

LC-MS/MS Analysis of 31 Restricted Pesticides in *Chrysanthemum morifolium* and *Ophiopogon japonicus*

Following Method VI in General Rule 2341 of ChP 2025

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Abstract

An UHPLC-MS/MS method was developed and validated for the simultaneous determination of 31 restricted pesticide residues in *Chrysanthemum morifolium* and *Ophiopogon japonicus* in accordance with Method VI, "Determination of Multiple Pesticide Residues in Relevant Medicinal Materials and Decoction Pieces," specified in the General Rule 2341 of the 2025 Chinese Pharmacopoeia. Following matrix-specific sample preparation and solvent extraction, chromatographic separation was achieved using a Purospher® STAR RP-C18 endcapped column (2 µm, Hibar® HR 100–2.1 mm). Method validation was performed in both herbal matrices by evaluating linearity, recovery, precision, and sensitivity. All target analytes exhibited excellent linearity, with correlation coefficients (R^2) equal or greater than 0.995. Mean recoveries ranged from 77.6–114.9%, meeting the acceptance criteria specified in the Chinese Pharmacopoeia. Relative standard deviations (RSDs) were below 9.4% at reporting level, demonstrating good method precision. These results demonstrate that the method is suitable for the routine determination of restricted pesticide residues in *Chrysanthemum morifolium* and *Ophiopogon japonicus*.

Introduction

Chrysanthemum morifolium and *Ophiopogon japonicus* are two medicinal herbs widely used in traditional Chinese medicine (TCM) due to their diverse therapeutic functions.¹ Phytochemical studies have shown that

Chrysanthemum morifolium contains flavonoids, volatile oils, and polysaccharides, which contribute to its antibacterial, anti-inflammatory, and antioxidant effects.² *Ophiopogon japonicus* contains saponins, steroids, carbohydrates, and various amino acids and has been reported to exhibit multiple pharmacological effects, including anti-inflammatory, immunomodulatory, and cardiopulmonary protective activities.^{3,4} Owing to their medicinal importance and growing demand, both herbs are cultivated extensively.

The large-scale cultivation of *Chrysanthemum morifolium* and *Ophiopogon japonicus* requires effective pest and disease management to maintain crop quality and yield. Consequently, pesticides are often used during cultivation to protect the crops and ensure consistent production. While the appropriate use of pesticides can effectively control pests and diseases, excessive or improper application may result in the accumulation of pesticide residues in plant tissues. These residues can enter the human body through the consumption of the herbal products and may pose potential health risks.⁵ Therefore, reliable analytical methods for monitoring pesticide residues in medicinal herbs are essential to ensure product quality, consumer safety, and regulatory compliance.

The analysis of pesticide residues in traditional Chinese medicinal materials received increasing attention since the release of the 2020 edition of the Chinese Pharmacopoeia.^{6,7} The 2025 edition further strengthens regulatory oversight through General Rule 2341, which introduces more comprehensive and stringent requirements on pesticide residue determination in Chinese herbal medicines. Compared with the 2020 edition, the list of banned pesticides has been expanded from 33 to 47 compounds, and the maximum residue limits for several highly toxic pesticides, including phorate, carbofuran, dicofol and metsulfuron-methyl,



Chrysanthemum morifolium



Ophiopogon japonicus

have been lowered. In addition, the 2025 edition establishes a dedicated regulatory framework for restricted pesticides via General Chapter 2341, covering 15 medicinal materials, including *Chrysanthemum morifolium* and *Ophiopogon japonicus*, and specifying 41 restricted pesticide residues, thereby filling the gap in the 2020 edition. Among the prescribed analytical procedures, Method VI—"Determination of Multiple Pesticide Residues in Relevant Medicinal Materials and Decoction Pieces", specifies multi-residue pesticide

detection methods for 15 types of medicinal materials, including *Chrysanthemum morifolium* and *Ophiopogon japonicus*.⁸ The method includes the determination of 10 pesticide residues by GC-MS/MS and 31 pesticide residues by LC-MS/MS.⁸ Based on Method VI of General Chapter 2341 in the 2025 Chinese Pharmacopoeia, an LC-MS/MS method was developed and evaluated for the determination of 31 restricted pesticide residues in *Chrysanthemum morifolium* and *Ophiopogon japonicus*.

