Chapter

Liquid Chromatography Tandem Mass Spectrometry after the QuEChERS Method for Determining 20 Herbicide Residues in Wheat and Flour

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Abstract

Agriculture is the backbone of the economy and social structure, and it plays a critical part in each country's overall growth. Because of the significant food gap that exists in several vital crops, wars, and the continual expansion in the population, the role of agriculture products has recently become critical. The world is currently experiencing a severe food shortage, estimated to be over 60% of its strategic food requirements. As a result, there is a need to increase the area of farmed land in order to satisfy the growing population and raise food demand by eliminating weeds that can reduce agricultural output. Weed is an unwanted plant (one that grows in the incorrect area) that reduces crop output. Herbicides are a type of pesticides that are used to kill weeds and increase crop output. As a result, herbicide residues on food, particularly cereals, must be determined. In this study, the QuEChERS approach for determining herbicides in wheat and corn by direct injection to Exion HPLC coupled with a SciexQtrap API 6500+ LC–MS/MS system using an electrospray positive ionization (ESI+) at lower concentrations without utilizing acids or clean-up is evaluated, optimized, and validated in this work.

Keywords: QuEChERS, LC–MS/MS, agriculture, herbicides, cereals

1. Introduction

As a result of the continuing expansion in the world's population, the use of pesticides in contemporary agriculture has become one of the most critical necessities for meeting society's food needs, and millions of tons of pesticides are used annually for this purpose [1]. Pesticides are one of the most commonly utilized substances on the planet. Despite their usefulness, pesticides are one of the most dangerous compounds that damage humans, animals, and surface water in particular [2]. When pesticides are used in large quantities in the environment, they have the potential to harm the environment, especially human health [3]. Weeds are any

unwanted plants that grow in a field and threaten crops, animals, or human health. Herbicides are a type of pesticide that kills weeds to protect plants and boost crop output [4]. Herbicides are frequently employed in agriculture and turf management in the landscape. They account for almost 70% of all agricultural pesticide use worldwide [5]. Herbicides can cause everything from skin rashes, nausea, and weariness to headaches, chest pain, and even death in some cases.

Pesticides are used in roughly 2 million tons over the world, with 47.5% being herbicides, 29.5% being insecticides, 17.5% being fungicides, and 5.5% being other pesticides [6]. China, the United States, Argentina, Thailand, Brazil, Italy, France, Canada, Japan, and India are the top ten pesticide-using countries in the world [7]. Furthermore, it is predicted that by 2020, global pesticide usage will have increased to 3.5 million tons [8]. Africa's economy is heavily reliant on agriculture, with approximately 59% of the population relying on it for a living [9]. Despite this, the African continent contributes 2–4% of the global pesticide market share and has the lowest pesticide usage rate in the world [9]. Food demand is expected to rise rapidly in the next three decades as a result of the rising population, and demand for pesticides, herbicides, and fungicides are also expected to rise [10].

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach was used to detect this chemical and estimate its concentration [11–18]. In terms of analysis costs and turnaround time, multiresidue methods are the most efficient way for herbicide analysis. The majorities of the procedures have multiple steps and use a lot of different solvents and reagents. In terms of good recovery, short duration of analysis, cheap cost, and safety, the QuEChERS approach combined with liquid chromatography–tandem mass spectrometry (LC–MS/MS) was determined to be the optimal combination for determining herbicides in some foods. Because of the more ionized herbicides, LC–MS/MS is now commonly employed [12–14, 19, 20].

Controlling herbicide residues in food items through monitoring and a maximum residue limit (MRL) setting is critical for consumer safety. The Codex Alimentarius Commission (CAC) and the European Commission determined MRLs based on residues in food that must be found at safe levels for consumers [21, 22]. In the European Union (EU) legislation, the lowest limit of analytical quantitation (LOQ) is specified as the MRL that equals 0.01 mg/kg if the MRL obtained by different trials is not safe for consumers [22].

