Determination of Coumoxystrobin Residue in Vegetables by QeEChERS-UPLC-MS/MS

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Abstract [**Objectives**] This study was conducted to establish a method for determining residual coumoxystrobin in vegetables using QeEChERS-liquid chromatography-tandem mass spectrometry (QeEChERS-LC-MS/MS). [**Methods**] The sample was extracted by acetonitrile, and the extract was purified by QeEChERS, concentrated by nitrogen blowing, and then detected. [**Results**] Coumoxystrobin had a good linear relation in the range of 0.01 - 10.0 mg/kg, and the linear equation was $y = 4\,686.92 \times x + 5\,683.28$, $R^2 = 0.999$. The limit of detection was 0.001 mg/kg, and the limit of quantitation was 0.003 mg/kg. [**Conclusions**] The method has the advantages of convenient and fast operation and stable detection process, and can provide technical support for the supervision and monitoring of coumoxystrobin.

Key words Vegetable; UPLC-MS/MS; Coumoxystrobin; Residual amount

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Coumoxystrobin is a methoxyacrylate fungicide having a chemical name of (E)-methyl 2-(2-(((3-butyl-4-methyl-2-oxo-2H-chromen-7-yl) oxy) methyl) phenyl)-3-methoxyacrylate and a molecular formula of $C_{26}H_{28}O_6$. In 2016, coumoxystrobin was officially registered by the Ministry of Agriculture of China, and its registered purpose was to prevent and control apple rot. Coumoxystrobin is effective against downy mildew, late blight, scab, anthracnose and leaf mold of fruits, vegetables and fruit trees, and also effective against ring rot, cotton fusarium wilt, rice blast, sheath blight, wheat common rot and southern blight of corn[1-4]. Bu *et al.*[5] analyzed the residues of coumoxystrobin and tebuconazole in corn, and made a dietary risk assessment. Wang *et al.*[6] established a QuEChERS-ultra-performance liquid chromatography-tandem mass spectrometry method (QeEChERS-UPLC-MS/MS) to determine the content of coumoxystrobin in soil. Wei *et al.*[7] studied the quantitative detection of coumoxystrobin residue in litchi. Jiao *et al.*[8] studied the determination of coumoxystrobin residue in soil by ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS/MS). Luo *et al.*[9] analyzed coumoxystrobin residue in the soil of orange orchards. QuEChERS has been widely used in the detection of chemical pollutant residues. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been widely studied in the detection of pesticide residues[10-11]. In this study, coumoxystrobin residue was investigated by combining QuEChERS and LC-MS/MS.

Materials and Methods

Reagents and equipment

High performance liquid chromatograph-tandem mass spectrometer: Waters XEVO TQ-S micro (Waters Corporation, USA); ultra-high speed freezing centrifuge (Xiangyi Centrifuge Instrument Co., Ltd.); nitrogen blower (Reeko Instrument Co., Ltd.); vortex oscillator (Shanghai Maiqi Environmental Protection Technology Co., Ltd.); electronic balance (0.000 1 g) (Mettler-Toledo Instruments (Shanghai) Co., Ltd); 0.22 μm organic filter membrane (Dikma Technologies).

Methanol (chromatographically pure); formic acid (99%); acetonitrile (chromatographically pure); sodium chloride (analytically pure); magnesium sulfate (analytically pure); N-(N-propyl) ethylenediamine (PSA); graphitized carbon black (GCB); standard substance of coumoxystrobin.

Standard solution

Standard stock solution was prepared with coumoxystrobin standard, CAS: 850881-70-8, purity≥99%. A certain amount of coumoxystrobin standard substance was accurately weighed into a 10 ml volumetric flask, and added with acetonitrile for dissolution, and then, the solution was diluted to constant weight to obtain a standard stock solution with a concentration of 100.00 μg/ml. The prepared stock solution was frozen at -18 ℃.

Standard working solution: Accurately, a certain amount of the standard stock solution was transferred to different 10 ml volumetric flasks, and diluted with acetonitrile to prepare standard working solutions with different concentrations. The prepared working solutions were prepared freshly for use.