Table 1. Alphabetical listings of the 31 pesticides in scope and peak IDs

Compound	Peak ID	Compound	Peak ID	Compound	Peak ID
Abamectin	31	Epoxiconazole	12	Propiconazole	19
Acetamiprid	5	Fenpyroximate	29	Pymetrozin	1
Boscalid	8	Fluazinam*	25	Pyraclostrobin	21
Carbendazim	3	Fludioxonil*	9	Pyrethrins II	24
Chlorpyrifos	26	Flusilazole	14	Pyrethrins I	28
Cyprodinil	11	Hexaconazole	20	Pyridaben	30
Diazinon	18	Imidacloprid	4	Pyrimethanil	6
Difenoconazole	23	Kresoxim-methyl	16	Tebuconazole	17
Dimethomorph - Isomer 1 & 2	10 a & b	Metalaxyl	7	Tebufozide	15
Ethionothion benzoate	27	Propamocarb	2	Thifluzamide*	13

Experimental

In accordance with Method VI of General Rule 2341 in the 2025 edition of the Chinese Pharmacopoeia, the 15 medicinal materials specified in this general chapter are categorized into three groups, each with a dedicated sample pretreatment workflow. *Chrysanthemum* (Group 1) and *Ophiopogon japonicus* (Group 2) were selected as representative matrix samples for this study.

Standard Preparation

Pesticide Stock Solutions (500 µg/mL): 10 mg of each of the 31 pesticide reference standards were accurately weighed into separate 20 mL volumetric flasks. The solutions were diluted to volume with acetonitrile and mixed thoroughly.

Mixed Standard Stock Solution (500 ng/mL): An aliquot of 10 µL from each of the 31 pesticide stock solutions, was transferred to a single 10 mL volumetric flask. The solution was diluted to volume with methanol and mixed thoroughly.

Blank Matrix Solution: The blank matrix solution was prepared by processing a blank matrix sample according to the same preparation procedure described below. Note: both matrices showed background of certain target compounds (see below).

Matrix-Matched Mixed Standard Solutions 1–7 (2.5, 5, 12.5, 25, 60, 125, and 250 ng/mL): Aliquots (2.0 mL) of the blank matrix solution were transferred into eight nitrogen evaporator vials and evaporated to dryness in a 40 °C water bath. The residues were reconstituted with 0.4 mL of methanol and spiked with 10 µL, 20 µL, 50 µL, 100 µL, 240 µL, 500 µL, and 1000 µL of the mixed standard stock solution, respectively. Subsequently,

0.6 mL of mobile phase A was added, and the solutions were brought to a final volume of 2.0 mL with methanol. The resulting solutions were vortex-mixed, filtered through a 0.2 µm PVDF membrane, and collected for LC-MS/MS analysis.

Sample Preparation

Preparation of *Chrysanthemum morifolium* sample solution:

1. *Chrysanthemum morifolium* powder (5.0 g, passed through a size No. 3 sieve), was accurately weighed and transferred to a 100 mL stoppered centrifuge tube.
2. 10 mL of water was added, and the mixture was shaken thoroughly.
3. The mixture was allowed to stand for 30 minutes.
4. 5 g of sodium chloride was added and the mixture was immediately shaken to ensure uniform dispersion.
5. 40 mL of acetonitrile was added, and the sample was homogenized at 12,000 rpm for 2 minutes.
6. The mixture was centrifuged at 4,000 rpm for 5 minutes.
7. The supernatant was collected. Acetonitrile (50 mL) was added to the residue, and the mixture was homogenized for 1 minute.
8. The mixture was centrifuged again at 4,000 rpm for 5 minutes.
9. The two supernatants were combined and diluted to 100 mL with acetonitrile.
10. The resulting solution was mixed thoroughly to obtain the *Chrysanthemum morifolium* sample solution.

11. An aliquot (2.0 mL) of the above solution was evaporated to dryness under a nitrogen stream at 40 °C. The residue was reconstituted with 0.4 mL of methanol, mixed with 0.6 mL of mobile phase A, and diluted to a final volume of 2.0 mL with methanol. The solution was filtered through a 0.2 µm PVDF membrane, and the filtrate was transferred to an autosampler vial for LC-MS/MS analysis.

Preparation of *Ophiopogon japonicus* sample solution:

1. *Ophiopogon japonicus* powder (3.0 g, passed through a size No. 3 sieve), was accurately weighed and transferred to a 50 mL stoppered centrifuge tube.
2. A 1% glacial acetic acid aqueous solution (15 mL) was added, and the mixture was vortexed to ensure uniform dispersion.
3. The mixture was allowed to stand for 30 minutes.
4. 15 mL of acetonitrile was added, and the mixture was vortex-mixed thoroughly.
5. The tube was placed on an oscillator and shaken at 500 cycles per minute for 5 minutes.
6. The tube was placed in an ice bath and allowed to stand for 20 minutes.
7. A mixture of anhydrous magnesium sulfate and anhydrous sodium acetate (7.5 g, 4:1) was added, and the mixture was immediately shaken to ensure uniform dispersion.
8. The tube was shaken on the oscillator for 3 minutes.
9. The mixture was centrifuged at 4,000 rpm for 5 minutes.
10. An aliquot (5 mL) of the supernatant was transferred to a volumetric flask, diluted to 20 mL with acetonitrile, and mixed thoroughly to obtain the *Ophiopogon japonicus* sample solution.
11. An aliquot (2.0 mL) of the above solution was evaporated to dryness under a nitrogen stream at 40 °C. The residue was reconstituted with 0.4 mL of

