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Response Surface Methodology-Optimized QuEChERS Combined with Liquid Chromatography–Quadrupole-Time-of-Flight Mass Spectrometry for Simultaneous Screening of Pesticides and Mycotoxins in Astragalus

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Abstract

This study used the QuEChERS method in combination with liquid chromatography–quadrupole-time-of-flight mass spectrometry (LC-Q-TOF/MS) to develop a method for simultaneous detection of 187 pesticides and 10 mycotoxins in Astragalus. The samples were extracted using an acetonitrile–water solution containing 5% formic acid, and the amount of purification materials was optimized through response surface methodology. The results show that 197 compounds exhibit good linear relationships within their respective linear ranges ($R^2 > 0.995$). The screening detection limits (SDLs) and the limits of quantification (LOQs) ranged from 0.001 to 0.02 mg/kg and 0.002 to 0.02 mg/kg, respectively. At the spiked levels of 1, 2, and 10 times LOQ, compound recoveries ranged from 61.5% to 118.9%, 67.1% to 119.6%, and 72.0% to 119.3%, respectively, with relative standard deviations (RSDs) all less than 20.0%. The intra-day precision and inter-day precision are less than 10% and 20%, respectively. This method was applied to detect 20 batches of commercially available Astragalus samples. Six compounds (three pesticides and three mycotoxins) were detected; the residues of aflatoxin and ochratoxin A in two batches exceeded the maximum residue limits and required attention. The established method is simple, rapid, and highly sensitive. It is also reproducible and meets the requirements for the accurate quantitative analysis of multiple pesticide residues and mycotoxins in Astragalus.

Keywords: astragalus; pesticide residues; mycotoxins; response surface methodology; QuEChERS; LC-Q-TOF/MS

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1. Introduction

Astragalus is a dried root from a perennial leguminous herb and possesses a range of medicinal properties, including immunomodulatory, cardiovascular-protective, anti-inflammatory, and antioxidant activities. It is widely used in pharmaceuticals, dietary supplements, and food products [1]. In 2023, Astragalus was recognized in China as a medicinal and food-homologous substance, further broadening its applications across both the food and pharmaceutical industries. In recent years, due to its significant medicinal value, Astragalus has experienced rapid growth in the global herbal medicine market [2]. Therefore, ensuring the quality and safety of Astragalus is crucial for safeguarding public health.

Medicinal Astragalus typically requires two years from planting to harvest. During cultivation, it is highly susceptible to pests and diseases, including root rot, powdery mildew, bean pod borer, and aphids. Growers commonly apply various pesticides, including fungicides and insecticides, to prevent pest and disease damage [3]. The indiscriminate and excessive use of pesticides not only pollutes the environment but also leads to excessive pesticide residues in Astragalus, posing safety risks to Astragalus and its related products [4]. Furthermore, it may lead to pests and diseases developing resistance to pesticides [5]. The Chinese Pharmacopoeia (2025 ed.) has specified 47 prohibited pesticides for Chinese herbal medicines and decoction pieces [6], while the Chinese national standard GB 2763-2021 also establishes maximum residue limits for pesticides in medicinal plants [7]. Literature reviews reveal that pesticide residue issues in Astragalus are becoming increasingly severe [8–10]. These residues pose potential risks to human health, including carcinogenicity, neurotoxicity, and endocrine disruption, and may also adversely impact environmental ecosystems [11]. Therefore, to ensure the quality and safety of Astragalus, it is of significant importance to develop high-throughput pesticide detection technology for Astragalus.

Herbal materials are directly exposed to soil during cultivation, making them susceptible to mycotoxin contamination. Improper handling during subsequent processing and storage stages may also lead to mold growth and the production of mycotoxins [12]. Hu et al. tested 35 batches of Astragalus samples for 10 mycotoxins and reported 100% detection rates for aflatoxin B_2, aflatoxin G_1, and T-2 toxin. One sample even contained ochratoxin A (OTA) at 1151.4 μg/kg [13]. The presence of these mycotoxins not only diminishes the medicinal efficacy of Astragalus [14] but may also pose risks to human health. Furthermore, for traditional Chinese medicines (TCM) like Astragalus, which possess liver detoxification properties, contamination with mycotoxins may conflict with their inherent liver metabolic mechanisms, potentially exacerbating liver damage [15]. In summary, pesticide residues and mycotoxins have become critical factors affecting the quality and safety of Astragalus in China and its export to international markets. Therefore, developing high-throughput detection technologies for pesticide residues and mycotoxins in Astragalus is a crucial step in ensuring the healthy development of the industry.

QuEChERS is a commonly used pretreatment technique for extracting various contaminants from matrices. Characterized by its simplicity, efficiency, and eco-friendliness, it is widely applied in the analysis of pesticides and mycotoxins in fruits, vegetables, and grains [16]. Due to the complex composition of TCM, detecting pesticide residues and mycotoxins in these samples can be difficult. Consequently, some researchers have modified the QuEChERS method for use with complex matrices such as TCM [17]. Currently, the primary methods for detecting pesticide residues and mycotoxins are liquid chromatography–tandem mass spectrometry (LC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS) [18–20]. Tölgyesi et al. developed a simultaneous multi-residue detection method for foods such as wheat using LC-MS/MS. This method can simultaneously detect 266 pesticides, 12 mycotoxins, 14 alkaloid toxins, and 3 Alternaria toxins [21]. However, these methods often face challenges, such as high false-positive rates and insufficient throughput in trace analysis, which make traceability analysis difficult and hinder the simultaneous fulfillment of high sensitivity and high throughput requirements [22,23]. Liquid chromatography–high-resolution mass spectrometry technology offers advantages such as high selectivity and sensitivity. Among these, quadrupole time-of-flight mass spectrometry (Q-TOF/MS) can generate a large number of accurately massed ions, providing high resolution, fast scanning speeds, and full-scan mass spectra. This significantly enhances the identification and detection of compounds while enabling the high-throughput qualitative and quantitative analysis of hundreds of pesticides and my-

cotoxins. Currently, this technology has been applied to the high-throughput analysis of multiple residues in complex matrices, such as vegetables, fruits, and grains, in agricultural production [24–26]. However, no studies have been reported on the simultaneous detection of pesticide residues and mycotoxins in Astragalus using the improved QuEChERS method combined with LC-Q-TOF/MS. [8,13], and existing methods exhibit significant shortcomings in detection and quantification limits, as well as the number of target compounds detectable in Astragalus [9,12].

Therefore, this study developed a novel QuEChERS extraction method for Astragalus, combining hydration and extraction steps into a single phase to shorten sample preparation time and incorporating multi-walled carbon nanotubes (MWCNTs) as a novel purification material, effectively mitigating matrix effects. By optimizing the sample preparation method using response surface methodology and combining it with LC-Q-TOF/MS, an analytical method was established for the simultaneous, accurate, and efficient determination of 187 pesticides and 10 mycotoxins in Astragalus for the first time. To evaluate the method's validity and applicability, its methodological parameters—including screening limits, quantification limits, accuracy, and precision—were validated. The method was then applied to analyze 20 batches of Astragalus samples. This method not only enables precise detection of quality and safety indicators in Astragalus, thereby contributing to public health protection, but also provides a scientific basis for quality control research on other TCMs.