Yingying et al. [23] improved and validated a QuEChERS technique for determining florasulam and pyroxsulam residues in wheat grain and straw using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The approach was tested on cereals such as oat, millet, corn, and rice. Average recoveries ranged from 76 to 113%, with RSDs ranging from 2 to 15%. TAO et al. [24] developed an efficient method for determining various phenoxy acid herbicide residues in grains. The study of phenoxy acid herbicides in rice, corn, and wheat was optimized using a QuEChERS approach combined with high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Renata Raina et al. [25] developed pesticide residue testing procedures for a wide range of foods, including cereal-based foods, nutraceuticals and associated plant products, and infant feeds. Many processed consumer products are made from these grain, fruit, vegetable, and plant-based components. A modified QuEChERS approach has been applied for cereal and nutraceuticals, which are dry sample products, with additional steps to allow wetting of the dry sample matrix and subsequent cleanup using dispersive or cartridge format SPE to eliminate matrix effects.

Wheat is a widely cultivated crop whose seed is a grain that is consumed as a staple food all over the world. The most important wheat types are common wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), and club wheat (*T. aestivum*) (*T. compactum*). Wheat is grown as a commercial crop because it generates a high yield per unit area, thrives in a temperate climate with a short growing season, and produces versatile, high-quality flour. Wheat flour is used to produce bread, pasta, cereal, pastries, cookies, crackers, muffins, tortillas, and pitas, among other things. Wheat is the second most widely grown cereal grain after maize, and its global trade volume exceeds that of all other crops combined. The total global wheat production in 2020 was 760 million tons. China, India, and Russia are the world's three greatest individual wheat producers, accounting for over 41% of global wheat production. Individually, the United States is the world's fourth-largest wheat producer. If the European Union were counted as a single entity, it would produce more wheat than any other country save China [26].

The current study's technique describes the examination of a mixture of herbicides in various matrices after extraction using the QuEChERS technology. The QuEChERS technique is evaluated, optimized, and validated for the determination of 20 herbicides in wheat and flour by direct injection to LC–MS/MS at lower concentrations without the use of acids or clean-up in this study. Exion HPLC paired with the SciexQtrap API 6500+ LC–MS/MS System was used to determine these chemicals utilizing electrospray positive ionization (ESI+).

2. Experimental method

2.1 Instrumentation and analysis

1. LC–MS/MS system, ExionLC AC coupled with Qtrap API 6500+ MS/MS system from AB Sciex, USA.

2. Chromatographic column, Infinity lab Poroshell 120 EC-C18 3.0 × 50 mm, 2.7 μm particle size (Agilent, USA).

The injection volume was 2 μL and the column temperature was 40°C. The pesticides are separated using a Gradient mixing program of 10% 50 mM ammonium format in deionized water, which is mostly used for positive ionization mode, with 0.1% formic acid as eluent A and methanol as eluent B at 300 μL/min flow rate starting by A bottle 60% for 1 min, changed continuously till 11.5 min to be 10% for 0.5 min, changed progressively till 12 min to be 0% for 2 min and returned to 60% from A in min 14 for 2 min to be 16 min complete run time for every one of the 20 pesticides. Electrospray ionization in the positive ion mode with multiple reactions monitoring (MRM) mode was used to complete the MS/MS analysis.

The LC mobile phase stock solution was 50 mM ammonium formate solution in methanol/water (1:9), and the LC mobile phase was 10 mM ammonium formate solution in methanol/water (1:9), dilute 200 mL of LC mobile phase stock solution with 800 mL methanol/water (1:9), adjust the pH to about 3.78 ± 0.02 with ammonia solution (33%), and then add 100 mL methanol and LC mobile phase was 10 mM ammonium formate solution in methanol/water (1:9), dilute 200 mL of LC mobile phase stock solution with 800 mL methanol/water (1:9), the pH should be 4 ± 0.1, adjust as needed.

