

Determination of Pesticide Residues in Wine Using the Relative Correction Factor Method

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Abstract [Objectives] To establish a method for the simultaneous determination of five pesticide residues in wine, including metalaxyl, captan, kresoxim-methyl, tebuconazole, and difenoconazole, using QuEChERS combined with gas chromatography-tandem mass spectrometry (GC-MS/MS), and investigate the feasibility of applying the relative correction factor for multiple evaluations within a single test. [Methods] The target compounds were extracted using the QuEChERS method. Kresoxim-methyl served as the internal standard to calculate the relative correction factor for metalaxyl, captan, tebuconazole, and difenoconazole. Chromatographic peaks were identified based on their relative retention time to assess the stability of the correction factors. Method validation was performed under optimized GC-MS/MS conditions. [Results] The linearity of the five pesticides was satisfactory within the injection volume range of 0.02 to 0.8 ng. The correction factor showed robust tolerance across varying injection volumes, linear velocities, and chromatographic column conditions. The recovery rate of the added standards ranged from 79.6% to 92.7%, with a precision relative standard deviation (RSD) between 0.9% and 1.7%. The detection limits were between 0.002 and 0.006 mg/kg, while the quantification limits ranged from 0.007 to 0.02 mg/kg. [Conclusions] This method displays strong specificity, high sensitivity, and operational simplicity. When combined with the relative correction factor, it enables accurate and quantitative detection of residues of five pesticides in wine.

Key words Wine, Correction factor, GC-MS/MS, Pesticide residue

1 Introduction

The application of various pesticides is inevitable in grape cultivation. National laws and regulations have established strict guidelines regarding the permissible scope and dosage of these pesticides^[1]. However, due to insufficient popularization and understanding of these regulations, some fruit farmers do not adhere to the prescribed pesticide usage or commence harvesting before the expiration of the stipulated safe interval following pesticide application. This operation leads to excessive or out-of-range pesticide residues in the raw materials. As a consequence, these pesticides are inevitably introduced into the winemaking process, and prolonged consumption of such products may pose risks to human health^[2].

Currently, various methods are used for the detection of pesticide residues, including gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and enzyme-linked immunosorbent assay (ELISA). Among these techniques, mass spectrometry has increasingly become the preferred analytical approach due to its high sensitivity and selectivity. This study presents a detection method for the simultaneous determination of multiple pesticide residues in wine, utilizing a correction factor method based on GC-MS combined with QuECh-

ERS pretreatment. This method offers a technical solution for the monitoring of pesticide residues in such products^[3–8].

2 Materials and methods

2.1 Materials

2.1.1 Consumables and reagents. Standard solutions of metalaxyl, captan, kresoxim-methyl, tebuconazole, and difenoconazole at a concentration of 100 µg/mL were obtained from Beijing Manhage Biotechnology Co., Ltd. Acetonitrile and acetone, both of chromatographic grade, were procured from Shanghai Aladdin Biochemical Technology Co., Ltd. PSA, C₁₈, and GCB powders were acquired from Bonna-Agela Technologies Co., Ltd.

2.1.2 Instruments. The GC-MS/MS system, model TQ-8040, was manufactured by Shimadzu Enterprise Management (China) Co., Ltd., while the high-speed refrigerated centrifuge, model TGL-16, was produced by Changsha Xiangzhi Centrifuge Instrument Co., Ltd.

2.2 Methods

2.2.1 Standard working solution. The five pesticide residue standards were diluted with acetone to prepare a standard stock solution at a concentration of 10 µg/mL and stored under refrigeration for future use. The standard stock solution was prepared using a blank sample extract to create standard working solutions of varying concentrations, which were subsequently employed to construct the standard calibration curve.