methanol, mixed with 0.6 mL of mobile phase A, and diluted to a final volume of 2.0 mL with methanol. The solution was filtered through a 0.2 µm PVDF membrane, and the filtrate was transferred to an autosampler vial for LC-MS/MS analysis.

Spiked samples-recovery/precision solution (0.25 mg/kg)

Chrysanthemum morifolium

Spiked solutions were prepared in accordance with the blank *Chrysanthemum morifolium* pretreatment procedure described above. After weighing blank powder in Step 1 and before extraction, 2.5 mL mixed standard stock solution (500 ng/mL) was added to 5.0 g blank powder to obtain a fortification level of 0.25 mg/kg, followed by 30 min equilibration. All subsequent extraction, dilution, nitrogen evaporation, reconstitution and filtration procedures were identical to Steps 1–11 described above.

Ophiopogon japonicus

Spiked solutions were prepared in accordance with the blank *Ophiopogon japonicus* pretreatment procedure described above. After weighing blank powder in Step 1 and before extraction, 1.5 mL mixed standard stock solution (500 ng/mL) was added to 3.0 g blank powder to obtain a fortification level of 0.25 mg/kg, followed by 30 min equilibration. All subsequent extraction, dilution, nitrogen evaporation, reconstitution and filtration procedures were identical to Steps 1–11 described above.

Spiked samples-recovery/precision solution (0.05 mg/kg)

The reporting limit (RL) spiked samples of *Chrysanthemum morifolium* and *Ophiopogon japonicus* were prepared following the same procedure as the 0.25 mg/kg fortified group. Before extraction, 0.5 mL (*Chrysanthemum*) and 0.3 mL (*Ophiopogon*) of the 500 ng/mL mixed standard stock solution was added to the weighed blank matrix to obtain a spiking level of 0.05 mg/kg.

LC-MS/MS Analysis

The matrix-matched calibration standards and samples were analyzed by LC-MS under the chromatographic

and mass spectrometric conditions summarized in **Tables 2 to 4.**

Table 2. LC-MS conditions for the analysis of 31 restricted pesticide residues

LC Conditions				
Instrument:	Waters Acquity UPLC I-class plus			
Column:	Purospher® STAR RP-C18 endcapped (2 µm) Hibar® HR, 100 x 2.1 mm I.D. (1.50648)			
Mobile phase:	[A] 0.1% Formic acid (FA) solution with 5 mmol/L ammonium formate; [B] Methanol – 0.1% FA solution with 5 mmol/L ammonium formate (95:5)			
Gradient:	Time (min)	A (%)	B (%)	Curve
	0.00	70	30	6
	1.00	70	30	6
	12.00	0	100	6
	14.00	0	100	6
	14.10	70	30	6
	16.00	70	30	6
Flow rate:	0.3 mL/min			
Column temp.:	40 °C			
Detector:	MS/MS, MRM (for conditions see Tables 3 and 4)			
Injection:	2 µL			
Sample(s):	See experimental section			

Table 3. MS conditions

MS conditions	(ESI+)	(ESI-)
Instrument:	Waters Xevo TQ-S	Waters Xevo TQ-S
Polarity:	Positive	Negative
Spray voltage:	3.0 kV	2.8 kV
Capillary temp:	250 °C	250 °C
Desolvation temp:	400 °C	400 °C
Desolvation:	800 L/h	800 L/h
Cone:	150 L/h	150 L/h
Nebuliser:	7 bar	7 bar

Table 4. MRM parameters used and retention times (sorted as listed in Chinese Pharmacopeia)

