinder, and centrifuged for five minutes at 4000 rpm (3430 rcf). After centrifugation, the cleaned extract is filtered through syringe filters (0.45 µm) and placed into a Polypropylene (PP) vial. Ultimately, the liquid sample was introduced into an LC–MS/MS apparatus.

Estimation of Measurement Uncertainty and Data Visualization

Measurement uncertainty at the LOQ level (0.01 mg/kg) was estimated for each analyte in both wheat and flour matrices based on the principles outlined in the European guidance document SANTE/2020/12830 Rev.2 (European Commission, 2015). The expanded uncertainty (U, k=2) was calculated by combining two main components: the uncertainty due to precision (calculated from %RSD at LOQ) and the uncertainty due to bias (derived from the deviation of recovery from 100%).

(Calculated from %RSD at LOQ) =

$$\sqrt{2(Recovery - 100|}/100) + (RSD/100)2; n$$

Visual representations of method performance were created using the Python programming language (version 3.9) and libraries including Matplotlib, Seaborn, and Pandas. A heatmap of recovery percentages was generated for 35 herbicides across three spiking levels (0.01, 0.05, 0.1 mg/kg) in wheat and flour. Boxplots of relative standard deviation (%RSD) were also created to illustrate the distribution of precision at each level and in each matrix.

RESULTS AND DISCUSSION

The developed analytical method successfully identified and quantified 35 herbicides in a single run using the QuEChERS–LC–MS/MS approach. The selection of target herbicides was based on their registration by local authorities for crop protection and their frequent reporting in the literature. Acidification with citrate buffer salts (trisodium citrate dihydrate and disodium citrate sesquihydrate) enhanced recovery by converting specific herbicide conjugates into their neutral forms and adjusting the pH to 5-5.5, thereby increasing sensitivity. Under the optimized conditions, electrospray ionization in positive mode (ESI+) coupled with multiple reaction monitoring (MRM) provided highly selective detection, as all analytes were ionized into the (M+H)+ form. Each herbicide exhibited a unique retention time within a 16-minute run. The corresponding chromatograms demonstrated high accuracy and precision (Figure 1). A methanol mixture containing the 35 herbicides at 0.1 µg/mL was injected into the LC-MS/MS system to determine optimal precursor and product ions as well as collision energies and declustering potentials, the details of which are summarized in Table 1.

Method validation confirmed that the technique complied with SANTE/2020/12830 guidelines. Calibration curves constructed across the concentration range of 0.001-0.10 µg/mL demonstrated excellent linearity, with correlation coefficients (r^2) exceeding 0.99 for all analytes (Table 2). These findings confirm that the method is suitable for reliable quantitative analysis of multiple herbicide residues in wheat and flour, representative examples shown in Figure 2.

Table 1. List of herbicides and MRM parameters in LC-MSMS-ESI positive mode

No.	Acidic herbicides	Q1	DP	Q3	EP	CE	CXP
1	Ametryn	228	80	116	10		
2	Amidosulfuron	370.1	87	261	10	19	10
3	Azimsulfuron	425	62	156	10	47	8
4	Beflubutamid	356	97	162	10	39	24
5	Butachlor	312.3	42	162	10	33	10
6	Carbetamide	237.2	42	192.1	10	11	10
7	Chlorbromuron	293	62	182.1	10	21	30
8	Chlorbufam	224	77	153.9	10	25	8
9	Chloridazon	222	102	104	10	41	10
10	Chloroxuron	291	91	218	10	37	12
11	Chlorsulfuron	358	71	167	10	23	12
12	Cinosulfuron	414.1	72	157	10	33	6
13	Clethodim	360	115	268	10	20	10
14	Clomazone	240.1	57	89.1	10	73	6
15	Cyhalofop butyl	375	51	120	10	43	12
16	Dimethachlor	256	37	148	10	39	18
17	Dimethenamid	276.1	47	168.2	10	33	24
18	Diuron	233.001	76	72	10	33	4
19	Florasulam	360	106	129	10	29	20
20	Fluazifop p butyl	384.1	120	282.1	10	35	10
21	Flufenacet	364.1	47	194.1	10	17	8
22	Fluroxypyr	254.9	95	208.9	10	21	16
23	Foramsulfuron	453.1	71	182.2	10	27	7
24	Halosulfuron methyl	434.9	61	182	10	29	14
25	Mefenpyr diethyl	373	92	160	10	59	22
26	Metazachlor	278.3	22	134	10	29	8
27	Methabenzthiazuron	222.1	71	165.2	10	21	4
28	Oxadiargyl	340.8	172	151	10	33	10
29	Oxadiazon	345.3	172	303	10	17	20
30	Oxasulfuron	407	77	150	10	39	22
31	Propachlor	212.1	52	169.9	10	25	24
32	Simetryn	214	87	144	10	33	20
33	Thifensulfuron methyl	388	101	167	10	23	10
34	Tralkoxydim	330.3	112	138.1	10	29	8
35	Trietazine	230.1	66	99.1	10	33	7