2.2 Reagents and materials

Atrazine (99%), clodinafop (free acid) (99%), clodinafop-propargyl ester (99%), cycloxydim (98.8%), diphenamid (99%), fenoxaprop-P-ethyl (R-enantiomer) (99%), haloxyfop-2-ethoxyethyl ester (99%), haloxyfop (free acid) (99%), imazamethabenz-methyl (97.4%), imazethapyr (99%), mesosulfuron-methyl (98%), metolachlor (98.5%), metribuzin (99.5%), metsulfuron-methyl (99.5%), pendimethalin (98.8%), quizalofop-ethyl (99.3%), quizalofop-P-ethyl (98.4%), simazine (98%), sulcotrione (99%), and triclopyr butotyl (99.1%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Methanol (99.9%) HPLC grade was purchased from J.T. Baker (PA, USA). Acetonitrile 99.9% -HPLC grade was purchased from J.T. Baker (Pennsylvania, USA). Deionized water (<18M_cm resistivity) was performed in the laboratory using a Millipore (Billerica, MA, USA) MilliQ water purification system. Ammonia solution (33%) was purchased from Riedel-de Häen (Seelze, Germany). Formic acid (98–100%) was purchased from Riedel-de Häen. QuEChERS extraction kits.—5982–5650 was purchased from Agilent {Agilent QuEChERs salts and buffers are prepackaged in anhydrous packages(4 g MgSO4; 1 g NaCl; 1 g trisodium citrate dihydrate;0.5 g disodium citrate sesquihydrate)} (Santa Clara, CA, USA).

2.2.1 Standard preparation

Stock solutions (1000 μg/mL) of each pesticide standard were prepared by dissolving atrazine in toluene, clodinafop (free acid) in toluene, clodinafop-propargyl ester in toluene, cycloxydim in toluene, diphenamid in toluene, fenoxaprop-P-ethyl (R-enantiomer) in toluene, haloxyfop-2-ethoxyethyl ester in toluene, haloxyfop (free acid) in toluene, imazamethabenz-methyl in toluene, imazethapyr in methanol/toluene (3:7 v/v), mesosulfuron-methyl in toluene/acetone (7:3 v/v), metolachlor in toluene, metribuzin in toluene, metsulfuron-methyl in toluene, pendimethalin in toluene, quizalofop-ethyl in toluene/acetone (8:2 v/v), quizalofop-P-ethyl in toluene, simazine in acetone, sulcotrione in toluene/acetone (9:1 v/v), and triclopyr butotyl in toluene/acetone (9:1 v/v). All stock solutions were prepared and kept at −20 ± 2°C. Working mixtures of the examined pesticides (5 g/mL each) and calibration mixtures of concentration levels 0.01, 0.05, 0.1, and 0.5 g/l were made by diluting suitable aliquots of the stock solutions with methanol kept at 4 ± 2°C.

2.2.2 Spiked samples preparation

The flour and wheat were purchased at the local market. The samples were thoroughly ground before being homogenized in an electric mill. In recovery experiments, wheat and flour samples were spiked with a suitable amount of working mixture standard solution.

2.3 Extraction procedure

Herbicide residues in wheat and flour were extracted using the QuEChERS technique for herbicide residue analysis. Initial single-phase extraction of 2 g of homogenized sample with deionized water in a 50 mL PFTE centrifuge tube, 10 mL deionized water added, tube closed and shaken vigorously by geno grinder at 500 rpm for 1 min, and then with acetonitrile in a 50 mL PFTE centrifuge tube, 10 mL acetonitrile added, tube closed and shaken vigorously by geno grinder at 500 rpm for 1 min.

After that, a mixture of Agilent QuEChERs salts and buffers is added to the tube, which is then closed and rapidly shaken for 1 min at 500 rpm with a geno grinder, then centrifuged for 5 min at 4000 rpm (3430 rcf). The cleaned extract is filtered using syringe filters (0.45 m) and transferred to a PP vial after centrifugation. Finally, the liquid sample was injected into a liquid chromatography-mass spectrometry (LC–MS/MS) apparatus.