Peak ID	Compound	RT (min)	Parent ion (m/z) Qualifier Quantifier	Daughter ion (m/z) Qualifier Quantifier	Cone (V) Qualifier Quantifier	Collision energy (V) Qualifier Quantifier
1	Pymetrozin	1.15	218.0	105.0	34	18
			218.0	78.0	34	40
2	Propamocarb	1.36	188.9	102.1	38	16
			188.9	144.0	38	12
3	Carbendazim	2.33	192.0	160.0	28	16
			192.0	132.1	28	26
4	Imidacloprid	3.09	256.0	209.0	28	14
			256.0	175.0	28	22
5	Acetamiprid	3.84	222.9	126.0	42	20
			222.9	90.0	42	32
6	Pyrimethanil	8.82	200.0	107.1	54	24
			200.0	183.1	54	22
7	Metalaxyl	7.62	280.0	248.1	34	10
			280.0	220.1	34	14
8	Boscalid	8.82	342.9	307.0	32	18
			342.9	140.0	32	30
9	Fludioxonil*	8.92	247.0	126.0	66	30
			247.0	180.0	66	28
10 a & b	Dimethomorph (2 isomers)	9.01	388.0	301.0	52	20
			388.0	165.0	52	30
11	Cyprodinil	10.48	226.0	108.0	58	30
			226.0	93.1	58	34
12	Epoxiconazole	9.77	330.0	121.1	32	20
			330.0	141.5	32	18
13	Thiifluzamide*	9.66	526.8	124.9	52	42
			526.8	165.9	52	24
14	Flusilazole	9.87	316.0	247.0	48	16
			316.0	165.0	48	26
15	Tebufenozide	9.85	353.1	133.0	18	18
			353.1	297.0	18	8
16	Kresoxim-methyl	10.19	314.0	267.0	12	6
			314.0	222.1	12	14
17	Tebuconazole	10.17	308.1	125.0	26	20
			308.1	70.0	26	32
18	Diazinon	10.51	305.0	169.0	26	20
			305.0	96.9	26	28
19	Propiconazole	10.42	342.0	159.0	40	28
			342.0	205.0	40	16
20	Hexaconazole	10.47	314.0	70.1	28	16
			314.0	159.0	28	30
21	Pyraclostrobin	10.61	388.0	296.0	20	12
			388.0	194.0	20	14
22	Tridemorph	9.94	298.1	130.0	70	26
			298.1	98.0	70	30

Table 4. MRM parameters used and retention times (sorted as listed in Chinese Pharmacopeia) – (continued)

Peak ID	Compound	RT (min)	Parent ion (m/z) Qualifier Quantifier	Daughter ion (m/z) Qualifier Quantifier	Cone (V) Qualifier Quantifier	Collision energy (V) Qualifier Quantifier
23	Difenoconazole	10.90	406.0	251.0	46	26
			406.0	337.0	46	16
24	Pyrethrins II	11.05	373.1	161.0	38	8
			373.1	143.0	38	16
25	Fluazinam*	11.56	462.8	415.9	44	20
			462.8	397.9	44	18
26	Chlorpyrifos	12.01	349.8	198.0	22	20
			349.8	97.0	22	30
27	Emamectin benzoate	11.28	886.4	158.0	40	36
			886.4	302.0	40	30
28	Pyrethrins I	12.22	329.1	161.0	26	10
			329.1	133.0	26	18
29	Fenpyroximate	12.53	422.0	138.0	22	30
			422.0	366.0	22	16
30	Pyridaben	12.67	365.0	147.1	22	26
			365.0	309.0	22	12
31	Abamectin	12.68	895.3	751.3	94	46
			895.3	327.0	94	54

Note: Pesticides marked with “*” were analyzed in negative ion scanning mode, while all other pesticides were analyzed in positive ion scanning mode.

Results

Pesticide residues in *Chrysanthemum morifolium* and *Ophiopogon japonicus* were extracted according to the sample preparation procedures described in Method VI in the 2025 edition of General Rule 2341 and quantified by LC-MS/MS using matrix-matched external calibration. Method performance was evaluated through the assessment of matrix-matched calibration curves, precision, recovery, and sensitivity in both *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples.

The chromatograms of *Chrysanthemum morifolium* and *Ophiopogon japonicus* matrix matched standards containing 31 pesticides at a concentration of 12.5 ng/mL are shown in **Figures 3 and 4**. Chromatograms of the unspiked *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples are shown in **Figures 5 and 6**, respectively.

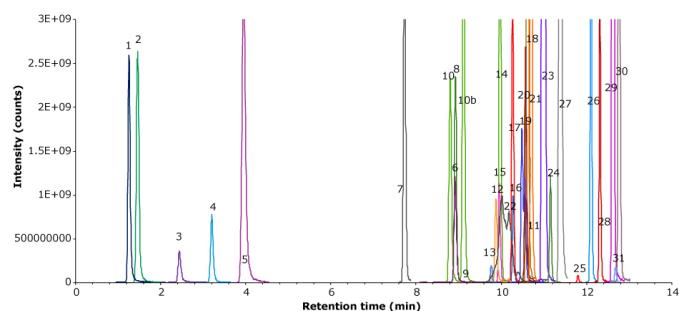


Figure 3. LC-MS/MS chromatogram (qualifier) of the *Chrysanthemum morifolium* matrix mixed standard (12.5 ng/mL each).