2. Materials and Methods

2.1. Chemicals and Reagents

Standards of 187 pesticides, each at 1000 μg/mL, were purchased from Tianjin Alta Technology Co., Ltd. (Tianjin, China). All solutions had a purity greater than 98%. 10 mycotoxin standard solutions obtained from Tianjin Alta Technology Co., Ltd. had purities greater than 95% (Tianjin, China). Except for deoxynivalenol (DON), stachybotrylactam (ST), and tentoxin (TEN), with a concentration of 100 μg/mL, the remaining 7 mycotoxins (aflatoxin B_1, B_2, G_1, G_2 (AFB_1, AFB_2, AFG_1, AFG_2), ochratoxin A (OTA), T-2 toxin (T-2), zearalenone (ZEN)) were all at a concentration of 1000 μg/mL.

Formic acid and ammonium acetate (mass spectrometry grade, Agilent Technologies, Santa Clara, CA, USA); acetonitrile and methanol (chromatography grade, Thermo Fisher, Waltham, MA, USA); formic acid, sodium chloride, and anhydrous magnesium sulfate (analytical purity, Sinopharm Chemical Reagent Co., Shanghai, China); primary-secondary amine (PSA), octadecylsilane (C_{18}), multi-walled carbon nanotubes (MWCNTs) (ANPEL Scientific Instrument Co., Ltd. Shanghai, China); and the water used in the experiments was high-purity water purified by a Milli-Q ultrapure water system (Merck, Darmstadt, Germany).

2.2. Apparatus

The analysis of target compounds was performed using an Agilent 1290–6550 liquid chromatography–quadrupole time-of-flight mass spectrometer equipped with a Dual AJS ESI source (Agilent Technologies, Santa Clara, CA, USA). An Auto EVA 80 automatic parallel concentrator (Raykol Instrument Co., Ltd., Xiamen, China) was employed for solvent evaporation. A TRIO TM-N vortex mixer (AS ONE Corporation, Osaka, Japan) and an SR-2DS horizontal shaker (Taitec Corporation, Tokyo, Japan) were employed to ensure thorough mixing and extraction during the sample preparation process. Centrifugation was performed using an Allegra X-30 R centrifuge (Beckman Coulter, Pasadena, CA, USA). High-purity water was obtained from a Milli-Q ultrapure water system (Millipore, Merck, Darmstadt, Germany). A PL602-L electronic balance (Mettler-Toledo, Zurich, Switzerland) was used for accurate weighing. An ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd., Kunshan, China) was used to assist dissolution.

2.3. Preparation of Standard Solutions

A total of 187 pesticides and 10 mycotoxins were divided into two groups. Standard solutions from each group were accurately transferred into separate 10 mL volumetric flasks. The solutions were then diluted to the mark with methanol, mixed thoroughly, and a 10 mg/L mixed standard intermediate solution was prepared. These intermediate solutions were stored at 4 °C protected from light. Subsequently, 1 mL of each of the two sets of mixed standard stock solutions was accurately transferred into a 10 mL volumetric flask. A final concentration of 1 mg/L in methanol was achieved by dilution, resulting in the mixed standard working solution. This working solution was also stored at 4 °C protected from light.

2.4. Real Samples

This study collected 20 batches of Astragalus samples from Gansu Province, including 10 from Min County, 5 from Weiyuan County, and 5 from Longxi County. The sample was ground to a fine powder by a pulverizer, sieved through a 50-mesh sieve, and stored at 4 °C under refrigeration.

2.5. Sample Preparation

Accurately weigh 2.0 g of sample into a 50 mL centrifuge tube. Add 15 mL of acetonitrile/water/formic acid (80/15/5, $v/v/v$) solution to the centrifuge tube. Vortex horizontally for 3 min, then let stand for 20 min to ensure thorough mixing. Add 4 g anhydrous $MgSO_4$, 1 g NaCl, and one ceramic homogenizer bead. Vortex for 3 min, then centrifuge at 5000 rpm for 5 min. Transfer 5 mL of the supernatant to a 15 mL purification tube (containing 400 mg $MgSO_4$, 194 mg PSA, 100 mg C_{18}, and 5 mg MWCNTs). Vortex for 3 min, then centrifuge at 5000 rpm for 5 min. Transfer 3 mL of the supernatant to a 10 mL glass test tube. Evaporate to dryness under nitrogen at 40 °C in a water bath. Redissolve in 1 mL of methanol/water (3:2, v/v) solution. Sonicate, vortex for 10 s, and filter through a 0.22 μm organic filter membrane for LC-Q-TOF/MS analysis.

2.6. Instrumentation

Chromatographic Conditions: The separation was performed on a ZORBAX SB-C18 column (100 mm × 2.1 mm, 3.5 μm particle size; Agilent, California, CA, USA). The column temperature was maintained at 40 °C to optimize peak shape and resolution. The injection volume was 5 μL. The mobile phase consisted of two components: Phase A, which was water containing 0.1% formic acid and 5 mmol/L ammonium acetate, and Phase B, which was methanol containing 0.1% formic acid. A gradient elution program was used to separate the target analytes effectively. The gradient program was as follows: 0–3 min, 1% B; 3–6 min, 1–30% B; 6–9 min, 30–40% B; 9–15 min, 40% B; 15–19 min, 40–60% B; 19–23 min, 60–90% B; 23–23.01 min, 90–1% B; 23.01–27.00 min, 1% B. The flow rate was maintained at 0.4 mL/min.

Mass Spectrometry Conditions: Mass spectrometric detection was performed using a Q-TOF/MS system with a Dual AJS ESI source operating in positive ion mode. The full scan range was set to m/z 50–1000. The capillary voltage was 4000 V. Nitrogen was used as the atomization gas at 0.14 MPa. The sheath gas temperature was 375 °C, and the sheath gas flow rate was 11.0 L/min. The drying gas flow rate was 12.0 L/min, and the drying gas temperature was 225 °C. The fragmentation voltage was 345 V. For All Ions MS/MS analysis, the collision energy was initially set to 0 V at 0 min. At 0.5 min, the collision energy was sequentially set to 0 V, 15 V, and 35 V, respectively. Mass spectrometry data for 197 compounds and the total ion current (TIC) of the standard solution are detailed in Table 1 and Figure 1.

Table 1. MS information for 187 pesticides and 10 mycotoxins.