3. Result and discussion

The analysis technique used in this study was created with the goal of detecting and quantifying as many herbicides as feasible in a single run. When deciding which herbicides to include, two criteria were used: (1) herbicides registered for crop protection by local authorities, and (2) searching the literature for commonly studied compounds. Acidification was used in this method in the form of buffer citrate salt (trisodium citrate dihydrate and disodium citrate sesquihydrate), which served two purposes: (1) improving extraction by converting conjugate of some herbicide to neutral form, thereby increasing recovery, and (2) adjusting pH 5–5.5, thereby increasing herbicide sensitivity. The herbicides were determined using LC–MS/MS with an ESI source and MRM mode, which offered a highly selective and sensitive technique. All of the target analytes were ionized to $(M + H)^+$ form in the positive mode, according to the physicochemical parameters of the target. The positive mode was chosen since it works well for the majority of analytes. Herbicides can be quantified directly using the LC–MS/MS approach, which does not require any derivatization and requires minimal cleaning. A QuEChERs approach was used to design the method for 20 herbicides. The chromatograms obtained for each compound, as shown in **Figure 1**, were determined with sufficient precision and accuracy. The approach was tested on a total of 20 herbicides, each with a distinct retention time of 16 min. Although an excellent summary of the LC–MS/MS methods used for herbicides was offered, it did not cover all herbicides discussed in this study, and only a few studies for determining several classes of herbicides in wheat and flour in a single multiresidue approach were published.

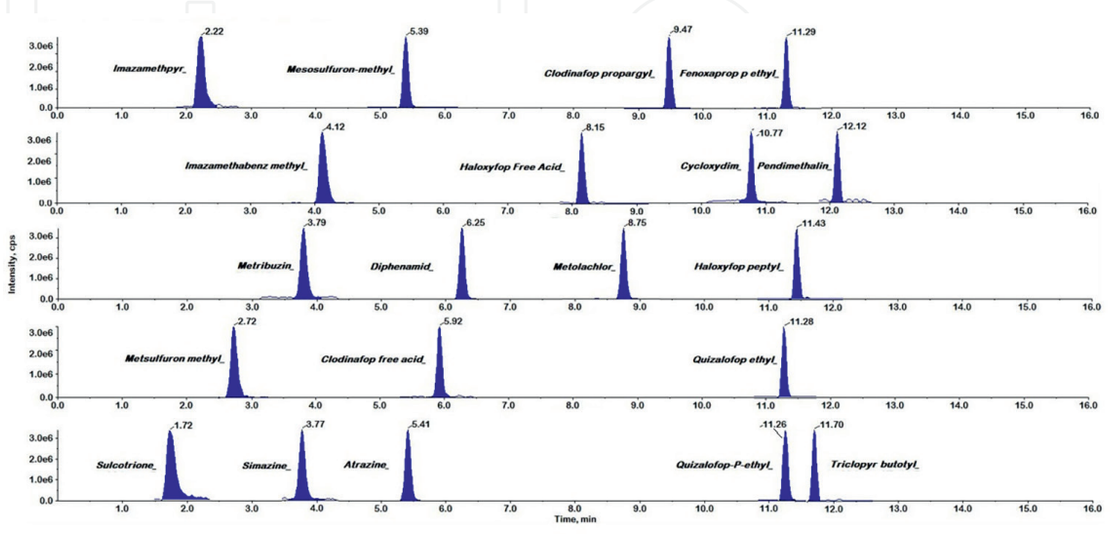

Figure 1.
The approach was validated using chromatograms produced by liquid chromatography tandem mass spectrometry (LC–MS/ MS) with electrospray ionization (ESI) in positive mode and MRM mode for 20 herbicides used in the study.

3.1 Mass spectrometry study of 20 herbicides

To discover the best precursor, product ions, and operating conditions, 20 herbicides were injected directly into the LC–MS/MS system in 1:1 methanol at a concentration of 0.1 μg/mL. **Table 1** summarizes the precursor and product quantification and confirmation ion pairs, as well as the declustering potential and collision energies.

3.2 Method validation

The developed method was validated in compliance with the document's method validation standards SANTE/2020/12830 document [27].