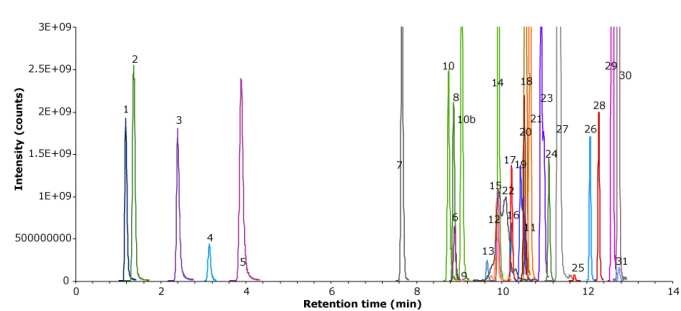


Figure 4. LC-MS/MS chromatogram (qualifier) of the *Ophiopogon japonicus* matrix mixed standard (12.5 ng/mL each).

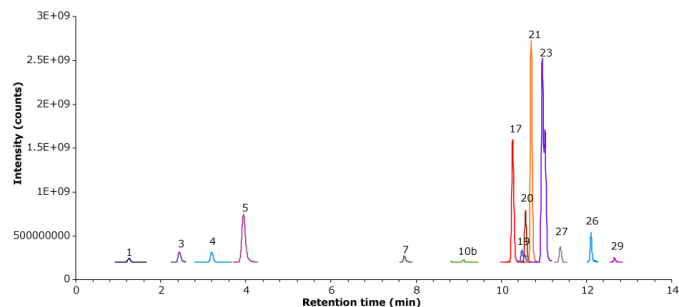


Figure 5. The MRM chromatogram (quantifier) of the unspiked *Chrysanthemum morifolium* sample.

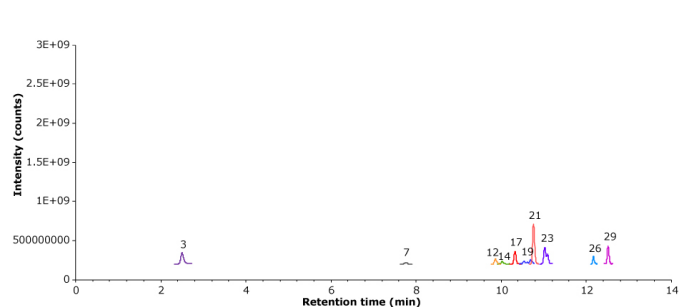


Figure 6. The MRM chromatogram (quantifier) of the unspiked *Ophiopogon japonicus* sample.

Calibration

Matrix-matched mixed standard solutions containing 31 pesticides at concentrations of 2.5, 5, 12.5, 25, 60, 125, and 250 ng/mL were prepared in *Chrysanthemum morifolium* and *Ophiopogon japonicus* matrices as described above, representing sample concentration of 0.05 to 50 mg/kg. Calibration performance data

are summarized in **Tables 5** and **6**, respectively. For three pesticides - diazinon, emamectin benzoate, and fenpyroximate - detector saturation was observed at higher concentrations and therefore, a reduced calibration range was used for these analytes.

Table 5. Calibration data of 31 pesticides in *Chrysanthemum morifolium* matrix-spiked samples