No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)	No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)
	Pesticides (187)					96	Metconazole	18.2	320.1524	70.0400	125.0153
1	Acetamiprid	5.4	223.0745	126.0105	90.0338	97	Methamidophos	1.9	142.0086	94.0052	124.9821
2	Acetochlor	17.2	270.1255	224.0837	148.1121	98	Methidathion	13.7	302.9691	145.0066	85.0396
3	Alachlor	17.2	270.1255	238.0993	162.1277	99	Methiocarb	15.2	226.0900	169.0678	121.0650
4	Aldicarb-sulfone	3.2	223.0747	86.0600	148.0427	100	Methoxyfenozide	16.7	369.2173	149.0597	313.1547
5	Ametryn	13.0	228.1277	186.0808	68.0243	101	Metribuzin	8.2	215.0961	187.1012	84.0808
6	Atrazine	12.2	216.1010	174.0541	104.0010	102	Metsulfuron-methyl	9.9	382.0816	167.0564	135.0441
7	Avermectin	20.5	895.4818	789.9903	896.4840	103	Monocrotophos	4.1	224.0682	127.0155	193.0260
8	Azaconazole	12.3	300.0301	230.9974	158.9763	104	Myclobutanil	16.6	289.1215	125.0153	70.0400
9	Azinphos-ethyl	17.0	346.0443	132.0444	232.9491	105	Napropamide	17.2	272.1645	171.0804	199.0754
10	Azoxystrobin	15.9	404.1241	372.0979	329.0795	106	Nicosulfuron	9.3	411.1081	182.0560	213.0322
11	Benalaxyl	18.1	326.1751	294.1489	208.1332	107	Omethoate	2.8	214.0297	182.9875	154.9926
12	Bitertanol	18.4	338.1863	269.1536	251.1417	108	Oxadiazon	19.2	345.0767	303.0298	219.9563
13	Boscalid	16.0	343.0399	307.0633	271.0866	109	Oxadixyl	7.8	279.1339	219.1128	133.0886
14	Bromuconazole	16.8	375.9614	158.9763	306.9273	110	Paclobutrazol	16.2	294.1368	70.0400	125.0153
15	Buprofezin	19.1	306.1635	201.1056	106.0651	111	Paraoxon-ethyl	12.8	276.0632	220.0002	174.0076
16	Cadusafos	18.6	271.0950	158.9698	130.9385	112	Paraoxon-methyl	7.3	248.0319	202.0389	109.0049
17	Carbofuran	9.1	222.1125	165.0910	123.0441	113	Penconazole	12.6	284.0716	158.9763	70.0400
18	Carbofuran-3-Hydroxy	5.2	238.1074	163.0754	181.0859	114	Phenthoate	17.7	321.0379	247.0011	163.0754
19	Carboxin	9.9	236.0740	143.0161	93.0573	115	Phorate-Sulfone	12.5	293.0097	247.0215	171.0230
20	Chlordimeform	4.0	197.0840	117.0573	152.0262	116	Phorate-sulfoxide	12.2	277.0150	199.0011	170.9698
21	Chlorfenvinphos	18.1	358.9768	155.0468	204.9371	117	Phosalone	18.3	367.9941	181.9998	110.9996
22	Chlorpyrifos	19.4	349.9336	321.9023	197.9268	118	Phosfolan	6.8	256.0217	227.9907	139.9563
23	Chlorsulfuron	11.3	358.0371	167.0564	141.0771	119	Phosfolan-Methyl	4.1	227.9911	167.9873	109.0044
24	Clothianidin	4.6	250.0160	169.0542	131.9669	120	Phosmet	14.5	318.0018	160.0393	133.0284
25	Coumaphos	18.1	363.0217	226.9926	306.9591	121	Phosphamidon	8.1	300.0762	174.0678	127.0155
26	Cyproconazole	17.4	292.1211	125.0153	70.0400	122	Phoxim	17.7	299.0614	129.0447	124.9821
27	Cyprodinil	17.2	226.1339	93.0573	210.1026	123	Picoxystrobin	17.7	368.1104	205.0859	145.0648
28	Cyromazine	1.9	167.1036	85.0511	125.0821	124	Piperonyl Butoxide	19.3	356.2423	177.0898	178.0940

Table 1. *Cont.*

No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)	No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)
29	Dicrotophos	4.5	238.0839	112.0757	127.0155	125	Pirimicarb	7.8	239.1503	182.1288	72.0444
30	Diethofencarb	15.3	268.1543	226.1074	152.0706	126	Pirimiphos-ethyl	19.2	334.1349	198.1059	170.0746
31	Difenoconazole	18.6	406.0721	408.0690	251.0021	127	Pirimiphos-methyl	18.2	306.1036	164.1182	108.0556
32	Diflubenzuron	13.9	311.0393	158.0412	141.0146	128	Pretilachlor	18.9	312.1725	252.1150	176.1434
33	Diflufenican	18.7	395.0813	266.0412	246.0350	129	Prochloraz	18.3	376.0381	307.9988	309.9951
34	Dimethoate	5.0	230.0069	198.9647	170.9698	130	Profenofos	19.0	372.9424	302.8642	344.9111
35	Dimethomorph	16.5	388.1310	301.0626	165.0546	131	Promecarb	15.9	208.1332	151.1117	109.0648
36	Diniconazole	18.4	326.0821	328.0799	327.0855	132	Prometryn	15.6	242.1434	158.0495	200.0964
37	Dioxacarb	8.2	224.0917	167.0703	123.0441	133	Propachlor	13.0	212.0837	170.0367	94.0651
38	Edifenphos	18.0	311.0324	283.0011	172.9821	134	Propamocarb	2.8	189.1598	102.0550	144.1019
39	Epoxiconazole	15.9	330.0806	332.0784	331.0837	135	Propiconazole	18.1	342.0771	158.9763	69.0699
40	Ethametsulfuron	6.9	397.0924	196.0823	168.0510	136	Pymetrozine	12.2	218.1036	105.0447	78.0338
41	Ethion	19.3	384.9949	199.0011	170.9698	137	Pyraclostrobin	18.3	388.1059	194.0812	163.0628
42	Ethoprophos	17.2	243.0637	172.9854	215.0324	138	Pyridaben	20.2	365.1449	309.0823	147.1168
43	Etofenprox	20.8	394.2378	399.2619	393.2476	139	Pyrimethanil	13.5	200.1182	168.0682	182.0838
44	Etrimfos	17.8	293.0719	265.0406	124.9821	140	Pyriproxyfen	19.3	322.1438	227.1067	96.0444
45	Fenamiphos	17.6	304.1131	217.0083	201.9848	141	Quinalphos	17.7	299.0614	271.0301	242.9985
46	Fenamiphos-sulfone	11.5	336.1029	308.0716	266.0247	142	Quinoxyfen	19.4	308.0040	196.9789	213.9821
47	Fenamiphos-sulfoxide	11.0	320.1080	233.0032	292.0767	143	Quizalofop-ethyl	19.0	373.0950	299.0577	255.0315
48	Fenarimol	17.2	331.0399	268.0524	259.0057	144	Simazine	8.5	202.0854	132.0323	124.0869
49	Fenazaquin	20.4	307.1805	161.1325	57.0699	145	Simeconazole	17.1	294.1432	70.0400	135.0605
50	Fenbuconazole	17.4	337.1219	339.1194	338.1248	146	Spirodiclofen	19.9	411.1124	313.0393	212.9505
51	Fenhexamid	17.0	302.0709	97.1012	55.0542	147	Spirotetramat	17.3	374.1962	330.2064	302.1751
52	Fenpropathrin	19.7	350.1751	125.0961	97.1012	148	Spirotetramat-enol	4.8	302.1758	216.1017	270.1487
53	Fenpropimorph	15.5	304.2625	305.2672	147.1160	149	Spirotetramat-enol-glucoside	4.8	464.2283	302.1750	324.1573
54	Fensulfothion	13.9	309.0379	252.9753	234.9647	150	Spirotetramat-keto-hydroxy	14.2	318.1702	300.1597	268.1333
55	Fensulfothion-oxon	7.3	293.0607	236.9980	218.9873	151	Spirotetramat-mono-hydroxy	9.2	304.1915	254.1537	211.1475

