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Simultaneous Determination of Chloroacetanilide Herbicide Residues in Crops with Gas Chromatography-Mass Spectrometry

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ABSTRACT

A rapid and sensitive method was developed for the simultaneous determination of eight chloroacetanilide herbicides in crops (soybean, rice, and wheat). Herbicides including alachlor, acetochlor, pretilachlor, butachlor, metolachlor, napropamide, diflufenican, and propanil were extracted from samples using a modified QuEChERS method. Acetonitrile containing 0.5% acetic acid was used as extraction solvent, and the extracts were analyzed by gas chromatography-mass spectrometry after being subjected to a simple clean-up procedure using C_{18} sorbent instead of the primary–secondary amine (PSA). The average recoveries for all eight herbicides were higher than 80% with relative standard deviations lower than 9% in the spiked concentration range of 16 ~800 µg kg^{-1} and limits of detection (LODs) ranged from 1 to 4 µg kg^{-1}. The method was applied to analyze some samples obtained from various sources (fields, markets, and families) and a lower residue level of chloroacetanilide herbicides in crops was found. The results showed that the new method is efficient, sensitive, and effective for monitoring residual chloroacetanilide herbicides in crops.

Keywords Chloroacetanilide herbicides, pesticide, multiresidue, QuEChERS, gas chromatography-mass spectrometry

INTRODUCTION

Herbicides have been widely used to control broadleaf weeds and annual grasses in row crops for more than 50 years [1]. Chloroacetanilide herbicides are one of the most important groups of modern chemical herbicides. These herbicides, which include alachlor, acetochlor, metolachlor, pretilachlor, and butachlor, are widely used to control grasses and some broadleaf weeds in various crops including corn and soybean [2]. In China, acetochlor, metolachlor, and butachlor

are the most widely used chloroacetanilide herbicides [3, 4], and are produced at approximately 20, 7, and 0.6 million kg per year, respectively [5, 6]. The effluents discharged from factories producing these herbicides have caused chloroacetanilide herbicide pollution in China in recent years [7]. Butachlor is a suspected carcinogen, which can stimulate cell proliferation and induce malignant growth *in vitro* [8]. Acetochlor shows strong genotoxicity *in vitro* but shows only weak activity *in vivo* [9]. Metolachlor also has relevant and irreversible toxicological effects and is a suspected carcinogen [10]. The World Health Organization (WHO) has assigned a hazard ranking of III (slightly hazardous) for acetochlor and metolachlor and a ranking of U (unlikely to be hazardous) for butachlor [11]. Some countries have developed strict maximum residue limits (MRLs) for chloroacetanilide herbicides. For example, in China the MRL for alachlor and acetochlor in corn is 0.02 mg kg^{-1}, and that for acetochlor in peanut is 0.01 mg kg^{-1}. In Japan, the MRL for alachlor is 0.01 mg kg^{-1} in spinach, cabbage, carrots, and broccoli, and that for napropamide is 0.1 mg kg^{-1}. In the USA and Australia, the MRL for metolachlor is 0.1 mg kg^{-1} in corn. Correspondingly, there is a need for accurate methods to analyze herbicide residues in food and environmental samples to evaluate possible risks to human health.

Some multi-residue methods have been established for the analysis of chloroacetanilide herbicide residues in water and soil [12-14]. The typical sample preparation methods involve liquid-liquid extraction (LLE) [15] and solid-phase extraction (SPE) [16]. However, LLE requires large volumes of organic solvents, and SPE requires complicated procedures including column conditioning and elution with organic solvents. Hence, these methods are expensive and time-consuming. In 2003, Anastassiades et al. developed a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method for the analysis of pesticides in foods [17-19]. In that method, residual pesticides were extracted from the sample with acetonitrile, and then water and proteins were removed from the raw extract by salting out with sodium chloride and magnesium sulfate. The dried crude extract was then cleaned by addition of a small amount of PSA. This step is similar to matrix solid-phase dispersion, where the matrix is homogenized with bulk sorbents. The use of the QuEChERS method resulted in outstanding recoveries of pesticides from several different pesticide classes [17], shorter analysis time, and less solvent consumption than the traditional methods.