2.2.2 Sample preparation methods. 5.00 g of the well-mixed sample was weighed and placed into a 50 mL centrifuge tube. After refrigeration for 20 min, 3 mL of chromatographic-grade acetonitrile was added, and the mixture was shaken for 5 min. Thereafter, 0.5 g of sodium chloride and 2.5 g of anhydrous magnesium

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sulfate were added, followed by vortexing for 1 min. The solution was then centrifuged at 8 000 rpm for 5 min. 1 mL of the supernatant was transferred to a centrifuge tube containing 50 mg of C_{18} , 50 mg of PSA, 10 mg of GCB, and 150 mg of anhydrous magnesium sulfate, and vortexed for 3 min before centrifugation. The resulting supernatant was concentrated to near dryness under a nitrogen stream, after which 1 mL of acetone was added. Finally, the solution was filtered through a 0.22 μm organic filter membrane.

2.2.3 GC-MS/MS conditions. The injection port was maintained at 250 $^{\circ}\text{C}$ with high-pressure splitless injection. A capillary column (Rxi-5Sil MS, 30 mm $\times$ 0.25 mm $\times$ 0.25 μm) was employed. The temperature program began at an initial temperature of 50 $^{\circ}\text{C}$, maintained for 1 min, followed by an increase to 280 $^{\circ}\text{C}$ at a rate of 20 $^{\circ}\text{C}/\text{min}$, which was then maintained for 10 min. The analysis was conducted under constant linear velocity mode. The injection volume was 1 μL , and helium (purity > 99.999%) was used as the carrier gas.

The solvent delay was set to 5 min. The interface temperature was maintained at 260 $^{\circ}\text{C}$, and the ion source temperature was 200 $^{\circ}\text{C}$. Ionization was performed using electron ionization (EI) mode with an ionization energy of 70 eV.

3 Results and analysis

3.1 Sample pretreatment Acetonitrile shows excellent compatibility with the majority of pesticides and effectively removes most impurities during extraction. Wine contains a large amount of interfering substances, such as sugars, pigments, and organic acids. In the QuEChERS method, sorbents such as C_{18} , PSA, and GCB are commonly used to mitigate the interference caused by these substances. Moreover, the addition of anhydrous magnesium sulfate and sodium chloride facilitates the more efficient transfer of target compounds into the organic phase. Based on prior experimental results, the optimal quantities of C_{18} , PSA, GCB, anhydrous magnesium sulfate, and sodium chloride were determined.

3.2 Optimization of chromatographic and mass spectrometry conditions The fragment ions produced by pesticide residues were monitored using scanning ion mode, with the results presented in Figs. 1 – 5. Collision voltage was optimized based on the abundance of each ion, leading to the establishment of the multiple reaction monitoring (MRM) method. The parameters for each target compound are detailed in Table 1.