Peak No.	Compound	Calibration curve	Linear range (ng/mL)	R ²
1	Pymetrozin	$y = 12,220.6025 x + 11,658.8071$	2.5–250	1.0000
2	Propamocarb	$y = 21,835.7230 x - 13,593.3540$	2.5–250	0.9999
3	Carbendazim	$y = 11,083.6007 x + 695.4017$	2.5–250	0.9999
4	Imidacloprid	$y = 3,266.0582 x + 3,269.0111$	2.5–250	0.9998
5	Acetamiprid	$y = 22,010.8146 x + 28,756.8057$	2.5–250	0.9999
6	Pyrimethanil	$y = 6,790.6234 x - 10,053.5059$	2.5–250	0.9993
7	Metaxyl	$y = 17,328.6691 x + 21,368.9937$	2.5–250	0.9999
8	Boscalid	$y = 10,175.3252 x + 5,749.8802$	2.5–250	0.9999
9	Fludioxonil*	$y = 102.1278 x + 66.6147$	2.5–250	0.9998
10 a + b	Dimethomorph	$y = 22,692.5457 x + 5,666.1364$	2.5–250	1.0000
11	Cyprodinil	$y = 10,105.9903 x + 13,114.1843$	2.5–250	0.9995
12	Epoxiconazole	$y = 984.5651 x - 251.3274$	2.5–250	0.9999
13	Thiifluzamide*	$y = 605.1057 x + 439.8980$	2.5–250	0.9997
14	Flusilazole	$y = 21,882.3032 x + 39,423.1192$	2.5–250	0.9996
15	Tebuconazole	$y = 4,153.3107 x + 14,818.2351$	2.5–250	0.9991
16	Kresoxim-methyl	$y = 3,613.1219 x + 5,440.2846$	2.5–250	0.9997
17	Tebuconazole	$y = 7,076.9697 x + 4,211.3894$	2.5–250	0.9999
18	Diazinon	$y = 55,119.5673 x + 60,788.6994$	2.5–125	0.9993
19	Propiconazole	$y = 11,023.3014 x + 5,581.4373$	2.5–250	1.0000
20	Hexaconazole	$y = 8,392.0574 x + 15,278.2662$	2.5–250	0.9994
21	Pyraclostrobin	$y = 20,333.9733 x + 35,736.6816$	2.5–250	0.9998
22	Tridemorph	$y = 22,567.9496 x - 8,395.2610$	2.5–250	0.9999
23	Difenoconazole	$y = 29,008.7988 x - 14,255.5661$	2.5–250	0.9999
24	Pyrethrins II	$y = 4,979.7133 x + 2,517.1361$	2.5–250	0.9999
25	Fluazinam*	$y = 682.4289 x - 2,044.9792$	2.5–250	0.9988
26	Chlorpyrifos	$y = 11,560.6428 x - 423.0877$	2.5–250	1.0000
27	Emamectin benzoate	$y = 100,484.0931 x + 147,863.0429$	2.5–60	0.9986
28	Pyrethrins I	$y = 11,031.0298 x - 6,441.5257$	2.5–250	0.9999
29	Fenpyroximate	$y = 58,454.8856 x + 50,225.2355$	2.5–125	0.9997
30	Pyridaben	$y = 28,854.0039 x + 11,118.9771$	2.5–250	1.0000
31	Abamectin	$y = 1,338.7225 x - 384.2759$	2.5–250	0.9999

Table 6. Calibration data of 31 pesticides in *Ophiopogon japonicus* matrix-spiked samples

Peak No.	Compound	Calibration curve	Linear range (ng/mL)	R ²
1	Pymetrozin	$y = 8,538.0043 x + 59.4074$	2.5–250	0.9999
2	Propamocarb	$y = 19,214.5521 x - 18,891.6102$	2.5–250	0.9998
3	Carbendazim	$y = 8,402.1395 x + 7,211.6338$	2.5–250	0.9999
4	Imidacloprid	$y = 2,315.2659 x + 813.4425$	2.5–250	0.9998
5	Acetamiprid	$y = 17,394.6445 x - 2,855.7408$	2.5–250	0.9998
6	Pyrimethanil	$y = 4,141.3038 x - 7,069.2858$	2.5–250	0.9997
7	Metaxyl	$y = 15,179.9060 x + 9,626.1591$	2.5–250	0.9993
8	Boscalid	$y = 8,902.0104 x + 973.4356$	2.5–250	0.9998

Table 6. Calibration data of 31 pesticides in *Ophiopogon japonicus* matrix-spiked samples (continued)

Peak No.	Compound	Calibration curve	Linear range (ng/mL)	R ²
9	Fludioxonil*	$y = 136.5956x + 122.1703$	2.5–250	0.9991
10 a + b	Dimethomorph	$y = 29,011.0700x - 5,668.7664$	2.5–250	0.9998
11	Cyprodinil	$y = 2,371.7246x - 4,720.2545$	2.5–250	0.9994
12	Epoxiconazole	$y = 4,116.3872x + 2,976.4308$	2.5–250	0.9998
13	Thifluzamide*	$y = 328.7999x + 2,217.5846$	2.5–250	0.9947
14	Flusilazole	$y = 16,590.7114x + 16,649.0577$	2.5–250	0.9997
15	Tebuconazole	$y = 4,789.1638x - 7,278.7183$	2.5–250	0.9970
16	Kresoxim-methyl	$y = 3,054.8739x - 3,935.1713$	2.5–250	0.9996
17	Tebuconazole	$y = 4,999.1344x - 8,537.6000$	2.5–250	0.9980
18	Diazinon	$y = 47,209.7351x - 6,793.7547$	2.5–125	0.9995
19	Propiconazole	$y = 10,227.6055x - 2,904.4770$	2.5–250	0.9998
20	Hexaconazole	$y = 8,520.6188x + 3,477.0698$	2.5–250	0.9997
21	Pyraclostrobin	$y = 18,427.8097x + 14,444.7541$	2.5–250	0.9999
22	Tridemorph	$y = 19,457.3432x - 11,552.9373$	2.5–250	0.9996
23	Difenoconazole	$y = 21,884.1495x + 9,992.8278$	2.5–250	0.9999
24	Pyrethrins II	$y = 5,776.2614x - 11,112.1365$	2.5–250	0.9987
25	Fluazinam*	$y = 785.4430x - 1,538.9246$	2.5–250	0.9997
26	Chlorpyrifos	$y = 5,249.1871x + 806.1808$	2.5–250	0.9999
27	Emamectin benzoate	$y = 96,746.0193x - 11,030.2029$	2.5–60	0.9984
28	Pyrethrins I	$y = 7,210.6241x - 12,094.8695$	2.5–250	0.9990
29	Fenpyroximate	$y = 49,997.6529x - 28,236.8267$	2.5–125	0.9998
30	Pyridaben	$y = 26,263.0806x - 6,474.3480$	2.5–250	0.9998
31	Abamectin	$y = 920.2853x + 548.0537$	2.5–250	0.9998