The techniques most frequently used for herbicide analysis include high-performance liquid chromatography (HPLC) [14], gas chromatography (GC) [20-22], gas chromatography-mass spectrometry (GC/MS) [12], liquid chromatography-mass spectrometry (LC/MS) [13], and immunoassay [23]. Several detectors, including electron capture detector (ECD) [20], flame ionization detector (FID) [21], nitrogen/phosphorous detector (NPD) [22] and mass spectrometry (MS), can be used. However, most of the currently used methods have been developed for analysis of environmental samples, such as water and soil, and only a few have been developed for analysis of herbicides in crops. In this paper, a rapid and inexpensive method was described for analysis of eight chloroacetanilide herbicides in crops using an optimized QuEChERS method and GC/MS.

MATEIRALS AND METHODS

Experimental

Chemicals and Reagents

Standards for propanil (99.0%), diflufenican (99.5%), and napropamide (99.5%) were obtained from Dr. Ehrenstorfer (Germany). Pesticide grade standards (certified purity from 92.0 to 97.0%) for acetochlor, alachlor, metolachlor, butachlor, and pretilachlor were obtained from commercial sources. PSA, C_{18}, and amino silica (NH_2) SPE bulk sorbents were obtained from Agilent

Technologies (USA). Acetonitrile (ACN), acetone, and *n*-hexane were HPLC grade and were purchased from J.T Baker (USA). Other reagents, including sodium chloride (NaCl), acetic acid (HAc) and anhydrous magnesium sulfate ($MgSO_4$) were analytical grade and were purchased from Beijing Chemical Works (China).

Apparatus

An Agilent 7890A GC/5975C MS equipped with a split-splitless injector and a 7683B autosampler was used for herbicide analyses. Chromatographic separation was carried out on an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, USA) with 5% phenyl polydimethylsiloxane as the nonpolar stationary phase. For extraction, samples were homogenized with an IKA T18 disintegrator (Germany). Sample extracts were separated by centrifugation in a TGL-16M centrifuge (Xiangyi Instrument Co., Ltd, China).

Sample Preparation

Soybean, rice, and wheat were crushed to powder with a blender (Jiuyang, China). A sample (10.0 g) was then weighed into a 50-mL polypropylene centrifuge tube, 20 mL ACN containing 0.5% HAc was added, and the mixture was vortexed for 2 min. Then, NaCl (2.0 g) and $MgSO_4$ (8.0 g) were added, and the mixture was homogenized for 2 min. The supernatant (2 mL) was transferred into a new 5-mL tube after centrifugation at 5000 r min^{-1} for 2 min, and then evaporated to dryness under nitrogen at 50°C. The residue was redissolved in 2 mL acetone/*n*-hexane (2:8, *v/v*), vortexed for 2 min with C_{18} sorbent (100 mg), and centrifuged for 2 min at 5000 r min^{-1}. The supernatant was filtered through a 0.45-µm filter into a sample vial for GC-MS analysis.

To evaluate the method, spiked samples containing different concentrations of herbicides were prepared by adding appropriate stock standard solution each herbicide to 10.0 g of blank crop samples. For the recovery study, the samples were fortified with each herbicide at the following concentrations: 0.016, 0.048, 0.096, and 0.48 mg kg^{-1} of diflufenican, butachlor, napropamide, and pretilachlor; 0.02, 0.06, 0.12 and 0.6 mg kg^{-1} of propanil; 0.026, 0.08, 0.12, and 0.8 mg kg^{-1} of acetochlor; 0.024, 0.072, 0.144, and 0.72 mg kg^{-1} of alachlor, and 0.01, 0.032, 0.064 and 0.32 mg kg^{-1} of metolachlor. The limits of detection (LOD) and limits of quantification (LOQ) were taken to be concentrations of spiked samples resulting in a signal-to-noise (S/N) ratio of 3 and 10, respectively.

Standard Solutions

Individual stock standard solution of each herbicide was prepared in acetone. A mixed standard solution containing 24 mg L^{-1} of each of diflufenican, butachlor, napropamide, and pretilachlor, 30 mg L^{-1} of propanil, 40 mg L^{-1} of acetochlor, 36 mg L^{-1} of alachlor, and 16 mg L^{-1} of metolachlor was prepared in acetone and stored in a dark vial at 4°C. Working standard solutions with concentration range of 0.005–2.7 mg kg^{-1} were prepared by dissolving the mixed standard solutions in solution of blank crop samples processed by the method described above.