3.2.1 Linearity of calibration curves

Plotting the detector response area ratio vs. the concentration of the analytical solutions at various concentration levels ranging from 0.001 to 0.1 μg/mL established the linearity of the calibration curve of 20 herbicides. The calibration curves were prepared using sex levels of calibration standards in the concentration ranges of 0.001, 0.002, 0.005, 0.01, 0.05, and 0.10 μg/mL. Plotting the peak area vs. concentration yielded a calibration curve. According to European guidelines, the analytes showed linear behavior in the studied concentration levels with a correlation

No.	Acidic herbicides	Q1	DP	Q3	EP	CE	CXP
1.	Atrazine	216.1	82	104	10	37	10
2.	Clodinafop (free acid)	312	41	237.9	10	33	4
3.	Clodinafop-propargyl ester	350	115	266	10	24	10
4.	Cycloxydim	326.3	61	280	10	19	16
5.	Diphenamid	240.1	31	134.1	10	25	4
6.	Fenoxaprop-P-ethyl (R- enantiomer)	362.1	71	288.1	10	23	7
7.	Haloxyfop-2-ethoxyethyl ester	434	92	288	10	49	10
8.	Haloxyfop (free acid)	362	81	316	10	25	18
9.	Imazamethabenz-methyl	289	117	161	10	37	10
10.	Imazamethpyr	290	81	177	10	41	18
11.	Mesosulfuron-Methyl	504	76	182	10	33	10
12.	Metolachlor	284.2	76	252.2	10	21	4
13.	Metribuzin	215.1	81	187.2	10	21	4
14.	Metsulfuron-methyl	382	76	167	10	21	6
15.	Pendimethalin	282	88	194	10	25	14
16.	Quizalofop-ethyl	373	120	299	10	20	10
17.	Quizalofop-P-ethyl	373.1	71	298.9	10	25	15
18.	Simazine	202.2	77	131.9	10	27	8
19.	Sulcotrione	329	86	111	10	39	10
20.	Triclopyr butotyl	356.2	122	237.7	10	15	14

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

Herbicide	R^2
Atrazine	0.9961
Clodinafop (free acid)	0.9982
Clodinafop-propargyl ester	0.9963
Cycloxydim	0.9997
Fenoxaprop-P-ethyl (R- enantiomer)	0.9986
Haloxyfop-2-ethoxyethyl ester	0.9992
Haloxyfop (free acid)	0.9991
Imazamethabenz-methyl	0.9976
Imazamethpyr	0.9966
Mesosulfuron-Methyl	0.9977
Metolachlor	0.9969
Metribuzin	0.9991
Metsulfuron-methyl	0.9999
Pendimethalin	0.9998
Quizalofop-ethyl	0.9987
Quizalofop-P-ethyl	0.9965
Simazine	0.9995
Sulcotrione	0.9978
Triclopyr butotyl	0.9999

Table 2.
R^2 values for the 20 herbicides.

coefficient (r2) greater than 0.99 as shown in **Table 2**, indicating that all analytes were within the acceptable range and the coefficient of variation (CV percent) for each calibration point was less than 20% [28].

3.2.2 Matrix effect

A matrix effect research was carried out on blank wheat and flour samples using a conventional herbicide mixture of 20 herbicides. To correct for matrix-induced suppression in LC–MS/MS, matrix-matched standard calculations were performed at 0.01, 0.05, and 0.1 mg/kg.

The following formula was used to make the calculations:

Matrix effect % = ((peak area STD in matrix/peak area STD in solvent) −1/100).

To compensate for the matrix effect suppression on the results, 450 μL of blank sample was fortified with 50 μL of 0.5 μ g/mL standard solutions to achieve 0.05 μg/mL concentration levels [29].

3.2.3 Quantification limit (LOQ)

The quantitation limit of all of the substances investigated was determined to be 0.01 mg/kg for all of them. The validity of this level has been established in accordance with the SANTE guidelines [28] and EU 396/2005 regulation [22].