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Table 1. *Cont.*

No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)	No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)
56	Fensulfothion-Oxon-Sulfone	7.5	309.0565	252.9932	175.0150	152	Spiroxamine	16.1	298.2741	144.1383	100.1121
57	Fensulfothion-sulfone	14.1	325.0328	268.9687	190.9914	153	Sulfentrazone	10.5	386.9891	306.9944	308.0038
58	Fenthion	17.8	279.0273	247.0011	169.0140	154	Sulfotep	17.8	323.0300	171.0239	294.9987
59	Fenthion-oxon	14.6	263.0501	231.0239	216.0005	155	Tebuconazole	17.9	308.1527	310.1498	309.1552
60	Fenthion-oxon-sulfone	5.9	295.0403	217.0624	296.0430	156	Tebufenpyrad	19.2	334.1681	117.0209	145.0522
61	Fenthion-oxon-sulfoxide	5.7	279.0451	264.0216	262.0423	157	Terbufos	16.0	289.0514	103.0576	57.0699
62	Fenthion-sulfone	11.6	311.0171	278.9909	124.9821	158	Terbufos-Sulfone	15.1	321.0412	171.0240	275.0536
63	Fenthion-sulfoxide	11.1	295.0222	279.9987	278.0195	159	Terbufos-Sulfoxide	15.3	305.0465	187.0010	158.9694
64	Flonicamid	3.6	230.0536	203.0427	148.0369	160	Tetrachlorvinphos	17.7	364.9065	203.9293	238.8982
65	Fluacrypyrim	18.6	427.1475	205.0859	145.0648	161	Tetraconazole	17.2	372.0290	374.0250	158.9752
66	Flucythrinate	19.6	469.1931	470.3460	468.3107	162	Thiabendazole	4.6	202.0433	175.0324	131.0604
67	Flufenacet	17.1	364.0737	194.0976	152.0506	163	Thiacloprid	6.3	253.0309	126.0087	90.0338
68	Flufenoxuron	19.5	489.0435	158.0401	141.0146	164	Thiamethoxam	3.8	292.0266	211.0648	181.0542
69	Flumioxazin	14.8	355.1088	327.1085	210.1506	165	Thidiazuron	8.7	221.0492	102.0120	77.0386
70	Flumorph	14.6	372.1606	285.0909	165.0530	166	Thifluzamide	17.4	526.8485	528.8456	488.8332
71	Fluroxypyr	7.3	254.9734	208.9679	180.9730	167	Thiobencarb	18.4	258.0714	125.0153	100.0757
72	Flusilazole	17.6	316.1076	247.0749	165.0697	168	Thiocyclam	2.0	182.0126	136.9523	73.0092
73	Flutriafol	12.9	302.1099	233.0773	123.0241	169	Tralkoxydim	19.6	330.2064	284.1645	285.1723
74	Fonofos	17.9	247.0375	137.0184	108.9871	170	Triadimefon	16.6	294.1004	197.0714	225.0659
75	Fosthiazate	11.9	284.0538	227.9909	104.0165	171	Triadimenol	16.8	296.1158	70.0405	298.1133
76	Heptenophos	13.7	251.0234	127.0155	109.0049	172	Triazophos	16.8	314.0723	162.0662	119.0604
77	Hexaconazole	18.1	314.0825	316.0790	315.0848	173	Trichlorfon	5.0	256.9299	220.9532	109.0051
78	Hexythiazox	19.4	353.1085	228.0240	168.0570	174	Tricyclazole	7.1	190.0433	136.0215	163.0324
79	Imazalil	12.7	297.0550	299.0526	158.9761	175	Trifloxystrobin	18.7	409.1370	186.0525	145.0260
80	Imidacloprid	4.6	256.0596	209.0589	175.0978	176	Triflumizole	18.8	346.0920	278.0547	280.0524
81	Imidaclothiz	4.9	262.0158	181.0537	265.1525	177	Trifluralin	17.8	336.1170	342.2657	345.2389
82	Indanofan	17.2	341.0941	348.2548	348.7550	178	Triflusulfuron-methyl	16.5	493.1112	264.0698	461.0850
83	Indoxacarb	18.7	528.0780	293.0319	218.0417	179	Uniconazole	17.4	292.1213	294.1191	293.1246
84	Ipconazole	19.2	334.1681	70.0400	125.0153	180	Zoxamide	18.0	336.0319	186.9712	158.9763
85	Isazofos	16.7	314.0490	162.0424	272.0020		Mycotoxins (10)				
86	Isocarbophos	13.6	312.0433	269.9950	236.0077	1	Aflatoxin B_1	9.6	313.0700	285.0741	269.0432

Table 1. *Cont.*

No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)	No.	Compound	Retention Time (min)	Precursorion (*m*/*z*)	Production 1 (*m*/*z*)	Production 2 (*m*/*z*)
87	Isoprothiolane	16.3	291.0719	231.0144	188.9675	2	Aflatoxin B_2	8.5	315.0862	259.0583	287.0891
88	Linuron	14.6	249.0192	159.9710	182.0241	3	Aflatoxin G_1	7.5	329.0638	243.0629	215.0682
89	Malaoxon	10.3	315.0662	127.0390	99.0077	4	Aflatoxin G_2	6.9	331.0807	313.0709	285.0760
90	Malathion	16.3	331.0433	127.0390	285.0015	5	Deoxynivalenol	3.4	297.1328	298.3453	249.1101
91	Mandipropamid	16.5	412.1310	328.1094	356.1043	6	Ochratoxin A	16.8	404.0895	239.0098	220.9990
92	Matrine	2.3	249.1967	148.1117	250.1991	7	Stachybotrylactam	18.2	386.2326	387.2331	178.0488
93	Mecarbam	17.2	330.0593	226.9960	142.9385	8	T-2 toxin	15.6	484.2541	215.1063	185.0960
94	Mepronil	16.3	270.1489	228.1019	119.0491	9	Tentoxin	14.3	415.2340	358.2125	302.1499
95	Metalaxyl	13.7	280.1543	220.1332	192.1383	10	Zearalenone	16.6	319.1540	283.1309	301.1413

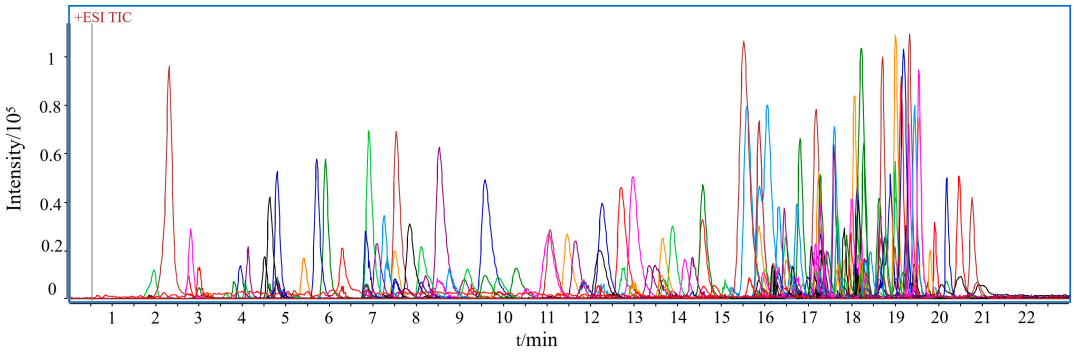


Figure 1. Total ion chromatogram (TIC) of 197 compound standard solutions.

2.7. Design and Optimization of Pretreatment

In qualitative identification, minimizing matrix interference is one of the most challenging tasks. To address this, pretreatment methods must first be optimized before quantitative validation can proceed. This study investigated factors potentially affecting the extraction efficiency of the QuEChERS method using single-factor experiments and multivariate response surface analysis. The response surface methodology is a powerful statistical technique that allows for the simultaneous optimization of multiple factors and the identification of factor interactions. Compared to single-factor experiments, response surface methodology can provide a comprehensive understanding of the relationships among factors and the response, leading to more efficient and robust optimization. The QuEChERS method was optimized by spiking at 50 μg/kg with pesticides and mycotoxins. After optimizing the extraction solvent and dosage, purification conditions were optimized, and a single-factor screening was conducted for the purification agent dosage. A three-factor, three-level response surface experiment was designed using the Box–Behnken model in Design-Expert software, with the dosages of PSA, C_{18}, and MWCNTs as the variable factors and the compound proportion within the recovery range as the optimization target.