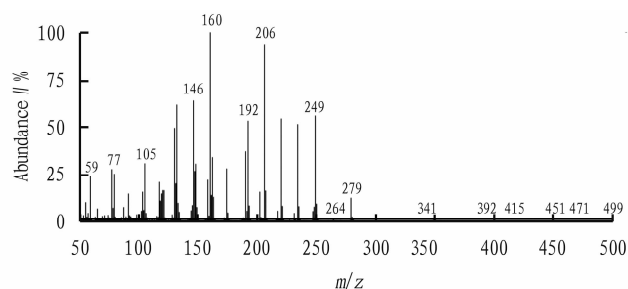


Fig. 1 Mass spectrometry of metalaxyl fragment ions

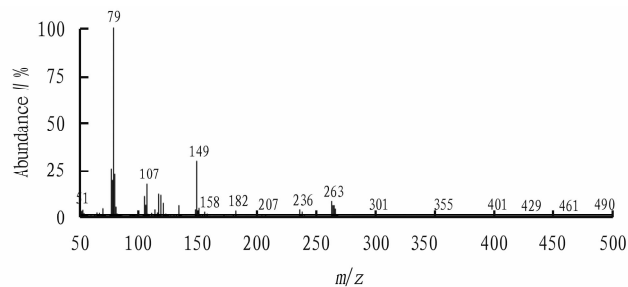


Fig. 2 Mass spectrometry of captan fragment ions

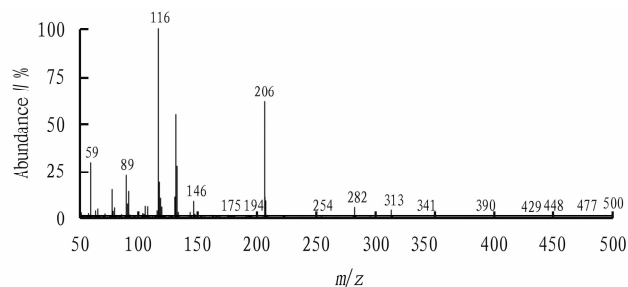


Fig. 3 Mass spectrometry of kresoxim-methyl fragment ions

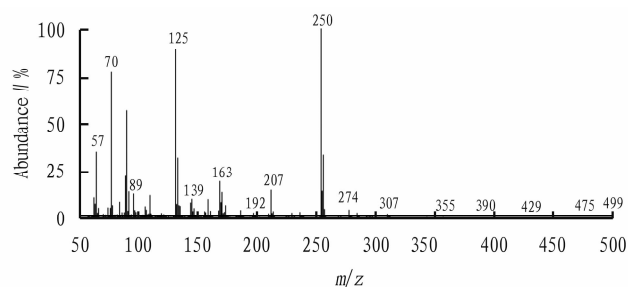


Fig. 4 Mass spectrometry of tebuconazole fragment ions

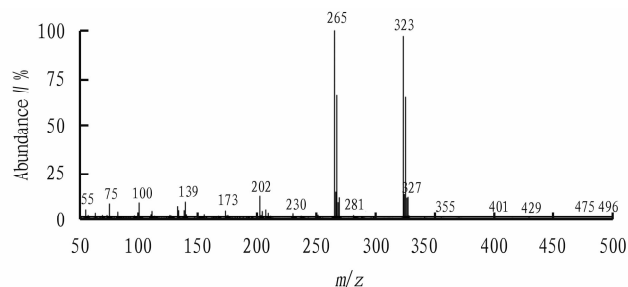


Fig. 5 Mass spectrometry of difenoconazole fragment ions

Table 1 Retention time of the target and MRM parameters

Target compound	Retention time//min	Quantitative ion pairs//m/z	CE	Quantitative ion pairs//m/z	CE
Metalaxyl	10.857	249 > 190	8	249 > 146	22
Captan	11.783	149 > 105	4	149 > 79	14
Kresoxim-methyl	12.237	206 > 116	6	206 > 131	14
Tebuconazole	13.209	250 > 125	22	250 > 153	12
Difenoconazole 1/2	18.383	323 > 265	14	323 > 202	28
	18.520				

3.3 Detection limit and quantification limit of the method

The detection limit was determined as 3 times the signal-to-noise ratio (S/N), while the quantification limit was established at

10 times the S/N. The corresponding results are presented in Table 2.

Table 2 Linear range and detection limit			mg/kg
Compound to be tested	Detection limit	Quantification limit	
Metalaxyl	0.002	0.007	
Captan	0.004	0.020	
Kresoxim-methyl	0.004	0.020	
Tebuconazole	0.002	0.007	
Difenoconazole 1/2	0.006	0.020	

3.4 Relative correction factor^[9] Experimental data demon-

Table 3 Tolerance of correction factors

Item		Compound to be tested			
		f metalaxyl/ f kresoxim-methyl	f captan/ f kresoxim-methyl	f tebuconazole/ f kresoxim-methyl	f difenoconazole/ f kresoxim-methyl
Injection volume//μL	0.8	0.834 7	0.120 6	1.185 7	1.324 4
	1.0	0.835 0	0.120 3	1.185 6	1.324 3
	1.2	0.835 2	0.120 4	1.185 2	1.324 6
Linear velocity//cm/sec	40	0.835 5	0.120 2	1.185 1	1.324 6
	42	0.835 0	0.120 3	1.185 6	1.324 3
	45	0.835 1	0.120 7	1.185 9	1.324 4
Chromatographic column	Rxi-5Sil MS	0.835 0	0.120 3	1.185 6	1.324 3
	DB-5 MS	0.835 5	0.120 3	1.185 5	1.324 6

The results in Table 3 indicated that variations in injection volume, linear velocity, and chromatographic column resulted in relatively minor fluctuations in the correction factor, demonstrating its good tolerance. Based on the determination results for kresoxim-methyl, the residue contents of the other four pesticides can be calculated simultaneously, thereby achieving the objective of multiple evaluations from a single test.