Compound	n	Wheat (0.01 mg/kg)		Flour (0.01 mg/kg)		Wheat (0.05 mg/kg)		Flour (0.05 mg/kg)		Wheat (0.1 mg/kg)		Flour (0.1 mg/kg)		Reproducibility
		Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Rec.%	CV%	Pooled CV%
Atrazine	6	85	5	79	4	89	3	97	4	101	7	95	6	4
Clodinafop (free acid)	6	80	7	77	6	91	2	93	3	94	7	96	7	5
Clodinafop-propargyl ester	6	80	10	82	5	95	2	99	8	103	7	82	7	5
Cycloxydim	6	78	13	78	6	98	2	98	12	106	6	95	5	11
Diphenamid	6	82	9	80	4	89	2	96	6	99	7	89	7	8
Fenoxaprop-P-ethyl (R-enantiomer)	6	79	9	79	7	93	2	97	8	103	7	84	7	5
Haloxyfop-2-ethoxyethyl ester	6	83	9	81	5	96	2	96	3	101	7	93	7	5
Haloxyfop (free acid)	6	84	8	80	5	91	2	93	3	99	7	96	6	7
Imazamethabenz- methyl	6	86	12	79	5	95	2	98	2	99	7	96	7	3
Imazamethpyr	6	83	11	85	15	83	2	94	3	102	7	83	8	4
Mesosulfuron-Methyl	6	87	9	82	3	89	2	103	6	102	6	98	6	4
Metolachlor	6	83	13	79	10	90	2	96	8	105	7	80	7	7
Metribuzin	6	82	7	71	5	93	2	98	3	101	7	116	6	4
Metsulfuron-methyl	6	83	8	82	4	96	2	103	10	98	5	88	6	4
Pendimethalin	6	82	15	74	14	86	2	91	9	104	6	103	5	8
Quizalofop-ethyl	6	80	15	82	5	95	2	100	10	103	7	81	7	6
Quizalofop-P-ethyl	6	80	13	81	5	93	2	100	13	104	7	74	7	5
Simazine	6	84	13	79	5	93	2	97	4	101	7	92	7	4
Sulcotrione	6	102	17	96	16	94	2	79	13	100	6	95	10	9
Triclopyr butotyl	6	90	6	82	7	90	2	93	6	97	7	85	7	8

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

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.

3.2.4 Accuracy and precision

Six replicate spiked wheat and flour samples were analyzed at three distinct levels (0.01, 0.05, and 0.1 mg/kg) to acquire accuracy and precision. The percentage of the money recovered ranged from 71–105%. The precision was based on the corresponding relative standard deviations, and the trueness was based on the mean recoveries (RSD). **Table 3** shows the recoveries, means, and RSD percent. Reproducibility (interday accuracy and precision) was tested over a two-month period at a fortification level of 0.05 mg/kg and found to be less than 12%.

4. Conclusion

The current study developed a multiresidue technique of testing for 20 herbicides with a limit of determination of 0.01 mg/kg, which meets the EU MRLs for wheat and flour farm goods. Two MRMs for quantification and conformation were chosen based on the optimal declustering potential and collision energy, and the mass spectrometric parameters were tuned to give the best sensitivity. In terms of approved recovery, short duration of analysis, cheap cost, and safety, the QuEChERS method followed by Exion HPLC and a SciexQtrap API 6500+ LC–MS/MS system using an electrospray positive ionization (ESI+) technology was shown to be the optimal combination for determining the 20 herbicides. Herbicides can be quantified directly using the LC–MS/ MS method, which does not require any derivatization and requires minimum cleanup with a total runtime of 16 min. The majority of the chemicals tested had recovery rates ranging from 71–105%, with relative standard deviations of less than 12%, indicating adequate precision. Recovery trials on six replicates of spiked blank wheat and flour samples at 0.01, 0.05, and 0.1 mg/kg were used to determine the method's precision and accuracy. The developed assay was linear over a concentration range of 0.01–0.5 μg/mL, with a correlation coefficient of more than 0.99 at the 0.01 μg/mL limit of quantification.

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Authors' contributions

The study's inception and design were aided by the author. Islam R. Ghoniem was in charge of material preparation, data collecting, and analysis. Islam R. Ghoniem wrote the first draught of the manuscript and provided feedback on prior draughts. The final manuscript was read and approved by the author.

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Data availability

On reasonable request, the corresponding author will provide the datasets used and/or analyzed during the current work.

Declarations

The author declares no competing interests.

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