2.8. Methodological Validation

This study conducted methodological validation of Astragalus, including screening detection limits (SDL), limit of quantification (LOQ), linear range, matrix effects (ME), accuracy, and precision. SDL evaluation was performed in accordance with the requirements of the guidance document SANTE/11312/2021 [27]. The LOQ is the minimum detectable amount of a target analyte in a sample that can be quantitatively determined with results that meet the requirements for accuracy (recovery within 70% to 120%) and precision (relative standard deviation (RSD) $\leq$ 20%). A matrix-matched standard curve is constructed by adding a series of standard solutions to a blank matrix. ME is evaluated by the slope of the matrix-matched calibration curve and the solvent calibration curve. To validate the accuracy and precision of the developed method, six replicate recovery experiments were performed for 197 targets in Astragalus at three spiking levels: 1 × LOQ, 2 × LOQ, and 10 × LOQ.

3. Results and Discussion

3.1. Optimization of Pretreatment Conditions

3.1.1. Optimization of Extraction Solvent

Typical solvents used for pesticide extraction include acetonitrile, methanol, and acetone. However, given the low solubility of mycotoxins in methanol and acetone and their tendency to extract interfering substances from matrices [28,29], acetonitrile, the most widely used solvent, was selected for this study to extract both pesticides and mycotoxins

simultaneously. Due to significant physicochemical differences among the compounds to be analyzed and Astragalus's low moisture content, it is crucial to add an appropriate amount of water to the extraction solvent. Water promotes cell swelling in Astragalus, enhancing the penetration of organic solvents and thereby improving the efficiency of compound extraction [30]. Furthermore, studies indicate that adding formic acid to the extraction solvent can enhance the stability of certain acid-sensitive compounds [31].

To enhance the extraction efficiency of compounds effectively, this study first compared five different solvent ratios at a spiking level of 50 µg/kg: acetonitrile/water/formic acid (50/47/3, $v/v/v$, I); (60/37/3, $v/v/v$, II); (80/17/3, $v/v/v$, III); (90/7/3, $v/v/v$, IV); and (90/7/3, $v/v/v$, V). Results indicated that as the proportion of water gradually decreased, the number of compounds meeting the recovery range first increased and then decreased (Figure 2A). At higher water ratios (I–III), the number of compounds meeting the recovery range was extremely low (16, 26, and 36, respectively), with most compounds exhibiting excessively high recoveries. When the water ratio decreased to 17%, the number of compounds meeting the requirements sharply increased to 178. However, when the water ratio decreased further to 7%, the excessively high acetonitrile content reduced the polarity of the extraction solvent, resulting in a lower proportion of some pesticides (cyromazine, metribuzin, spirodiclofen, and thidiazuron) being distributed to the organic phase. This may be due to the high water solubility and strong polarity of these pesticides, leading to lower recoveries when the aqueous solution volume is insufficient. Therefore, acetonitrile/water/formic acid (80/17/3, $v/v/v$) was selected for subsequent studies.

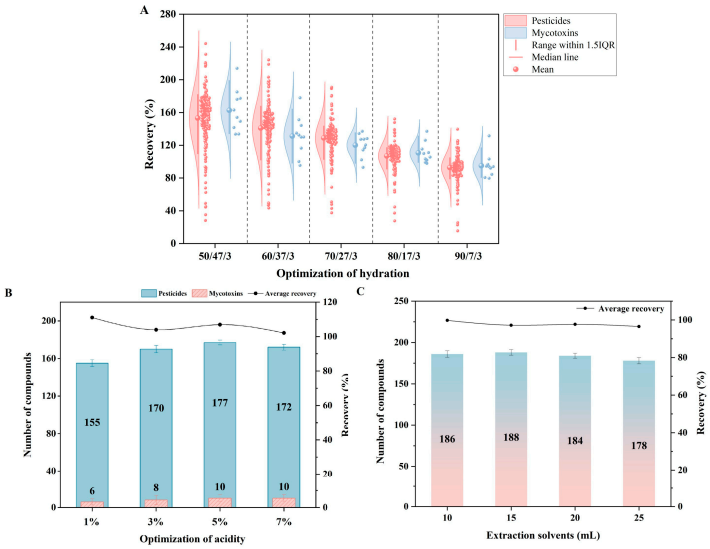


Figure 2. Effect of different extraction solvents on compound recovery rate ((**A**): different water ratios, (**B**): different formic acid ratios, (**C**): different extraction liquid volumes).

Compounds such as buprofezin, aflatoxin G_1, and T-2 toxin are unstable under alkaline conditions and are relatively sensitive to acids [32]. Therefore, an appropriate amount of formic acid must be added to adjust the pH of the extraction solvent, thereby enhancing its stability and extraction efficiency. This study investigated the extraction efficiency of compounds from Astragalus using four different concentrations of formic acid (1%, 3%, 5%, 7%). As shown in Figure 2B, as the proportion of formic acid in acetonitrile increases, the number of compounds that meet the recovery range first increases and then decreases, reaching 161, 178, 187, and 182, respectively. When the formic acid concentration reaches 7%, the proportion becomes excessively high, altering the solvent polarity. This change in polarity may cause lipophilic compounds, such as malaoxon and pyridaben, to be more readily distributed into the aqueous phase, thereby reducing recoveries. Therefore, an

acetonitrile–water solution containing 5% formic acid was selected as the extraction solvent for this study.

During extraction, the amount of extraction solvent used also affects the compound's extraction efficiency. Therefore, this study investigated the impact of different extraction solvent volumes (10 mL, 15 mL, 20 mL, 25 mL) on the recoveries of 197 compounds, as shown in Figure 2C. When the volume increased from 10 mL to 25 mL, the number of compounds with recoveries between 70% and 120% was 186, 188, 184, and 178, respectively. Simultaneously, increased solvent usage leads to reagent waste and environmental pollution. After careful consideration, the extraction solvent volume was ultimately determined to be 15 mL.

3.1.2. Single-Factor Test of Purification Conditions

Although steps such as salt precipitation and centrifugation can remove some macromolecular interfering substances due to the presence of abundant polysaccharides, proteins, lipids, and other substances in Astragalus [1], residual interferents may still cause matrix enhancement or suppression. This interferes with compound determination and contaminates the detection system. Therefore, selecting appropriate purification materials (PSA, C_{18}, MWCNTs) and their dosages during sample pretreatment is crucial for reducing the impact of interfering substances on target compounds and enhancing compound recoveries. PSA is a weak anion exchange resin capable of adsorbing certain polar interfering substances [33], while C_{18} can remove fats and nonpolar compounds [34]. The PSA and C_{18} combination purification materials demonstrate optimal purification performance across various solid matrices [35]. Concurrently, MWCNTs, as a novel purification material, effectively eliminate interfering substances such as fatty acids and pigments from samples, thereby enhancing purification outcomes [36]. In recent years, it has demonstrated advantages for detecting pesticide residues in high-pigment foods such as fruits, vegetables, and tea [37]. This experiment examined three purification materials (PSA, C_{18}, MWCNTs) at different dosages. By calculating the number of compounds with recoveries between 70% and 120% in each group, the response surface factor level parameters were determined. As shown in Figure 3, the number of compounds meeting the 70–120% recovery range in all three single-factor experiments first increased and then decreased with increasing dosage.