3.5 Recovery rate of the added standards Three blank wine samples were weighed, and standard solutions of metalaxyl, captan, kresoxim-methyl, tebuconazole, and difenoconazole were added. The samples were prepared following the established sample processing method, with an addition level of 0.02 mg/kg. The recovery rate and relative standard deviation (*RSD*) of the method were calculated, and the results are displayed in Table 4. Following spiking and extraction of the blank samples for detection, the separation of each compound was optimal, and both the recovery rate and precision satisfied the analytical criteria.

Table 4 Recovery rate of the added standards and its relative standard deviation (*n* = 3, %)

Target	Average recovery rate	<i>RSD</i>
Metalaxyl	87.9	2.2
Captan	90.4	1.8
Kresoxim-methyl	89.8	3.1
Tebuconazole	92.7	2.5
Difenoconazole 1/2	79.6	1.5

3.6 Precision A mixed standard solution containing five pesticides, each at a concentration of 0.3 μg/mL, was injected continuously 6 times. The *RSDs* for metalaxyl, captan, kresoxim-

strated a strong linear relationship when the injection volumes of metalaxyl ranged from 0.02 to 0.4 ng, captan from 0.1 to 0.8 ng, kresoxim-methyl from 0.02 to 0.4 ng, tebuconazole from 0.02 to 0.4 ng, and difenoconazole from 0.1 to 0.8 ng. The ratio of the slope of the linear regression equations for metalaxyl, captan, tebuconazole, and difenoconazole relative to kresoxim-methyl was used as the peak area correction factor. Given that variables such as injection volume, linear velocity, and different column brands may exert minor influences on the correction factor during the detection process, their tolerances were further examined. The results are presented in Table 3.

methyl, tebuconazole, and difenoconazole were 1.1% , 0.9% , 1.4% , 1.7% , and 1.2% , respectively. These results indicate that, under the specified measurement conditions, the instrument demonstrated good precision and satisfied the analytical requirements.

4 Conclusions

A sample pretreatment method was developed using the QuEChERS approach, and GC-MS was employed to simultaneously detect pesticide residues of metalaxyl, captan, kresoxim-methyl, tebuconazole, and difenoconazole in wine by targeting characteristic fragment ions. Meantime, the stability of metalaxyl, captan, tebuconazole, and difenoconazole was evaluated relative to the correction factor of kresoxim-methyl, with the objective of enabling a single test for multiple evaluations. The experimental results demonstrate that this method is characterized by simplicity, high sensitivity, and relatively stable correction factors, thereby satisfying analytical requirements and providing significant reference value for the analysis and detection of other pesticide residues in wine.

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tol strategies and rigorously managing all aspects of cultivation, economic losses caused by pests and diseases can be effectively reduced, thereby ensuring the yield and quality of under-forest *A. auricula* and promoting the green and sustainable development of this industry.

4 Conclusions

The under-forest cultivation model of *A. auricula*, recognized as an environmentally sustainable and efficient planting approach, has exhibited significant developmental potential. Forest ecosystems naturally provide shaded and humid conditions, which are advantageous for the cultivation of *A. auricula*. Utilizing these natural benefits not only conserves land resources and reduces production costs but also fosters economic development within forest areas^[10]. Nevertheless, to achieve high-quality yields in under-forest *A. auricula* production, cultivators must possess comprehensive techniques, including site selection, mushroom stick production, spawn running management, and ear emergence management, and effectively address key challenges such as pest and disease control, environmental regulation, and the enhancement of both yield and quality.

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