Recovery and Precision

Method recovery and precision were evaluated for both sample solutions fortified at reporting limit (RL) of 0.05 mg/kg and at 0.25 mg/kg, respectively. Recovery rates and relative standard deviations (RSDs) were determined from spiked recovery experiments and multiple replicate measurements ($n = 3$). The results are summarized in **Table 7** and **Table 8**. At RL the recoveries ranged from

77.6–109.8% and RSDs from 0.3–9.4% across both sample types; at spike concentration of 0.25 mg/kg recoveries were 91.8–114.9% and RSDs 0.2–6.6% (acceptance criterium specified in the Chinese Pharmacopoeia for recovery is 70–120%, the RSDs complied with the requirements of ChP General Chapter 9101).

Table 7. Mean recoveries ($n = 3$) and relative standard deviations (RSDs) for 31 Pesticides for *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples spiked at 0.05 mg/kg (RL)

Peak No.	Compound	<i>Chrysanthemum</i> mean recovery (%)	RSD (%)	<i>Ophiopogon</i> mean recovery (%)	RSD (%)
1	Pymetrozin	89.0	5.2	96.4	1.8
2	Propamocarb	100.1	1.9	98.9	0.9
3	Carbendazim	96.3	0.9	95.7	1.2
4	Imidacloprid	97.5	3.4	95.4	1.0
5	Acetamiprid	86.1	9.4	95.8	1.2
6	Pyrimethanil	81.8	3.0	86.8	3.3
7	Metaxyl	78.4	2.4	98.5	2.1
8	Boscalid	101.9	6.7	98.6	3.4
9	Fludioxonil*	100.3	4.3	77.6	1.6
10 a & b	Dimethomorph	98.6	3.8	97.4	2.0
11	Cyprodinil	94.8	3.9	87.0	2.0
12	Epoxiconazole	105.6	3.0	99.3	4.0
13	Thifluzamide*	95.6	5.6	93.2	6.0

Table 7. Mean recoveries ($n = 3$) and relative standard deviations (RSDs) for 31 Pesticides for *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples spiked at 0.05 mg/kg (RL) – (continued)

Peak No.	Compound	<i>Chrysanthemum</i> mean recovery (%)	RSD (%)	<i>Ophiopogon</i> mean recovery (%)	RSD (%)
14	Flusilazole	89.7	1.8	98.4	1.1
15	Tebufozide	92.5	4.0	92.1	2.8
16	Kresoxim-methyl	96.6	6.8	94.2	3.2
17	Tebuconazole	105.1	9.2	92.4	1.6
18	Diazinon	101.4	2.6	106.1	1.3
19	Propiconazole	100.1	6.1	96.2	2.0
20	Hexaconazole	99.4	6.2	97.3	1.3
21	Pyraclostrobin	88.6	6.1	96.4	0.8
22	Tridemorph	98.3	0.5	97.0	0.3
23	Difenoconazole	101.1	4.0	95.5	0.9
24	Pyrethrins II	102.0	4.0	94.1	1.3
25	Fluazinam*	109.8	5.8	93.0	1.8
26	Chlorpyrifos	99.1	3.2	96.1	1.3
27	Emamectin benzoate	100.6	6.2	89.8	2.6
28	Pyrethrins	105.1	3.5	90.6	1.8
29	Fenpyroximate	107.7	4.1	95.5	1.3
30	Pyridaben	103.8	4.6	84.4	1.3
31	Abamectin	100.5	6.9	97.7	1.1

Table 8. Mean recoveries ($n = 3$) and relative standard deviations (RSDs) for 31 Pesticides for *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples spiked at 0.25 mg/kg