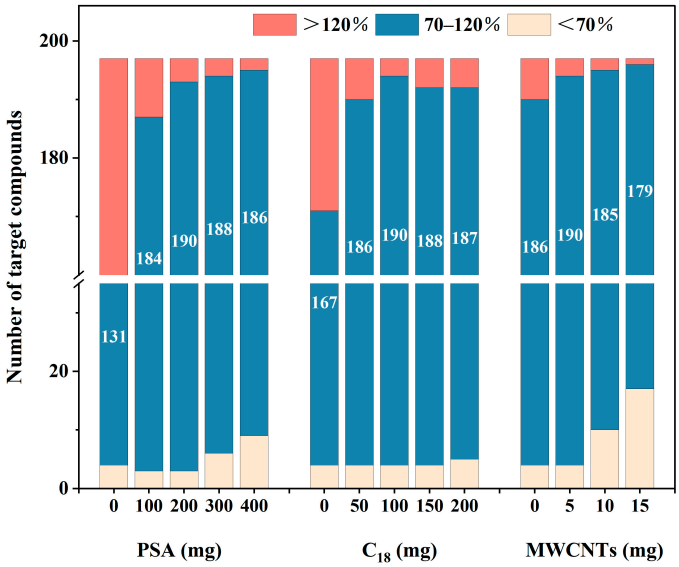


Figure 3. Effect of different purification agent dosages on the number of compounds meeting requirements.

Under fixed dosages of C_{18} (100 mg) and MWCNTs (5 mg), PSA was evaluated at different dosages (0 mg, 100 mg, 200 mg, 300 mg, 400 mg). Results indicate that when the PSA dosage was 0 mg, polar matrix interferences such as sugars and organic acids present in Astragalus were not sufficiently removed, resulting in suppressed recoveries for several target compounds. Notably, recoveries for polar pesticides such as dicrotophos and dioxacarb were significantly lower, indicating interference from polar matrix components. When the PSA dosage increased to 200 mg, the number of compounds meeting the recovery range was maximized. However, when the PSA dosage exceeded 200 mg, recovery of compounds such as indoxacarb and dicofol decreased significantly. This may be attributed to excessive PSA, which causes its amino groups to react with the carboxyl or phenolic hydroxyl groups of the compounds, leading to partial loss of the compounds. Therefore, 200 mg was selected as the optimal parameter for PSA dosage in the single-factor experiment for subsequent response surface optimization studies.

Under fixed dosages of PSA (200 mg) and MWCNTs (5 mg), C_{18} was evaluated at different dosages (0 mg, 50 mg, 100 mg, 150 mg, 200 mg). Results indicate that without C_{18} addition, non-polar interfering substances were not adsorbed, and recovery for certain compounds (e.g., carbofuran, dimethoate, and penconazole) exceeded 130%. When the C_{18} was 100 mg, the number of compounds meeting the recovery range was highest. However, as C_{18} increased to 150–200 mg, the number of compounds meeting the range gradually decreased. The recovery of certain heterocyclic compounds showed a downward trend, likely due to excessive adsorption by C_{18}. Therefore, 100 mg was selected as the optimal parameter for C_{18} dosage in the single-factor experiment for subsequent response surface optimization studies.

Under fixed dosages of PSA (200 mg) and C_{18} (100 mg), MWCNTs were evaluated at different dosages (0 mg, 5 mg, 10 mg, 15 mg). The results indicate that, due to the adsorption properties of MWCNTs toward certain planar structures or compounds with high specific surface areas [38], the recoveries of planar compounds containing benzene rings (such as carbofuran and thiabendazole) decrease as the MWCNT dosage increases. At a MWCNT dosage of 5 mg, not only was the highest number of compounds within the recovery range achieved, but the sample solution also showed a significantly lighter color than at 0 mg, demonstrating remarkable purification efficacy. Therefore, 5 mg was selected as the optimal MWCNT dosage in the single-factor experiment for subsequent response surface optimization studies.

3.1.3. Response Surface Optimization of Purification Conditions

A three-factor, three-level design was employed using response surface optimization, building upon single-factor experiments, to determine the optimal dosages of PSA, $C_{18,}$ and MWCNTs. The proportion of compounds with recovery ranging from 70% to 120% (Y) was used as the response value. A mathematical model was developed using a Box–Behnken centered-combinatorial experimental design to optimize responses. Results were visually presented through three-dimensional plots and contour maps. The experiment comprised 17 test points, with the results summarized in Table 2. Data regression and analysis of variance were performed using Design-Expert software, with the results shown in Table 3.

Table 2. Box–Behnken test results.

Number	A: PSA (mg)	B: C_{18} (mg)	C: MWCNTs (mg)	Y (%)
1	300	50	5	83.76
2	200	150	10	83.76
3	100	100	10	82.23
4	100	50	5	86.30
5	300	100	10	81.73
6	300	100	0	81.22
7	100	150	5	85.28
8	200	50	10	81.73
9	200	100	5	94.92
10	200	100	5	95.94
11	200	150	0	85.28
12	300	150	5	85.28
13	200	50	0	86.8
14	200	100	5	94.42
15	200	100	5	96.45
16	100	100	0	84.26
17	200	100	5	95.43

Table 3. Analysis of variance for the response surface quadratic regression equation model.

Source	Sum of Squares	df	Mean Square	F-Value	*p*-Value	Significance
Model	499.8	9	55.53	65.68	<0.0001	**
A-PSA	4.62	1	4.62	5.46	0.052	
B-C_{18}	0.1275	1	0.1275	0.1508	0.7093	
C-MWCNTs	8.22	1	8.22	9.72	0.0169	*
AB	1.61	1	1.61	1.91	0.2097	
AC	1.61	1	1.61	1.91	0.2097	
BC	3.15	1	3.15	3.73	0.0949	
A^2	159.5	1	159.5	188.63	<0.0001	**
B^2	71.55	1	71.55	84.62	<0.0001	**
C^2	201.47	1	201.47	238.27	<0.0001	**
Residual	5.92	7	0.8455			
Lack of fit	3.34	3	1.11	1.72	0.2994	
Pure error	2.58	4	0.6452			
Cor total	505.72	16				

"*" means a significant effect on the results ($p < 0.05$); "**" means a highly significant impact on the results ($p < 0.01$).

The analysis results indicate that the response surface model exhibits extremely significant effects ($p < 0.01$). The regression equation for the compound ratio (Y) with respect to each factor is: $Y = 95.43 - 0.76A + 0.1263B - 1.01C + 0.6350AB + 0.6350AC + 0.8875BC - 6.15A^2 - 4.12B^2 - 6.92C^2$. The lack of fit was not significant ($p = 0.2994 > 0.05$), indicating that the proportion of non-normal errors in fitting the obtained regression equation was small. The regression coefficient (R^2) was 0.9883, and the adjusted coefficient (R^2_{adj}) was 0.9732. These results demonstrate a strong correlation between the selected factors and the response values, indicating that the model is highly reliable and well-suited for explaining and predicting responses. The coefficient of variation (CV%) was 1.05%, indicating good experimental stability. The magnitude of the F-value reflects the order of influence of the three factors on the response value: MWCNTs (C) > PSA (A) > C_{18} (B). All of the quadratic terms (A^2, B^2, and C^2) significantly influenced the recoveries ($p < 0.01$).

Contour plots and 3D response surface plots illustrate the impact of factor interactions on the required compound ratio to meet the recovery range. Figure 4 shows contour plots and 3D response surface plots generated from the regression equation.