Q1 = Precursor ion, Q3: Product ion, DP = Decluster Potential [V], EP = Entrance Potential [V], CE = Collision Energy [V] and CXP = Collision Cell Exit Potential [V].

Table 2. R2 values for the 35 herbicides

Herbicide	R^2	Herbicide	R^2
Ametryn	0.9934	Diuron	1.0000
Amidosulfuron	0.9980	Florasulam	0.9975
Azimsulfuron	0.9904	Fluazifop-P-butyl	0.9981
Beflubutamid	0.9998	Fluroxypyr	0.9998
Butachlor	1.0000	Foramsulfuron	0.9978
Carbetamide	1.0000	Halosulfuron-methyl	0.9959
Chlorbromuron	0.9999	Mefenpyr-diethyl	1.0000
Chlorbufam	0.9996	Metazachlor	0.9999
Chloridazon	0.9986	Methabenzthiazuron	0.9997
Chloroxuron	0.9999	Oxadiargyl	0.9999
Chlorsulfuron	0.9933	Oxadiazon	0.9999
Cinosulfuron	0.9987	Oxasulfuron	0.9987
Clethodim	0.9992	Propachlor	0.9999
Clomazone	0.9997	Simetryn	0.9985
Cyhalofop-butyl	0.9977	Thifensulfuron-methyl	0.9978
Dimethachlor	0.9996	Tralkoxydim	1.0000
Dimethenamid	0.9998	Trietazine	0.9944

To minimize matrix-induced signal suppression, 50 µL of a 0.5 µg/mL standard solution was spiked into 450 µL of a blank sample to achieve a final concentration of 0.05 µg/mL, following the approach reported by Kwon et al. (2012). This adjustment ensured more accurate recovery and quantification across the tested analytes. The method demonstrated a quantification limit (LOQ) of 0.01 mg/kg for all investigated herbicides, confirming its high sensitivity and suitability for detecting trace levels in wheat and flour matrices.

At the quantification limit (LOQ, 0.01 mg/kg), the expanded uncertainty for individual analytes ranged from 9.96% to 77.66% in wheat (mean 36.83%, median 33.95%) and from 12.49% to 79.32% in flour (mean 36.06%, median 30.61%). The uncertainty budget was primarily driven by bias, reflecting deviations from 100% recovery, with a more minor contribution from precision (RSD) at this low level. These magnitudes are typical of method performance near the LOQ and remain compatible with routine residue monitoring when interpreted alongside identification criteria and matrix-matched calibration (Tables 3 and 4). Precision improved systematically with concentration: RSD values, though modest at LOQ, decreased further at 0.05-0.10 mg/kg, and dispersion remained low in both matrices, with only a slightly broader spread at the LOQ. Notably, the absence of significant outliers at higher levels confirmed stable instrument response and reproducible sample handling, reinforcing the method’s suitability for confirmatory quantitation.

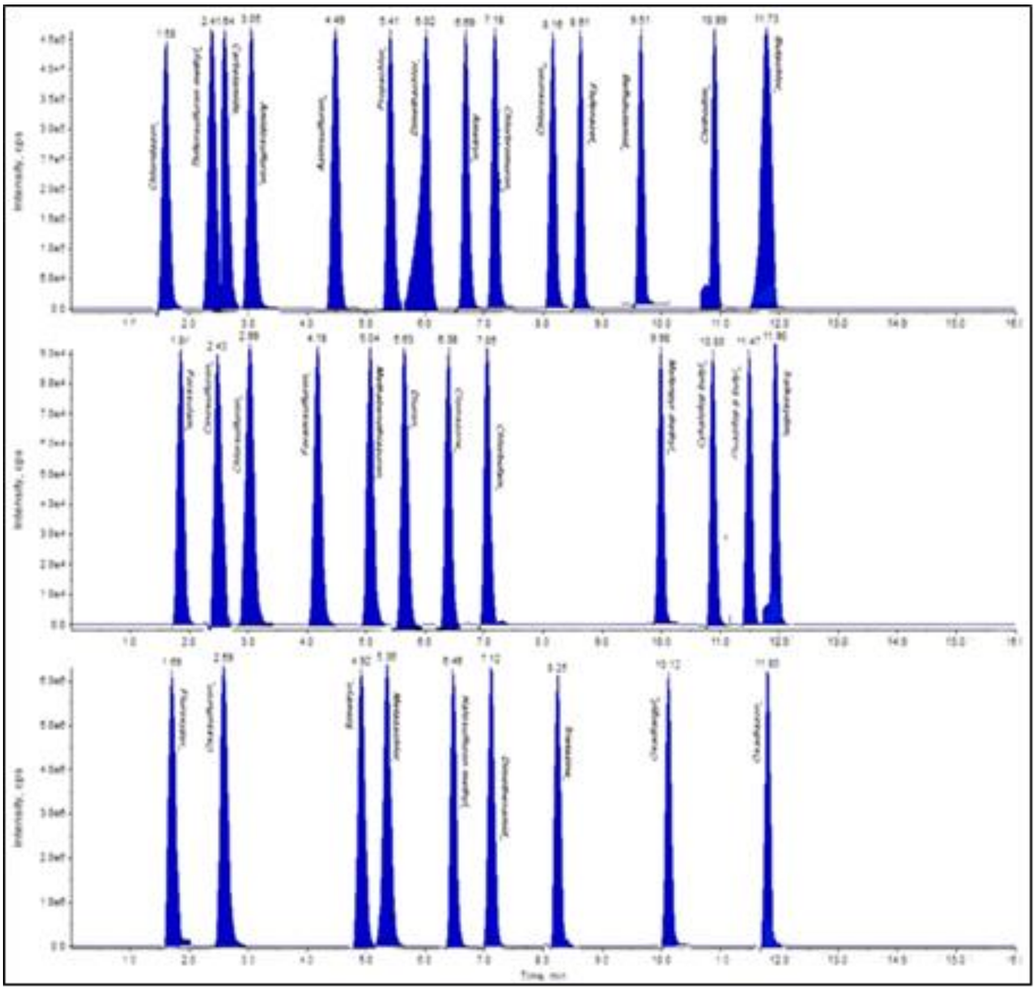

Figure 1. Chromatograms from liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) in both MRM and positive mode for 35 herbicides chosen for the study were used to validate the methodology

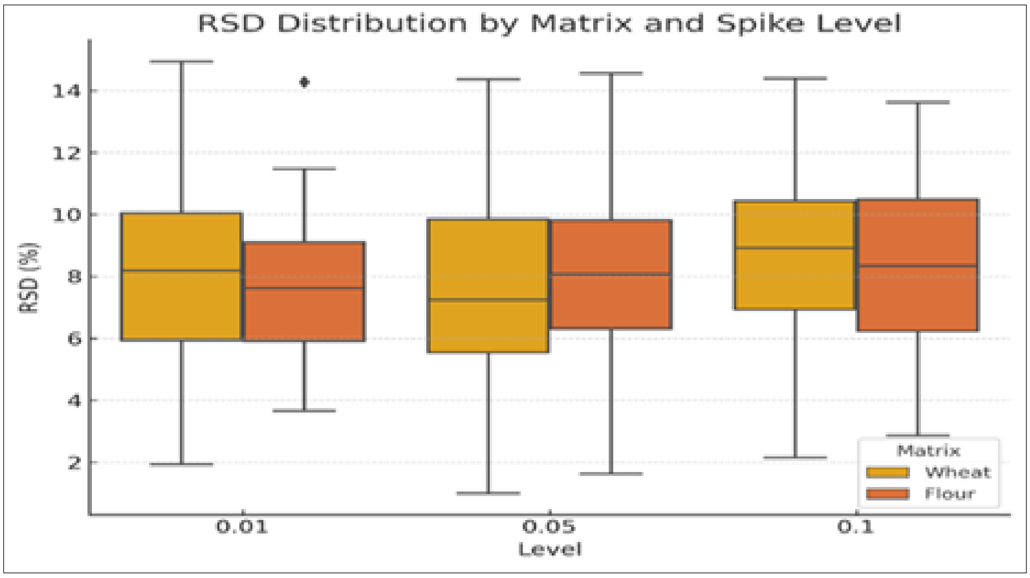

Figure 2. Boxplots of precision (RSD, %) by matrix (wheat, flour) and spike level (0.01, 0.05, 0.10 mg/kg)

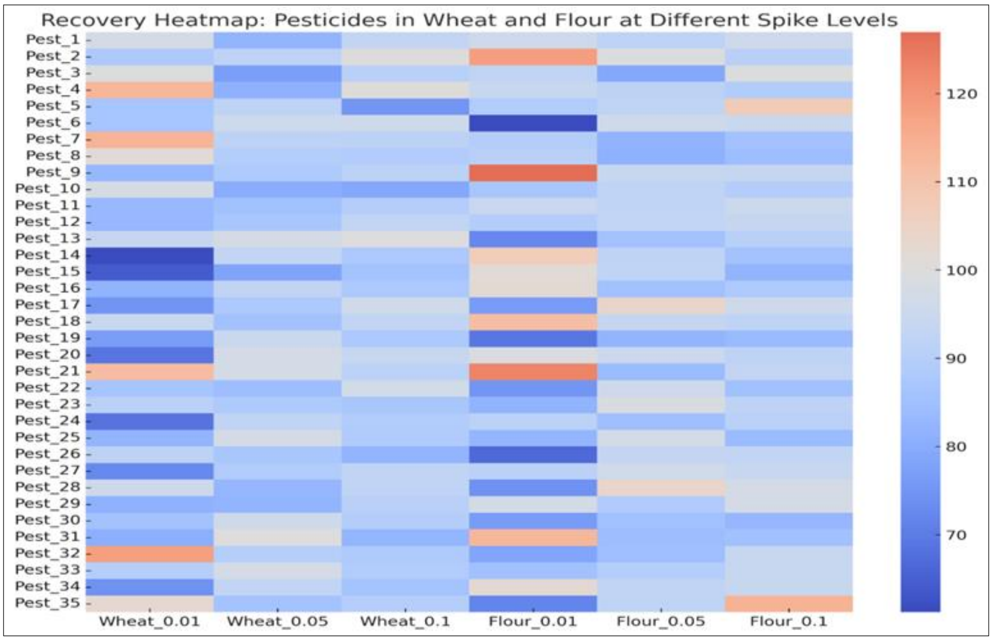

Figure 3. Recovery heatmap (%) for 35 herbicides in wheat and flour at 0.01, 0.05, and 0.10 mg/kg using the QuEChERS-LC-MS/MS method

Table 3. Average recoveries and coefficient of variation (CV %), on wheat and flour samples were spiked at 3 different concentration levels 0.01, 0.05 and 0.1 mg/kg

Compound	n	Wheat (0.01 mg/kg)		Flour (0.01mg/kg)		Wheat (0.05mg/kg)		Flour (0.05mg/kg)		Wheat (0.1mg/kg)		Flour (0.1 mg/kg)		Reproducibility
		Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Pooled CV%
Ametryn	6	79	4	84	9	99	3	92	4	94	11	83	5	5
Amidosulfuron	6	85	5	92	9	109	1	110	3	115	9	114	7	7
Azimsulfuron	6	82	2	84	10	114	2	105	5	105	9	99	6	3
Beflubutamid	6	81	5	85	10	100	3	92	6	93	6	87	3	4
Butachlor	6	83	5	82	8	95	3	88	2	89	5	82	4	3
Carbetamide	6	83	5	84	7	102	1	93	4	92	2	86	4	2
Chlorbromuron	6	77	6	79	11	101	1	88	5	97	1	82	3	3
Chlorbufam	6	97	2	95	11	90	2	90	7	110	8	106	10	4
Chloridazon	6	77	7	81	8	98	3	87	2	88	7	79	3	2
Chloroxuron	6	77	3	79	8	101	4	91	6	90	6	84	3	5
Chlorsulfuron	6	88	8	85	6	111	1	108	2	110	6	112	9	1
Cinosulfuron	6	82	5	85	7	109	1	110	1	96	8	102	9	2
Clethodim	6	75	12	85	12	107	2	104	3	113	8	116	10	2
Clomazone	6	77	5	82	8	96	2	86	7	94	10	82	4	5
Cyhalofop-butyl	6	80	6	85	11	102	1	93	4	95	9	80	3	6
Dimethachlor	6	78	4	86	9	95	4	87	7	94	5	85	4	10
Dimethenamid	6	79	5	78	10	97	3	88	4	92	7	82	4	3
Diuron	6	78	4	87	9	100	2	92	4	96	9	85	2	3
Florasulam	6	93	9	87	5	100	2	112	2	88	10	104	10	2
Fluazifop-P-butyl	6	81	5	78	10	103	4	98	5	85	10	85	3	5
Flufenacet	6	78	8	76	8	89	12	92	4	96	9	79	4	12
Fluroxypyr	6	78	6	75	8	92	6	88	7	89	8	86	10	7
Foramsulfuron	6	82	4	89	8	104	3	96	10	98	7	97	9	11
Halosulfuron-methyl	6	90	12	85	8	106	1	105	2	109	6	101	8	2
Mefenpyr-diethyl	6	80	6	77	10	97	3	87	10	91	10	80	6	9
Metazachlor	6	79	4	82	10	96	3	87	5	95	11	84	4	4
Methabenzthiazuron	6	80	4	81	8	104	1	89	4	98	11	83	6	3
Oxadiargyl	6	79	10	87	8	95	2	103	1	91	12	93	4	3
Oxadiazon	6	77	8	96	10	95	1	87	4	90	12	80	6	2
Oxasulfuron	6	87	3	86	12	110	1	107	2	116	10	111	11	3
Propachlor	6	78	5	84	11	98	2	88	5	91	5	82	7	4
Simetryn	6	78	4	80	10	97	1	82	6	92	4	79	7	3
Thifensulfuron-methyl	6	80	5	77	9	108	1	106	1	97	7	104	9	2
Tralkoxydim	6	79	11	88	10	102	5	111	5	100	7	105	8	4
Trietazine	6	78	6	84	11	100	3	90	2	91	3	83	2	3

n: No. of replicates, Rec.: Mean recovery, CV: Coefficient of variation.

Accuracy and precision studies, based on fortified wheat and flour samples at 0.01, 0.05, and 0.1 mg/kg, yielded recoveries ranging from 75% to 116%. Interday reproducibility was consistently below 13% at the 0.05 mg/kg spike level during a two-month evaluation. Heatmap analysis (Figure 3) highlighted generally satisfactory recoveries, with slight underestimation at LOQ and convergence toward 90-105% at higher concentrations. Wheat samples tended to yield marginally higher recoveries at upper spike levels, while flour samples performed better for specific analytes near the LOQ. Collectively, these outcomes validate the QuEChERS extraction and LC-MS/MS conditions, confirming the method's reliability for routine multiresidue herbicide quantification in wheat and flour.

Table 4. Summary of expanded measurement uncertainty at LOQ (U, k = 2)

Matrix*	Min U(%)	Max U(%)	Mean U(%)	Median U(%)
Wheat	9.96	77.66	36.83	33.95
Flour	12.49	79.32	36.06	30.61

*Statistics of U (k=2, %) per matrix at LOQ (0.01 mg/kg). Values are calculated from precision (RSD) and recovery bias for each analyte.

The validation outcomes observed here - recoveries primarily within the 70-120% acceptance band and RSDs ≤20% at decision-relevant concentrations - are consistent with the most recent European guidance for analytical quality control and method validation, which emphasizes transparent uncertainty budgets and clear identification criteria; as the European Commission points out in the AQC update (2023), such performance characteristics support quantitative use above the LOQ and provide defensible reporting paths for routine monitoring (Figures 1-3; Table 1) (European Commission, 2023a). As Kwon et al. (2012) demonstrated in a broad survey across multiple commodities following QuEChERS extraction, matrix-induced ionization effects can vary markedly by analyte-matrix combination and are a principal driver of trace-level bias in LC-MS/MS; this aligns with our LOQ behavior, where a subset of analytes displays modest recovery depression while precision remains acceptable (Figure 1; Table 1). Recent food-matrix work by Zhang et al. (2023) further disentangles the role of matrix species and mass-spectrometric conditions, concluding that even optimized workflows rarely eliminate matrix effects-hence the value of explicit ME% documentation and, where feasible, isotopically labeled internal standards for the most bias-prone compounds.

In terms of scope and throughput, our 16-minute scheduled MRM gradient for a 35-analyte panel aligns with current practice in cereal and legume matrices, where large panels are quantified without compromising selectivity. For example, Yuan et al. (2023) reported an LC-MS/MS method covering 380 pesticides in beans with a QuEChERS-based workflow and highlighted sample-preparation nuances that preserve quantitative performance across matrices; similarly, contemporary multi-residue applications in high-impact journals continue to validate rapid chromatographic programs against stringent quality targets, reinforcing the practicality of our acquisition settings (Figures 1-3).

Regarding measurement uncertainty, our expanded uncertainty at LOQ - with median U (k=2) around 31-34% (Table 1) - is typical of bias-dominated uncertainty budgets at quantification limits. As the European Commission guidance on analytical methods stresses, laboratories may report either uncorrected results accompanied by U (k=2) or recovery-corrected results with the correction factor's uncertainty propagated into U; the choice depends on policy and legal context near MRLs, but both routes are considered acceptable when supported by full method-validation evidence (Figures 1-3; Table 1). This interpretive framework provides a clear rationale for pairing per-analyte results with uncertainty, enabling transparent decision-making.

While our method achieved LOQs of 0.01 mg/kg and acceptable uncertainty profiles, comparable or superior to recent cereal-focused LC-MS/MS validations (e.g., Yuan et al., 2023; Zhang et al., 2023), the practical relevance of these analytical performances becomes clearer when placed against real dietary risk benchmarks. Reported Maximum Residue Limits (MRLs) for wheat and flour in international legislation typically range from 0.01 to 0.5 mg/kg; thus, the current method's sensitivity ensures reliable detection and quantification at or below these regulatory thresholds, supporting meaningful consumer risk assessments. The uncertainty levels observed near the LOQ, though bias-driven, remain compatible with transparent reporting and decision-making in food-safety contexts, especially when combined with %MRL and risk-quotient interpretations. The heatmap visualization (Figure 3) provides an integrative overview of recoveries across all analytes and matrices, highlighting modest depression at the LOQ and progressive convergence toward 90-105% at higher spike levels. This graphical representation reinforces the robustness of extraction and LC-MS/MS conditions and supports the selected calibration range for routine quantitation in wheat and flour (Atia et al., 2021; Omar et. al., 2021).

Nevertheless, one limitation of this study is its targeted scope: only 35 herbicides were validated, despite the broader diversity of pesticides applied in wheat cultivation and grain storage worldwide. Although this focused selection reflects both national registrations and compounds of frequent international concern, expanding the panel to include additional systemic fungicides, insecticides, and polar herbicides (e.g., glyphosate-class) would further strengthen applicability. Future work should therefore build on the current robust QuEChERS-LC-MS/MS framework to encompass these broader analyte classes and to compare incurred-residue data in real-world wheat and flour samples.

Beyond analytical validation, a practical next step is to translate measured residues into risk-relevant indicators that support regulatory decision-making and public health communication. In cereals, linking results to Maximum Residue Limits (MRLs) and dietary exposure models can contextualize findings for high-consumption subgroups and typical processing pathways (milling, baking). Following workflows exemplified by recent QuEChERS-LC-MS/MS applications that paired residue measurement with deterministic/probabilistic intake calculations (Su et al., 2024), laboratories can report %MRL, the proportion of samples above action thresholds, and risk quotients for chronic (ADI-based) and, where applicable, acute (ARfD-based) exposure. Incorporating the study's quantified measurement uncertainty directly into these exposure estimates strengthens transparency and helps risk managers weigh exceedances against analytical variability when prioritizing follow-up or enforcement.

A complementary avenue for future work is to extend the scope and robustness of the method toward analytically challenging herbicides and real-world sample conditions. Polar/acidic herbicides (e.g., specific phenoxy acids and glyphosate-class compounds) often require tailored extraction (acidified aqueous phases), derivatization (e.g., FMOC for glyphosate), or alternative chromatographic modes (HILIC/anion-exchange) and negative-ion ESI to achieve fit-for-purpose sensitivity (Raina-Fulton, 2014; Guo et al., 2019). Beyond matrix-matched calibration, the structured mapping of ionization suppression/enhancement - via post-column infusion or standard-addition experiments - can quantify analyte- and matrix-specific effects, guiding the selective use of isotopically labeled internal standards (Kwon et al., 2012; Williams et al., 2023). Finally, formal storage-stability and freeze-thaw assessments in both grain and flour (Bi et al., 2020) would document potential time-dependent loss or transformation, reduce bias at low-level spikes, and support confident interpretation of incurred-residue samples in routine surveillance. To strengthen regulatory-grade submissions, it is advantageous to document extraction efficiency and to present ion-ratio conformity and retention-time windows for all analytes. The extraction-efficiency note from the European Commission (2023b) offers a practical template for demonstrating that the sample-prep step does not under-recover certain classes, complementing the AQC and method-guidance documents and improving inter-laboratory comparability when positive findings are reported.

CONCLUSIONS

The multiresidue method developed for analyzing 35 herbicides demonstrates a detection limit of 0.01 mg/kg and adheres to EU MRLs for wheat and flour agricultural commodities. The method utilizes optimal collision energy and declustering potential, selecting two MRMs for quantification and confirmation, while tuning mass spectrometric parameters for enhanced sensitivity. The methodology synergizes the QuEChERS technique with an Exion HPLC and a Sciex Qtrap API 6500+ LC–MS/MS system employing electrospray positive ionization (ESI+). This setup delivers satisfactory recovery rates, a brief analysis duration, cost-effectiveness, and enhanced safety. Notably, the LC-MS/MS procedure permits direct herbicide quantification without derivatization and minimal cleaning, culminating in a total runtime of 16 minutes. The precision is deemed adequate, with relative standard deviations under 13% and recovery values between 75% and 116% for the majority of compounds assessed. Method validation included recovery evaluations through six replicates of spiked blank wheat and flour samples at concentrations of 0.01, 0.05, and 0.1 mg/kg, achieving a correlation coefficient exceeding 0.99 and linearity across the 0.01-0.5 µg/mL concentration range at the quantification limit of 0.01 µg/mL.

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