GC/MS Conditions

Helium was used as carrier gas at a constant column flow rate of 1 mL min^{-1}. The temperature program was as follows: the initial temperature of 120°C was increased to 200°C at 20°C min^{-1}, to 230°C at 5°C min^{-1}, to 260°C at 30°C min^{-1}, finally held for 2 min. The total run time was 13.00 min. The temperature of the injection port was 250°C and 1.0 µL of sample was injected into the GC in pulsed splitless mode. Electron impact ionization source with ionization energy of 70 eV and selected ion monitoring (SIM) mode were used. The SIM program was 6.0–7.3 min

for propanil (m/z 161, 163, 217), acetochlor (m/z 146, 162, 223) and alachlor (m/z 160, 188, 146), 7.3–8.8 min for metolachlor (m/z 162, 238, 240), 8.8–11.3 min for butachlor (m/z 176, 160, 188), napropamide (m/z 72, 128, 100), and pretilachlor (m/z 162, 238, 176), and 11.3–13.0 min for diflufenican (m/z 266, 267, 394). The ion source and MS Quad temperatures were 230°C and 150°C, respectively. The solvent delay was 6 min.

RESULTS AND DISCUSSION

Optimization of QuEChERS Method

The QuEChERS method is an increasingly popular method in the area of pesticide residue analysis, and it is still being refined to optimize the extraction and clean-up steps. It was used as sample preparation method in this paper to prepare the samples, and investigated the effects of the volume of extraction solvent, HAc, clean sorbent, and eluent on the extraction of pesticide residues from samples.

Volume of Extraction Solvent

The initial extraction solvent in the original QuEChERS method is ACN (1% HAc) [25], which is used at a ratio of 1 mL per 1 g of sample. Anastassiades [26] used 1% HAc as a protective agent. In that study, the authors conducted a theoretical analysis of protective agents and described six major advantages of HAc; simple processing, better peak shape, lower LOD, more accurate quantification, improved service life of the injection port, and lower cost. In the traditional method [27], the swelling of dry samples (<25% water content) with water was essential to allow the extraction solvent access to the sample and to increase the extraction efficiency. In this study, the effects of the volume of ACN (1% HAc) and water were compared on extraction efficiency by determining the average recovery of each herbicide using different volumes of ACN (1% HAc) for extraction (*Fig. 1*). The sample became too solid to mix sufficiently when 10 mL of solvent was used for extraction, but recovery was significantly improved using 20 mL of ACN (1% HAc). The volume of water did not affect the recovery of herbicides. Therefore, 20 mL of ACN (1% HAc) was selected as the optimum volume of extraction solvent.

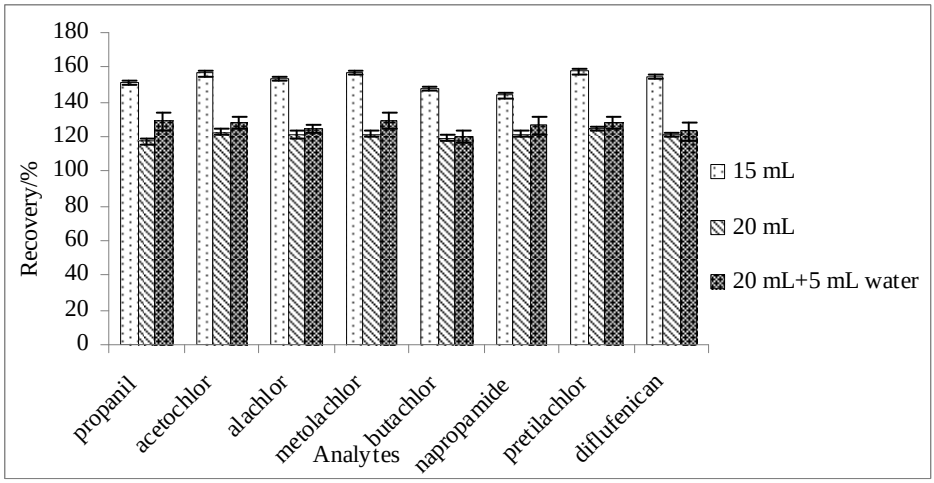

Fig. 1 Effects of solvent volume on herbicide extraction efficiency

Effect of HAc Concentration

Generally, HAc is used at a concentration of 1% in the QuEChERS method [25]. To optimize the HAc concentration, average recovery for each herbicide using different concentrations of HAc

were determined. As shown in *Fig. 2*, the best rates of recovery (94–100%) were obtained using 0.5% HAc.

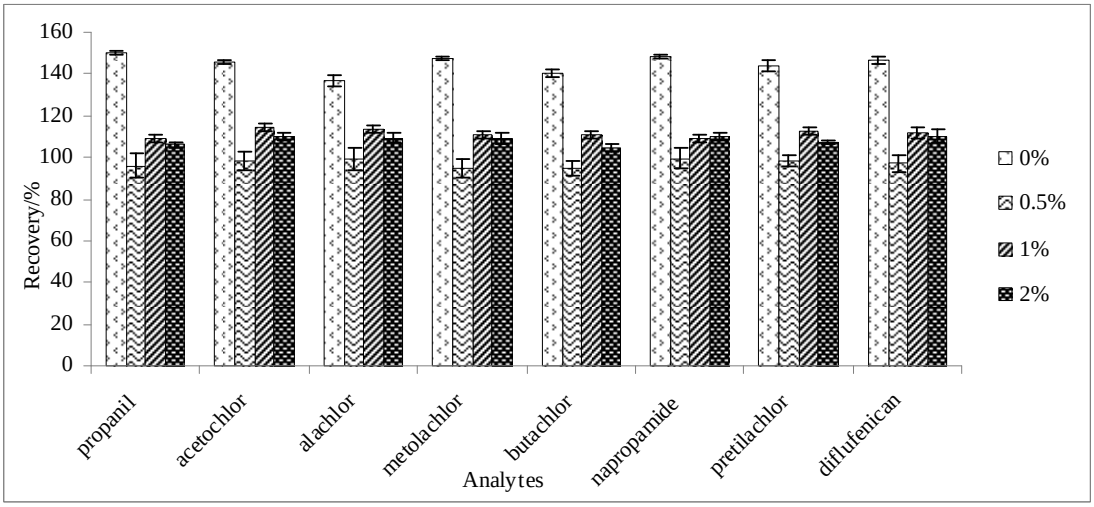

Fig. 2 Effects of concentration of HAc in ACN on herbicide extraction efficiency

Clean-up Step

In the original method, $MgSO_4$ is used in the dispersive SPE (d-SPE) procedure to remove trace amounts of water from the extract. PSA is a weak anion exchange sorbent that binds and retains carboxylic acids such as fatty acids from the ACN extracts. To increase the capacity to remove fatty acids from these types of sample extracts, Anastassiades et al. [17] increased the dosage of PSA from 25 to 150 mg per mL [28]. Lehotay et al. [29] found that the nonpolar sorbent C_{18} bound trace amounts of lipids more effectively, and that this was particularly effective in milk and egg extracts. Although C_{18} was not used in the original method, subsequent trials have shown that it is a useful clean-up sorbent, and that it does not affect pesticide recoveries. In this study, the investigation on using C_{18}, PSA, NH_2, and $MgSO_4$ as sorbents showed that there were no significant differences among the average recoveries of herbicides. In addition, $MgSO_4$ did not significantly improve the purification. Because some impurities dissolve readily in ACN, this solvent was not conducive to further purification and enrichment steps. Moreover, a weak polar solvent was required to protect the apparatus and the column. Therefore, ACN and $MgSO_4$ were replaced with acetone/*n*-hexane (2:8, *v/v*), which resulted in less interference from impurities. Consequently, the chromatograms of samples prepared using the C_{18} method showed more distinct herbicide peaks and fewer peaks from impurities than those of samples prepared using the NH_2 or PSA methods. Therefore, C_{18} was chosen as the sorbent for the clean-up step.

In addition, the results showed that recovery of the herbicide was better if the sample was homogenized, rather than vortexed. This is probably because homogenization results in smaller particles, increasing the ability of the solvent to extract the analytes from the matrix.

Matrix Effect

In present study, a significant matrix effect was observed; that is, the chromatographic response obtained from blank crop samples spiked with the chloroacetanilide herbicides was greater than that obtained from each respective stock solution. The matrix effect was first described by Erney et al. [24], and is known to be affected by the type of pesticide, the type of matrix, the pesticide-to-matrix ratio, and the GC system. To counteract the matrix effect, quantification was carried out using standard solutions that were dissolved with extracts of blank crop samples

processed by the method described above.

Method Evaluation

The performances of the modified QuEChERS method for detection of eight herbicides in soybean, rice, and wheat were validated by evaluating precision, linearity range, LOD, LOQ (*Table 1*), and recovery (*Table 2*).

Linearity

The linearity of the method was assayed by analyzing standard solutions dissolved in extracts of blank crop samples within a concentration range of 0.005–2.7 mg kg^{-1}. For all herbicides, the mean regression curves were linear with coefficients of ≥0.998 (*Table 1*). The typical GC/MS/SIM chromatograms of the reference standard, spiked, and measured crop samples are shown in *Fig. 3.*

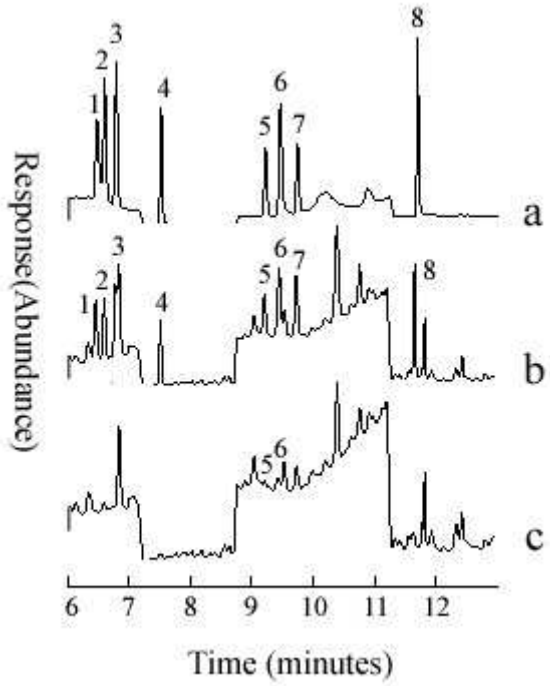

Fig. 3 Typical GC/MS/SIM chromatograms of reference standard (a), spiked (b) and measured (c) wheat samples (1. Propanil, 0.12 mg kg^{-1}; 2. Acetochlor, 0.16 mg kg^{-1}; 3. Alachlor, 0.144 mg kg^{-1}; 4. Metolachlor, 0.064 mg kg^{-1}; 5. Butachlor, 0.096 mg kg^{-1}; 6. Napropamide, 0.096 mg kg^{-1}; 7. Pretilachlor, 0.096 mg kg^{-1}; 8. Diflufenican, 0.096 mg kg^{-1} for spiked sample)

Repeatability

The repeatability of the chromatographic method was evaluated by analyzing a spiked sample containing 0.48 mg kg^{-1} of each of diflufenican, butachlor, napropamide, and pretilachlor, 0.6 mg kg^{-1} propanil, 0.78 mg kg^{-1} acetochlor, 0.72 mg kg^{-1} alachlor, and 0.3 mg kg^{-1} metolachlor. The sample was injected 20 times with an automatic injector. The repeatability expressed as relative standard deviations (RSDs) in *Table 1* ranged from 0.9 to 1.3% for all herbicides, demonstrating good repeatability.

Table 1 Validation parameters of the developed method

Pesticide	Equation	Linear (mg L^{-1})	Coeff. (r^2)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	MRL (mg kg^{-1})	Repeat. (RSDs, %) (*n*=20)
Propanil	y=23706x-1853	0.01-2.0	0.9997	1.5	5	2	1.3
Acetochlor	y=10787x-165.5	0.01-2.7	0.9999	2	6.5	0.02	1.0
Alachlor	y=13500x-1125	0.012-2.4	0.9999	4	12	0.02	1.1
Metolachlor	y=19456x-814.1	0.005-1.0	0.9999	1	3	0.2	1.1
Butachlor	y=10724x-836.5	0.008-1.6	0.9998	1.5	5	0.1	1.0
Napropamide	y=29326x-15199	0.008-1.6	0.9985	2	6	0.1	1.2
Pretilachlor	y=95941x-513.6	0.008-1.6	0.9998	3	8	0.1	1.4
Diflufenican	y=29489x-1599	0.008-1.6	0.9999	1	3	0.05	0.9

Table 2 Average recoveries of the herbicides from spiked samples (*n*=5)

Pesticide	Fortification levels (mg kg^{-1})	Soybean		Rice		Wheat	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Propanil	0.020	104.1	2.5	105.8	1.6	105.8	1.6
	0.060	100.0	1.3	101.5	1.4	103.5	1.1
	0.120	90.9	5.1	86.3	5.1	96.9	2.2
	0.600	95.6	5.1	78.7	5.1	104.1	0.6
Acetochlor	0.026	105.8	3.9	104.8	2.9	104.8	2.9
	0.080	105.0	3.9	104.4	2.6	97.2	3.7
	0.160	99.0	5.3	93.0	3.1	101.3	2.0
	0.800	100.2	2.5	98.2	0.7	106.1	1.1
Alachlor	0.024	101.7	1.9	100.3	2.8	100.3	2.8
	0.072	104.3	3.7	94.9	4.3	92.7	4.8
	0.144	103.1	7.1	86.0	3.4	99.2	3.3
	0.720	101.1	2.6	94.8	0.5	104.1	0.5
Metolachlor	0.010	99.6	3.0	105.6	2.6	105.6	2.6
	0.032	95.0	3.4	103.0	2.2	92.4	6.6
	0.064	91.7	7.1	89.1	3.2	98.0	3.2
	0.320	98.6	2.7	97.1	0.6	105.4	0.8
Butachlor	0.016	96.4	2.1	104.2	3.1	104.2	3.1
	0.048	101.9	2.7	94.3	3.7	92.1	1.5
	0.096	85.9	8.8	90.2	2.7	84.2	2.6
	0.480	88.5	3.1	79.3	2.6	104.0	1.8
Napropamide	0.016	95.3	3.9	90.6	3.4	90.6	3.4
	0.048	96.6	2.2	98.6	2.6	94.9	2.4
	0.096	105.1	6.9	84.1	2.7	98.0	1.9
	0.480	101.5	1.5	95.1	1.0	110.7	0.7
Pretilachlor	0.016	95.9	3.0	105.3	2.9	105.3	2.9
	0.048	104.5	2.7	93.8	4.3	94.5	2.9
	0.096	94.1	7.6	90.7	3.3	96.2	4.7
	0.480	98.4	3.0	97.9	1.0	105.3	1.1
Diflufenican	0.016	102.9	1.8	101.4	4.5	101.4	4.5
	0.048	101.9	2.4	106.0	2.9	97.8	3.1
	0.096	91.4	7.5	88.9	3.4	95.7	4.6
	0.480	98.4	3.1	96.2	0.8	106.3	1.1

Limits of Detection and Quantification

The LODs and LOQs for all herbicides in crop samples were defined as signal-to-noise ratios of 3 and 10, respectively. The LODs and LOQs for all herbicides were less than 0.01 and 0.02 mg kg^{-1}, respectively (*Table 1*). These concentrations are lower than the Chinese MRLs, indicating the high sensitivity of the method.

Recovery

The recoveries of the method were calculated by analyzing five replicates (n=5) of each type of sample (soybean, rice, and wheat) fortified with three concentrations of herbicides ranging from 0.01 to 0.8 mg kg^{-1}. For all herbicides, the recoveries (*Table 2*) were greater than 80% with RSDs of less than 9%.

Application of Method

The developed method was used to investigate herbicide residues in five soybean samples, 18 rice samples, and 10 wheat samples. These samples were collected from crop fields, markets, and families. The results showed that only one wheat sample contained detectable levels of butachlor (0.015 mg kg^{-1}) and napropamide (0.008 mg kg^{-1}), indicating that there were low levels of herbicide residues in crops.

CONCLUSIONS

A simple and sensitive analytical method for the simultaneous determination of eight chloroacetanilide herbicides in crops (soybean, rice, and wheat) was developed and validated. The method employed an optimized QuEChERS method, in which ACN containing 0.5% HAc was used as the extraction solvent. After extraction, samples were subjected to a simple clean-up step using C_{18} sorbent, and then analytes were detected by GC/MS/SIM in 13 min. The method showed good linearity, precision, and recovery, and low LODs and LOQs, indicating that it is sensitive and accurate. The method was used to analyze eight herbicides in 33 crop samples and low levels of butachlor and napropamide residues were found in only one sample. The results showed that this method is less time-consuming and high efficiency, making it suitable for monitoring of herbicide residues in crops.

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