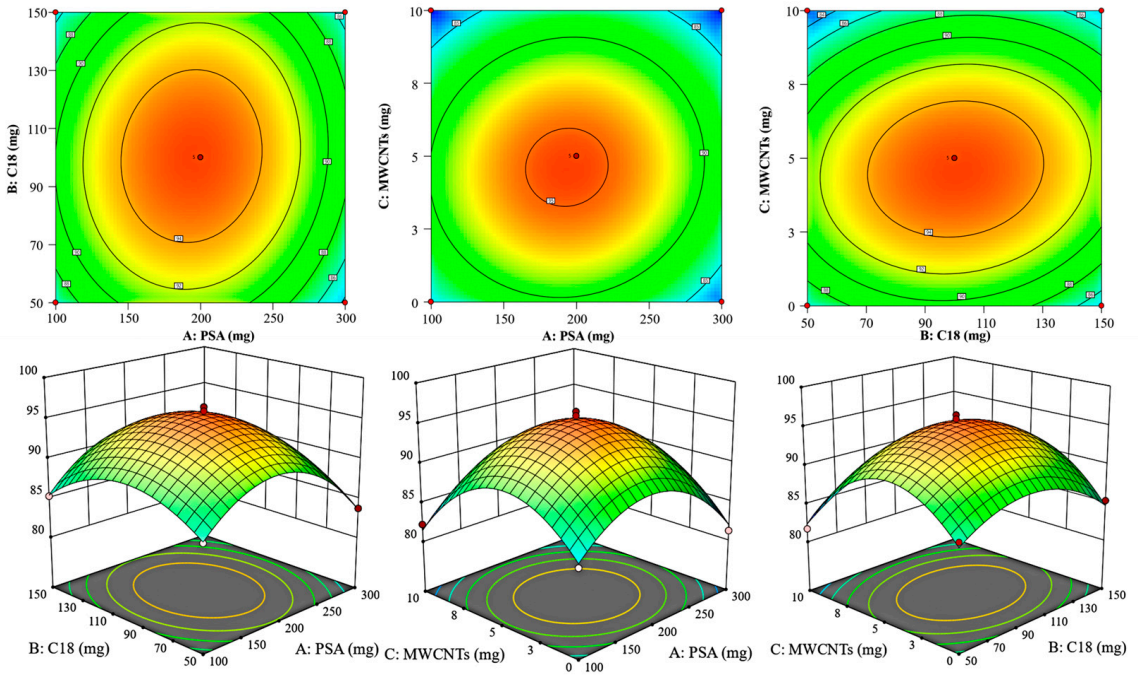


Figure 4. Response surface and contour plots of factor interactions.

As shown in Figure 4, all three 3D plots exhibit convex shapes with downward-facing openings, and their contour lines are nearly elliptical. This indicates that the selected factors exhibit strong interactions and reach their maximum values within the examined range. Among these, C_{18} and MWCNTs have the contour shapes that most closely resemble ellipses, with densely packed contours. Their 3D plots also exhibit steeper gradients. This indicates that the interaction between C_{18} and MWCNTs has the most significant impact on recoveries, followed by other factors. Furthermore, when the dosage of MWCNTs was treated as the independent variable while the dosage of C_{18} remained constant, the 3D plot exhibits a pronounced trend of first increasing and then decreasing. However, when C_{18} dosage was the independent variable, the 3D plot exhibited relatively gentle fluctuations, with a surface steepness lower than that of the MWCNTs dosage curve. This indicates that in the interaction experiment between these two factors, MWCNTs dosage exerts a greater influence on the compound ratio than C_{18} dosage. This finding is consistent with the conclusions drawn from the analysis of variance in Table 3.

Based on the analysis of the experimental data, the software recommends the following optimal parameters: PSA: 193.5 mg; C_{18}: 100.1 mg, and MWCNTs: 4.6 mg. Under these conditions, the theoretical compound ratio within the 70% to 120% range is 95.49%. Based on actual conditions, the optimal parameters selected are: PSA: 194 mg, C_{18}: 100 mg, and MWCNTs: 5 mg. Under these conditions, the compound ratio reached 96.45%. This shows that the pretreatment process parameters obtained through response surface optimization are reliable and possess practical value. Furthermore, although the conclusions from single-factor experiments and response surface analysis are similar, the former examine only the independent effects of individual factors. In contrast, response surface analysis considers the interactions among multiple factors, thereby enhancing the statistical reliability of the conclusions.

3.2. Validation of the Approach

3.2.1. Matrix Effect

The matrix effect (ME) refers to the phenomenon where interfering components in a sample affect the accuracy of compound concentration or mass determination, leading to increased or decreased response values for the target analyte. Different types of compounds and sample matrices can cause different MEs. Therefore, when detecting multiple pesticide residues and mycotoxins, ME is a critical factor in ensuring the accuracy and reproducibility of test results. ME = (slope of matrix curve/slope of standard curve − 1) × 100% [39].

When ME > 0, it indicates the presence of a matrix enhancement effect; when ME < 0, it indicates the presence of a matrix inhibition effect. Astragalus components are complex, and the matrix constituents and co-extractables readily interfere with target ions, leading to ion enhancement or suppression. This study calculated MEs by establishing matrix curves and solvent curves. The results are shown in Figure 5; most compounds exhibit matrix inhibition effects. Specifically, among the analyzed pesticides, 85% showed matrix inhibition, while 15% showed matrix enhancement; among the mycotoxins, 80% showed matrix inhibition and 20% showed matrix enhancement.

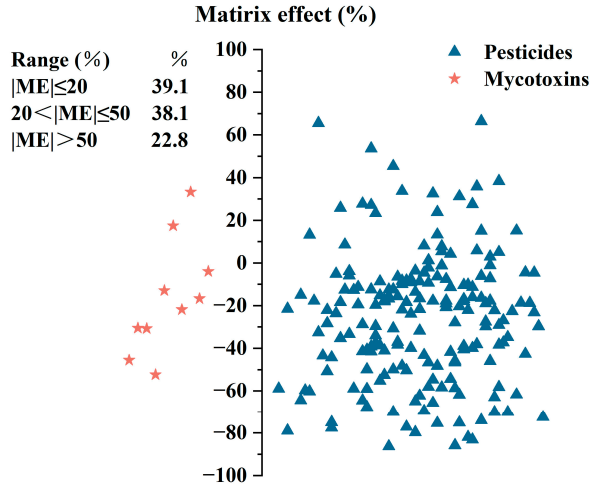


Figure 5. Distribution of matrix effects for 187 pesticides and 10 mycotoxins.

Based on the magnitude of the impact, it can be further classified into weak matrix effects (|ME| ≤ 20%), moderate matrix effects (20% < |ME| ≤ 50%), and strong matrix effects (|ME| > 50%). As shown in Figure 5, 39.0% of the pesticides and 40.0% of the mycotoxins in Astragalus exhibited weak MEs. In comparison, 37.4% of the pesticides and 50.0% of the mycotoxins showed moderate MEs, demonstrating the method's strong resistance to matrix interference in Astragalus.

3.2.2. Screening Detection Limits (SDLs), Limits of Quantification (LOQs), and Linearity

This study validated the screening limit (SDL) and limit of quantification (LOQ) in accordance with the SANTE/11312/2021 guideline. The SDL is defined as the lowest spiked concentration at which the detection rate reaches 95% in 20 spiking experiments conducted at multiple spiked concentrations. The LOQ is the lowest spiked concentration at which the analyte can be reliably and accurately determined, with recovery rates between 70% and 120% and a relative standard deviation (RSD) ≤ 20%.

Under optimal conditions, the method validation results are shown in Table S1. For 197 compounds in Astragalus, the SDLs and LOQs ranged from 1 to 20 and 2 to 20 μg/kg, respectively. Pesticides and mycotoxins with LOQs ≤ 10 μg/kg accounted for 82.4% and 70.0%, respectively. A series of mixed standard working solutions was added to the blank

matrix of Astragalus. The calibration curve's linear correlation coefficient (R^2) was used to assess the reliability of the regression line. Experimental results showed that the R^2 values for all target compounds within the linear range exceeded 0.995, indicating excellent linear relationships. Overall, this study requires less time, is easy to operate, and effectively enhances the sensitivity of compounds in the matrix.

3.2.3. Recovery and Precision

To evaluate the method's accuracy and precision, spiked recovery experiments were conducted in blank matrices at spiking levels of 1×, 2×, and 10× the LOQ, with six replicates at each level. The mean recoveries and RSDs for each level were calculated, and the results are presented in Table S1. The recoveries for 197 compounds at three spiking levels ranged from 61.5% to 118.9%, 67.1% to 119.6%, and 72.0% to 119.3%, respectively. The RSDs ranged from 0.7% to 17.7%. The method's precision was verified by repeating six analyses of the same sample within a single day and over three consecutive days. The intra-day RSD in Astragalus was below 10.0%, while the inter-day RSD was below 20.0%. These results show that the established method demonstrates good accuracy and precision and can simultaneously detect both pesticide residues and mycotoxins in Astragalus.

3.2.4. Comparison with Other Methods

The results of comparing this method with previously reported methods are shown in Table 4. Our method achieves a lower LOQ range compared to the methods reported by reference [8,39–42]. Additionally, our method achieves shorter analysis times compared to those reported in [8,40]. Compared to [43], our method detects more compounds. Furthermore, our method is the first to combine response surface methodology for the analysis of pesticides and mycotoxins in Astragalus, providing a novel and practical approach.

Table 4. Comparison of the proposed method with other analytical methods for determination.

Matrix	Number of Analytes	Extraction Method	Instrumentation	LOQ Range (μg/kg)	Total Analysis Time	References
Astragalus	33 pesticides	QuEChERS	LC-MS/MS, GC-MS/MS	10–100	50 min	[8]
Six edible and medicinal plants	31 mycotoxins	QuEChERS	LC-MS/MS	0.03–50	15 min	[39]
Two TCM	147 pesticides	QuEChERS	GC-MS/MS	10–50	30 min	[40]
Three TCM	108 pesticides	QuEChERS	UHPLC-MS/MS	0.01–50	21 min	[41]
Three rhizome TCM	16 mycotoxins	mPFC-QuEChERS	UPLC-MS/MS	0.5–50	17 min	[42]
Spices and herbs	134 pesticides and 11 mycotoxins	QuEChERS	HPLC-QqQ-MS/MS	2.4–12	15 min	[43]
Astragalus	187 pesticides and 10 mycotoxins	QuEChERS	LC-Q-TOF/MS	2–20	23 min	This work

3.3. Real Sample Analysis

The method established in this study was applied to screen and quantify pesticide residues and mycotoxins in 20 batches of real Astragalus samples. To avoid false positives during compound identification, we performed detailed confirmation of the compounds in accordance with SANTE/11312/2021 and the research by Tóth et al. [44]. The results are presented in Table 5. Three pesticides and three mycotoxins were detected among the

20 batches of samples. The most frequently detected pesticide was tebuconazole (15%), followed by azoxystrobin (10%) and difenoconazole (5%). None of these detected pesticides have maximum residue limits (MRLs) specified in GB 2763-2021. However, all three are organic nitrogen fungicides, which may be related to fungicides applied during Astragalus cultivation to prevent infections such as root rot. The mycotoxins detected were aflatoxin B_2, aflatoxin G_2, and ochratoxin A, with detection rates of 10%, 10%, and 5%, respectively. Among these, two batches of real samples were found to contain both aflatoxin B_2 and aflatoxin G_2, with aflatoxin sums of 22.62 µg/kg and 48.67 µg/kg, respectively. China has not yet established MRLs for aflatoxins in Astragalus. However, compared with the European Pharmacopoeia limit (total aflatoxins G_2, G_1, B_2, and B_1 not exceeding 4 µg/kg), these two batches of the real samples exceeded this requirement [45]. Ochratoxin A was detected at 45.1 µg/kg in one batch of the real samples, exceeding the maximum limit of 20 µg/kg specified in the Chinese Pharmacopoeia (2025 ed.). All detection results were consistent with previous reports [14,46]; representative chromatograms are shown in Figure 6. The aforementioned results indicate that improper pesticide application may occur during Astragalus cultivation. However, overall residue levels remain low, posing minimal associated risk. In contrast, contamination by mycotoxins resulting from improper drying or storage practices is a more significant concern and requires urgent attention.

Table 5. Detection of pesticide residues and mycotoxins in real Astragalus samples.

No.	Compound	Detected Quantity	Detection Rate (%)	Concentration Range (µg/kg)
1	Azoxystrobin	2	10.0	5.08–10.88
2	Difenoconazole	1	5.0	5.66
3	Tebuconazole	3	15.0	11.15–34.28
4	Ochratoxin A	1	5.0	45.10
5	Aflatoxin B_2	2	10.0	16.40–21.43
6	Aflatoxin G_2	2	10.0	6.22–27.24
	Sum of aflatoxins	2	10.0	22.62–48.67

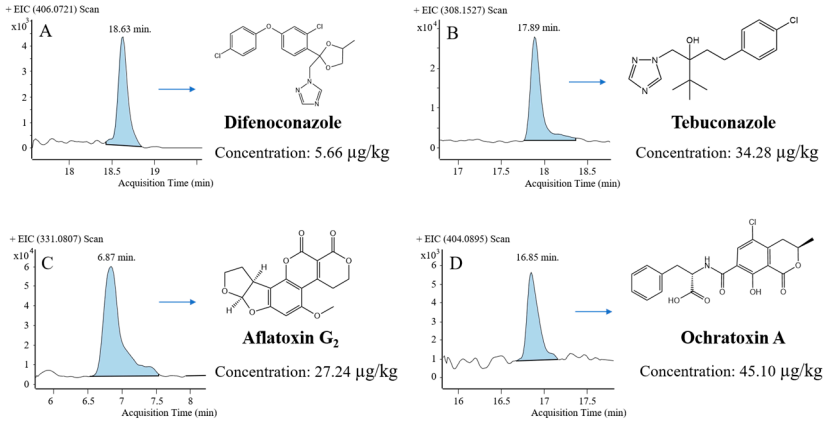


Figure 6. Chromatograms of difenoconazole (**A**), tebuconazole (**B**), aflatoxin G_2 (**C**), and ochratoxin A (**D**).

4. Conclusions

This study employed response surface methodology to optimize the QuEChERS method combined with LC-Q-TOF/MS detection technology, establishing a rapid multi-residue detection method for 187 pesticides and 10 mycotoxins in Astragalus. The results indicate that the established detection technology demonstrates high sensitivity for identifying pesticides and mycotoxins in Astragalus, meeting routine screening requirements. Both accuracy and precision were favorable. This method was applied to test 20 batches of real

Astragalus samples. The results indicate that while pesticide residues were detected at low levels in the samples, mycotoxin contamination exceeding standards was a particular issue. To effectively reduce the risk of mycotoxin contamination, it is recommended that control measures be strengthened at key stages such as processing and storage. This method is rapid and straightforward to operate, providing a technical reference for high-throughput screening of pesticide residues and mycotoxins in other root and rhizome-based medicinal and food homologous substances. It contributes to enhancing the safety of such materials and safeguarding public health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations13030076/s1, Table S1: SDL, LOQ, Linear range, R^2, Spiked recoveries, and precision of 187 pesticides and 10 mycotoxins.

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