Sample treatment

First, 2 g of sample was weighed and added with 4 g of sodium chloride and 20 ml of acetonitrile. After vortex-mixing for 1 min, ultrasonic treatment was performed for 30 min, and centrifugation was performed for 5 min at 4 000 r/min. Next, 8 ml of supernatant was added into a 15 ml plastic centrifuge tube containing 885 mg of magnesium sulfate, 150 mg of PSA and 15 mg of GCB. After vortex-mixing for 1 min, centrifugation was performed for 5 min at 4 000 r/min. Next, 5 ml of the supernatant was blown

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with nitrogen until it was almost dry, and 2 ml of acetonitrile was added. Finally, the sample solution was filtered with a 0.22 μm filter membrane, and determined by LC-MS/MS.

Instrument condition

LC condition Mobile phase A: 0.2% formic acid water; mobile phase B: acetonitrile, solution gradient elution (Table 1); chromatographic column: C_{18} (2.6 μm, 21 mm × 100 mm); flow rate: 0.3 ml/min; column temperature: 35 ℃; sample volume: 1 μl.

Table 1 Setting of mobile phase %

Time//min	Mobile phase A: 0.1% formic acid water	Mobile phase B: acetonitrile
0	90	10
0.4	90	10
0.9	80	20
2.2	10	90
3.5	10	90
4.0	90	10
5.0	90	10

MS conditions MS ion source: ESI; positive ion mode (Table 2); collision gas (CAD): 9.0; curtain gas (CUR): 30.0 psi; ion source gas (GSI): 55 psi; ion spray voltage (IS): positive ion 4 500 V; auxiliary heating gas (GSI): 55 psi, desolvation temperature (TEM): 550 ℃.

Table 2 Setting of positive ion mode

Name	Precursor ion	Daughter ion	Declustering potential (DP)//V	Collision energy//V
Coumoxystrobin	437.2	145.1*	65	13
		205.3	65	32

* Quantitation ion.

Results and Analysis

Determination of mobile phase conditions

Different mobile phase combinations were selected for comparison: acetonitrile-water, methanol-water, formic acid water-acetonitrile, and formic acid water-methanol. The separation degree between the target and impurities and the response value of the target at the same concentration were compared through the peak type of the target. The results showed that when acetonitrile: 0.2% formic acid solution was used as the mobile phase, the peak type of the target was the best, and the separation degree with impurities was good. It was finally chosen as the mobile phase for this method. The chromatogram of coumoxystrobin is shown in Fig. 1.

Linear range, standard curve, limit of detection and limit of quantitation

Standard working solutions with concentrations of 2, 5, 10, 20 and 50 ng/ml were prepared using the standard stock solution, respectively. A standard curve was drawn with the concentration of standard substance as the abscissa and the peak area of quantitation ion as the ordinate. The quantitative limit was calculated according to the signal-to-noise ratio S/N = 10, and the detection limit was calculated according to signal-to-noise ratio S/N = 3. Coumoxystrobin had a good linear relation in the range of 0.01 – 10.0 mg/kg, and the linear equation as $y = 4\,686.92 \times x + 5\,683.28$, $R^2 = 0.999$. The limit of detection was 0.001 mg/kg, and the limit of quantitation was 0.003 mg/kg.

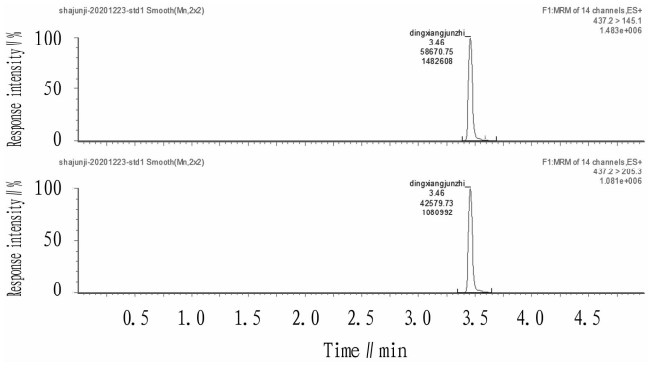

Fig. 1 Chromatogram of coumoxystrobin

Method accuracy test

The target was added to three substrates of cucumbers, Chinese cabbage and eggplants at three levels of 0.015, 0.030 and 0.100 mg/kg, respectively. The detection results of samples are shown in Table 3. The results in Table 3 showed that the recovery of coumoxystrobin in vegetables met the detection requirements.