Peak No.	Compound	<i>Chrysanthemum</i> mean recovery (%)	RSD (%)	<i>Ophiopogon</i> mean recovery (%)	RSD (%)
1	Pymetrozin	103.4	0.9	107.9	0.9
2	Propamocarb	102.0	1.7	109.2	1.1
3	Carbendazim	98.9	2.0	106.6	2.1
4	Imidacloprid	106.6	1.2	106.7	0.5
5	Acetamiprid	103.6	1.7	108.0	1.6
6	Pyrimethanil	91.8	1.3	95.0	4.9
7	Metalaxyl	105.4	1.1	110.3	0.9
8	Boscalid	104.0	3.4	110.4	5.6
9	Fludioxonil*	107.2	5.3	109.7	3.1
10 a & b	Dimethomorph	102.1	2.8	108.1	2.2
11	Cyprodinil	96.4	2.3	100.0	4.8
12	Epoxiconazole	96.7	3.5	107.8	2.7
13	Thifluzamide*	105.2	1.8	108.8	1.9
14	Flusilazole	104.5	1.7	109.8	1.5
15	Tebufozide	104.1	2.2	102.4	1.8
16	Kresoxim-methyl	105.3	3.5	103.8	1.7
17	Tebuconazole	103.8	2.6	106.9	2.9
18	Diazinon	103.7	0.3	112.2	0.2
19	Propiconazole	103.6	2.1	106.9	2.3
20	Hexaconazole	105.3	2.3	108.4	2.6
21	Pyraclostrobin	104.4	0.9	107.8	2.3
22	Tridemorph	101.6	0.5	105.4	0.4
23	Difenoconazole	104.5	1.4	106.3	2.3
24	Pyrethrins II	98.7	2.6	107.0	1.4

Table 8. Mean recoveries ($n = 3$) and relative standard deviations (RSDs) for 31 Pesticides for *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples spiked at 0.25 mg/kg (continued)

Peak No.	Compound	<i>Chrysanthemum</i> mean recovery (%)	RSD (%)	<i>Ophiopogon</i> mean recovery (%)	RSD (%)
25	Fluazinam*	95.8	3.5	108.6	4.8
26	Chlorpyrifos	103.1	1.2	108.4	0.8
27	Emamectin benzoate	114.9	6.6	105.5	3.0
28	Pyrethrins I	104.6	1.0	100.2	2.0
29	Fenpyroximate	105.6	1.7	113.3	0.9
30	Pyridaben	111.9	1.1	100.8	2.5
31	Abamectin	101.6	2.2	100.7	4.3

Analysis of Unspiked Samples

The concentrations of 31 pesticide residues in unspiked *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples were determined using the developed LC-MS/MS method. The results are summarized in **Table 9**.

Table 9. Results of 31 pesticide residues in unspiked *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples

Sample	Peak ID	Compound	Determined content (mg/kg)
Unspiked <i>Chrysanthemum morifolium</i>	3	Carbendazim	0.13
	17	Tebuconazole	0.23
	21	Pyraclostrobin	0.10
	23	Difenoconazole	0.13
	All other detected (Figure 5) <Reporting Level (0.05 mg/kg).		
Unspiked <i>Ophiopogon japonicus</i>	The residues detected (Figure 6) were all <Reporting Level (0.05 mg/kg).		

Conclusion

A UHPLC-MS/MS method based on triple quadrupole mass spectrometry was developed for the simultaneous determination of 31 pesticide residues in *Chrysanthemum morifolium* and *Ophiopogon japonicus* samples in accordance with the General Rule 2341, Method VI, of the Chinese pharmacopoeia. Sample preparation was performed using an extraction step involving solvent & salt addition and centrifugation, followed by chromatographic separation on a Purospher® STAR RP-C18 endcapped (2 µm) Hibar® HR 100–2.1 mm column.

The method demonstrated excellent linearity for all target analytes, with correlation coefficients

(R^2) equal or greater than 0.995 (Thifluzamide in *Ophiopogon* at 0.9947). The calibration range was 2.5–250 ng/mL for 28 pesticides, 2.5–125 ng/mL for diazinon and fenpyroximate, and 2.5–60 ng/mL for emamectin benzoate. The mean recoveries for samples spiked at 0.25 mg/kg ranged from 91.8–114.9%, while those obtained at the reporting limit concentration (0.05 mg/kg) were in the range of 77.6–109.8%, meeting the acceptance criteria of 70–120% specified in the Chinese Pharmacopoeia. The relative standard deviations (RSDs) ranged from 0.2–9.4%, demonstrating excellent method precision and compliance with the requirements of ChP General Chapter 9101.

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Flusilazole, PESTANAL®, analytical standard	45753
Hexaconazole, PESTANAL®, analytical standard	34348
Imidacloprid, PESTANAL®, analytical standard	37894
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