Table 3 Recovery data of different addition concentrations

Substrate	Addition concentration//mg/kg	Recovery//%
Cucumber	0.015	94.3
	0.030	88.9
	0.100	81.2
Chinese cabbage	0.015	89.5
	0.030	103.2
	0.100	99.1
Eggplant	0.015	94.8
	0.030	101.1
	0.100	93.7

Conclusions and Discussion

A method for the determination of coumoxystrobin residue in vegetables by LC-MS/MS was established. The sample was extracted by acetonitrile, and the extract was purified by QeEChERS, concentrated by nitrogen blowing, and then detected. Coumoxystrobin had a good linear relation in the range of 0.01 – 10.0 mg/kg, and the linear equation was $y = 4\,686.92 \times x + 5\,683.28$, $R^2 = 0.999$. The limit of detection was 0.001 mg/kg, and the limit of quantitation was 0.003 mg/kg. The method has the advantages of high precision, convenient and fast operation and stable detection process, and can provide technical support for the supervision and monitoring of coumoxystrobin.

References

[1] LI M, LIU CL, ZHANG MX, *et al.* Design, synthesis and fungicidal activity of coumoxystrobin (SYP-3375)[J]. Chinese Journal of Pesticide Science, 2010, 12(4): 453 – 457. (in Chinese).

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Conclusions

In conclusion, the extraction process of MSP was optimized using single factor experiments and the response surface design. The optimal extraction conditions of MSP were as follows: extraction time 2 h, extraction temperature 40.5 ℃, solid-liquid ratio 1∶15 (g/ml), and enzyme dose 2.02%. The extraction yield reached 15.89%.

References

[1] ZHAO R, LIU SX, MA C. Development status and market analysis of *Morchella* industry in our country[J]. Chinese Edible Fungi, 2022, 39(2): 7 – 10.

[2] DAI YC, YANG ZL. Revision of the list and some names of medicinal fungi in China[J]. Journal of Bacteriology, 2008, 27(6): 801 – 824.

[3] CHEN Y, PAN J, ZHOU LW. Study on anti-tumor effect of exopolysaccharides from *Morchella* sp. [J]. Food Science, 2008, 29(9): 553 – 556.

[4] LI LB. Study on anti-fatigue effect of *Morchella* sp. [J]. Wild Plant Resources in China, 2001, 20(1): 17 – 18, 32.

[5] YANG F, WANG XF, WENG L. Antioxidant activity of two kinds of *Morchella* polysaccharide *in vitro*[J]. Food Science, 2010, 31(23): 76 – 78.

[6] LONG ZH, JI QX, ZHOU HY. Antibacterial activity of *Morchella* polysaccharide *in vitro*[J]. ShiZhen National Medicine, 2006, 17(6): 902 – 903.

[7] JIAO ML. Extraction of polysaccharides from *Morchella* esculenta and analysis of monosaccharide composition[J]. Western forestry science, 2016, 45(4): 130 – 135.

[8] LI MH, SHUN YX, ZHENG Y. Study on extraction technology of polysaccharide and exopolysaccharide from *Morchella pulcherrima* mycelium [J]. Study of fungi, 2017, 15(3): 195 – 200.

[9] ZHANG J, ZHAO H, LIU C. Optimization of extraction process of *Morchella* polysaccharide by osmotic pressure[J]. The food industry, 2015, 36(11): 167 – 171.

[10] ZHANG Y, LIU C. Extraction and purification of *Morchella* polysaccharide by high voltage pulsed electric field[J]. The food industry, 2016, 37(12): 54 – 58.

[11] ZHAO SH, NONG GZ, MENG LL. Study on enzy matic extraction process of polysaccharide from *Urena lobata* L. and investigation on its antioxidant activity[J]. Chin J Phar Toxicol, 2018(4): 341.

[12] WU P, TAN HY, ZHAN JF, *et al.* Optimization of bioprocess extraction of *Poria cocos* polysaccharide (PCP) with *Aspergillus niger* β-glucanase and the evaluation of PCP antioxidant property[J]. Molecules, 2020, 25(24): 5930 – 5948.

[13] XU TR, ZHANG HM, WANG SG, *et al.* A review on the advances in the extraction methods and structure elucidation of *Poria cocos* polysaccharide and its pharmacological activities and drug carrier applications [J]. International Journal of Biological Macromolecules, 2022(217): 536 – 551.

[14] XU HS, JIANG JP, XU P. Antioxidant activity of polysaccharide from *Radix Ginseng Rubra*[J]. Journal of Zhejiang Chinese Medical University, 2011(35): 909 – 912.

[15] XU HY, WANG S, PENG XJ. Comparison of polysaccharide content in *Gentiana apiata* before and after flowering and optimization of extraction process and antioxidant activities[J]. Journal of Tropical and Subtropical Botany, 2022(27): 1 – 10.

[16] FENG SL, CHENG HR, FU L. Ultrasonic-assisted extraction and antioxidant activities of polysaccharides from *Camellia oleifera* leaves[J]. International Journal of Biological Macromolecules, 2014(68): 7 – 12.

[17] DU XJ, MU HM, ZHOU S. Chemical analysis and antioxidant activity of polysaccharides extracted from *Inonotus obliquus* sclerotia[J]. International Journal of Biological Macromolecules, 2013(62): 691 – 69.

[18] LI Y, ZHU CP, ZHAI XC, *et al.* Optimization of enzyme assisted extraction of polysaccharides from pomegranate peel by response surface methodology and their anti-oxidant potential[J]. Chin. Herb. Med., 2018(10): 416 – 423.

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[2] SI NG, LIU JL, CHEN L. Application techniques of a new fungicide coumoxystrobin[J]. Pesticide Market News, 2010. 2010-24-029. (in Chinese).

[3] ZHANG L. Determination of coumoxystrobin TC by HPLC[J]. Modern Agrochemicals, 2017, 16(4): 30 – 31. (in Chinese).

[4] DING Q, JIA FY, CHEN G, *et al.* Residue analysis of coumoxystrobin in apple and soil[J]. Agrochemicals, 2012, 51(1): 53 – 54. (in Chinese).

[5] BU QZ, ZHANG L, HAO XL, *et al.* Residue analysis and dietary risk assessment of coumoxystrobin and tebuconazole in corn[J]. Agrochemicals, 2022, 61(11): 822 – 826. (in Chinese).

[6] WANG LF, LI CM, XU JB, *et al.* Simultaneous determination of 11 strobilurin fungicides in different vegetation types of soil by QuEChERS-ultra performance liquid chromatography-tandem mass spectrometry[J]. Chinese Journal of Chromatography, 2018, 36(8): 730 – 737. (in Chinese).

[7] WEI LQ, PAN XF, JIANG YB, *et al.* Quantitative detection method for four pesticide residues, namely flufiprole, coumoxystrobin, chlorodincarb and cyetpyrafen in lychee[J]. Guangxi Plant Protection, 2023, 36(4): 23 – 28. (in Chinese).

[8] JIAO B, XU J, LIU XG, *et al.* Simultaneous determination of pyrisoxazole and five other pesticides in typical types of Chinese soil using ultra-performance liquid chromatography and tandem mass spectrometry[J]. Journal of Agricultural Resources and Environment, 2020, 37(3): 424 – 431. (in Chinese).

[9] LUO HL, ZHOU JR, ZHOU S, *et al.* Residue analysis of coumoxystrobin in orchard soil[J]. Agrochemicals, 2020(2): 113 – 116. (in Chinese).

[10] YUAN X, LI Q, LI LJ, *et al.* Quantitative analysis of saflufenacil residue in food samples with HPLC-MS/MS[J]. Hunan Agricultural Sciences, 2022(9): 49 – 53.

[11] ZHENG M, ZHANBIN LI, LIU Y, *et al.* Determination of coumoxystrobin residues in milk[J]. Agricultural Biotechnology, 2021, 10(5): 12 – 13, 36.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU