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Screening of 258 Pesticide Residues in Silage Using Modified QuEChERS with Liquid- and Gas Chromatography-Quadrupole/Orbitrap Mass Spectrometry

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Abstract: A method for the simultaneous screening of 258 pesticide residues in silage using modified QuEChERS combined with liquid chromatography (LC)- and gas chromatography (GC)- quadrupole-Orbitrap mass spectrometry (Q-Orbitrap/MS) has been developed. After hydration, the silage was homogenized with a 1% acetic acid–acetonitrile solution, and the extract was purified using C_{18}, PSA, and anhydrous magnesium sulfate. Finally, the sample was detected using LC/GC-Q-Orbitrap/MS, and quantified using an external standard method. The results showed that 258 pesticides had an excellent linear relationship in the range of 0.1–50 μg L^{-1}, and that the coefficients of determination (R^2) were more than 0.99. The screening detection limit (SDL) of silage was in the range of 0.5–50 μg kg^{-1}, and the limit of quantitation (LOQ) was in the range of 1–50 μg kg^{-1}. The accuracy and precision of the method were verified at the spiked levels of 1-, 2- and 10-times LOQ, and the recovery of 258 pesticides was in the range of 66.5–119.8%, with relative standard deviations (RSDs) of less than 20% ($n = 6$). This method was simple, rapid, and reliable, and could be applied to screen and quantify multi-pesticide residues in silage.

Keywords: silage; pesticides; QuEChERS; Orbitrap

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Citation: Xie, Y.; Wu, X.; Song, Y.; Sun, Y.; Tong, K.; Yu, X.; Fan, C.; Chen, H. Screening of 258 Pesticide Residues in Silage Using Modified QuEChERS with Liquid- and Gas Chromatography-Quadrupole/Orbitrap Mass Spectrometry. *Agriculture* **2022**, *12*, 1231. https://doi.org/10.3390/agriculture12081231

Academic Editor: Fabio Sciubba

Received: 9 July 2022
Accepted: 13 August 2022
Published: 15 August 2022

1. Introduction

As a bulk livestock product, milk yield and quality is of widespread concern. "Grass-raising livestock" has been proven to be a reliable source of high-quality dairy products [1]. Silage is fermented from fresh plants as a necessary fundamental feed for dairy farmers [2], and its production process mainly includes the steps of harvesting, processing, transportation, filling, compaction, and capping [3–5]. As high-quality roughage, silage is rich in nutrients after 45 days of fermentation. It is an essential source of fermentable carbohydrates for ruminants, and is known as the "king of feed" [4,6,7]. The silage of interest in this study mainly uses corn stover as the raw material, which has high nutritional value and a wide market demand.

The demand for silage is growing with the rise of the livestock industry in China. To increase silage yield, the irrational application of pesticides may exist during maize cultivation [8]. Pesticide residues in silage are mainly derived from pesticide spraying during planting to prevent diseases and pests [9]. According to the literature, the main pesticides involved in maize cultivation are organophosphorus, organochlorine, and carbamate [10,11]. In addition to the incorrect use of pesticides, pollution in the environment can also cause silage to contain a small number of pesticide residues, such as water and soil that is contaminated by pesticides [12,13]. At the same time, the farmer sprays the pesticide to kill insects and bacteria before silage fermentation. Once an application is deemed unreasonable, it will result in residues, compromising the quality and safety of silage [14]. When contaminated silage is fed to cows, pesticides accumulate in their bodies and are transferred to the milk during lactation, which can harm milk quality [15]. The quality of silage has been the primary focus of safety supervision, but the silage contains numerous impurities (e.g., pigments, sugars, fatty acids, and proteins) that make pesticide residue identification difficult [15]. Therefore, it is necessary to figure out how to efficiently eliminate matrix background interference and to develop a method for screening pesticide residues in silage.

Solid-phase extraction (SPE) [16] and QuEChERS [17] were the most common forage pretreatment methods. The QuEChERS method is easy to use, requires fewer reagents, and is quick to process. It meets the requirements of green chemistry and is receiving increasing attention. However, the pretreatment methods of hundreds of pesticide residues in silage have not been reported. Viera et al. [18] detected 72 pesticide residues in 10 agricultural items (including corn silage) using QuEChERS with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The limit of detection of this method was from 4.8 $\mu g\ kg^{-1}$ to 48 $\mu g\ kg^{-1}$. The recovery was in the range of 70–120%. The literature research shows that the current published QuEChERS method has achieved satisfactory stability and recovery, but the number of pesticides detected and the sensitivity of the method need to be improved. It is vital to apply sensitive and reliable techniques to detect pesticide residues in forage. The current analytical methods for the detection of multi-pesticide residues are mainly GC-MS [19], GC-MS/MS [19,20], and LC-MS/MS [19,21]. These chromatography-mass spectrometry techniques have become an essential analytical tool. Still, they suffer from a lack of sensitivity, low resolution, and the inability to avoid false positives [22]. High-resolution mass spectrometry (HRMS), such as electrostatic field Orbitrap MS, has been widely used in food analysis due to its high mass accuracy at the ppm level, ultra-high resolution, and accurate mass information, enabling high-throughput information acquisition and retrospective analysis without additional injection [23–25].

This work aims to screen multi-pesticide residues that may have remained from planting to fermentation, based on the QuEChERS with LC/GC-Q-Orbitrap/MS method. This work is the first reported to screen and quantify compounds in silage using the HRMS methods. Because of the difficulties encountered during pretreatment, several typical cases, such as the hydration volume, extraction volume, salting-out agent type, and purification filler, were optimized to minimize the matrix effects and to improve recovery. The samples were detected using LC/GC-Q-Orbitrap/MS to achieve sensitivity for pesticides with different physico-chemical properties. A methodological validation was carried out, and the qualitative and quantitative analysis of various pesticide residues in forage was successfully completed. Additionally, the validated method was applied to the actual silage samples.

2. Materials and Methods

2.1. Instrumentation

The ultra-high-performance liquid chromatography quadrupole Orbitrap mass spectrometry system used was the Ultimate 3000 UHPLC system (Dionex Corporation, Sunnyvale, CA, USA), in conjunction with the Q-Orbitrap mass spectrometer from Thermo Fisher Scientific (Bremen, Germany). A Trace 1310 GC coupled to a quadrupole Orbitrap mass spectrometry with a TriPlus RSH automatic sampler was purchased from Thermo Fisher Scientific (Bremen, Germany). A PL602-L electronic balance was purchased from Mettler-Toledo (Zurich, Switzerland); an AH-30 Fully Automatic Homogenizer was obtained from Raykol Instrument Co., Ltd. (Xiamen, China); an N-112 Nitrogen evaporator concentrator was obtained from Organomation Associates (EVAP 112, Worcester, Massachusetts, USA); as well as an SR-2DS oscillator (Taitec, Japan), a KDC-40 low-speed centrifuge (Zonkia, China), and a Milli-Q ultrapure water machine from Millipore Corporation (Milford, MA, USA).

2.1.1. Chromatographic and MS Conditions of LC-Q-Orbitrap/MS

Chromatographic Conditions: Chromatographic separation was achieved under chromatographic conditions: a reversed-phase chromatography column (Accucore aQ 150 × 2.1 mm, 2.6 μm; Thermo Fisher Scientific, Santa Clara, CA, USA); mobile phase A is 5 mM ammonium acetate–0.1% formic acid–water; mobile phase B is 0.1% formic acid–methanol; gradient elution program, 0 min: 1% B, 3 min: 30% B, 6 min: 40% B, 9 min: 40% B, 15 min: 60% B, 19 min: 90% B, 23 min: 90% B, 23.01 min: 1% B, and run after 4 min; the flow rate is 0.4 mL min^{-1}; column temperature: 40 °C; injection volume: 5 μL.

MS Conditions: An HESI-II electrospray source was used on the Q-Orbitrap in positive ionization mode. The conditions for electrospray ionization were set as follows: scan mode: full MS/dd-MS^2 (full scan/data dependent secondary scan); full MS scan range: 80–1100 m/z; resolution: 70,000 FHWM, full MS; 17,500 FHWM, MS^2; maximum injection time: full MS, 200 ms; MS^2, 60 ms; automatic gain control: full MS, 1×10^6; MS^2, 2×10^5; loop count: 1; multiplex count: 1; isolation width: 2.0 m/z; under fill ratio: 1%; stepped normalized collision energy: 20, 40, 60; apex trigger: 2–6 s; dynamic exclusion: 8 s.

2.1.2. Chromatographic and MS Conditions of GC-Q-Orbitrap/MS

Chromatographic Conditions: Chromatographic separation was achieved under chromatographic conditions: TG-5 SILMS chromatography column (TG-5 SILMS 30 m × 0.25 mm (i.d.) × 0.25 μm); gas chromatographic heating procedure: 40 °C for 1 min, 30 °C min^{-1} to 130 °C, 5 °C min^{-1} to 250 °C, 10 °C min^{-1} to 300 °C, 7 min; injector type and temperature: SSL, 250 °C; injection volume: 1 μL; carrier gas: helium; flow rate: 1.2 mL min^{-1}.

MS Conditions: Ion source: EI source; electron energy: 70 eV; ion source temperature: 280 °C; mass spectrum end transmission line temperature: 280 °C; solvent delay time: 4 min; scan range: 50–600 (m/z); resolution: 60,000 FHWM (200 m/z); automatic gain control: 1×10^6; scan mode: full scan.

The mass spectrum information for 258 pesticides is shown in Table 1.

Table 1. HRMS parameters and validation parameters for all target analytes in silage.

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
1	1-(2-chloro-4-(4-chlorophenoxy)phenyl)-2-(1H-1,2,4-triazol-1-yl)ethanol	Fungicides	17.45	350.0453	118.0412	0.9994	50	50	85.7	6.7	88.1	6.2	86.2	1	LC-Q-Orbitrap/MS
2	1-(2-Chloro-pyridin-5-yl-methyl)-2-imino-imidazolidine hydrochloride	Insecticides	3.03	211.0744	126.0105	0.9983	10	10	85.5	5.3	90.4	7.7	87.5	6	LC-Q-Orbitrap/MS
3	1-methyl-3-(tetrahydro-3-furylmethyl) urea	Insecticides	2.8	159.1127	102.0914	0.9995	20	20	87.8	6.8	83.1	1.9	83.4	3	LC-Q-Orbitrap/MS
4	2,4-D butylate	Herbicides	17.03	185	186.997	0.9991	2	2	71.2	8.7	104.7	6.4	85.3	5	GC-Q-Orbitrap/MS
5	2,4′-DDD	Insecticides	22.62	235.0076	165.0699	0.9995	2	5	90.7	9.2	97.7	2.1	97.8	2	GC-Q-Orbitrap/MS
6	2,4′-DDE	Insecticides	21.17	245.9999	176.062	0.9991	1	1	99.2	9.1	81	6.5	100.9	2	GC-Q-Orbitrap/MS
7	3-(Trifluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide	/	3.37	194.0536	134.0349	0.9986	5	5	95.2	6	96.5	5.7	97.6	2	LC-Q-Orbitrap/MS
8	4,4′-DDD	Insecticides	23.91	235.0077	165.0699	0.999	1	2	87.5	10	89.1	6.5	88.2	2	GC-Q-Orbitrap/MS
9	5-hydroxy Imidacloprid	Insecticides	4.02	272.0541	225.0539	0.9988	50	50	86.1	5.1	88.3	4.4	75.4	4	LC-Q-Orbitrap/MS
10	Acetamiprid	Insecticides	5.46	223.0744	126.0106	0.9999	5	5	99.3	3.8	94.3	4.2	79.1	5	LC-Q-Orbitrap/MS
11	Acetamiprid-N-desmethyl	Insecticides	5.17	209.059	126.0105	0.9997	5	5	94.7	7.7	97	6.2	81	3	LC-Q-Orbitrap/MS
12	Acetochlor	Herbicides	16.9	146.0965	162.0914	0.9996	2	5	106.7	4.4	86.6	6.5	94.2	3	GC-Q-Orbitrap/MS
13	Acrinathrin	Insecticides	29.35	181.0647	180.081	0.999	5	5	89	6	99	6.3	99.7	5	GC-Q-Orbitrap/MS
14	Alachlor	Herbicides	17.22	188.107	146.0965	0.9996	5	5	98.2	8.2	113.5	3.4	95.1	2	GC-Q-Orbitrap/MS
15	Aldicarb-sulfone	Insecticides	3.44	240.101	86.06002	0.9997	50	50	93.5	4.2	94	4	77.9	3	LC-Q-Orbitrap/MS
16	Allethrin	Insecticides	19.33	303.195	135.0805	0.9994	10	10	95.8	6	92.4	6.4	94.9	8	LC-Q-Orbitrap/MS
17	Allidochlor	Herbicides	6.9	174.0678	98.09644	0.9998	0.5	1	102.1	5.6	102.5	4.4	99.8	4	LC-Q-Orbitrap/MS
18	alpha-HCH	Insecticides	13.53	180.9373	218.911	0.9996	5	10	96.4	4.4	89.7	2.3	109.1	12	GC-Q-Orbitrap/MS
19	Ametryn	Herbicides	17.72	227.1199	170.0493	0.9983	5	5	100.7	1.9	98.4	1.3	84.5	4	GC-Q-Orbitrap/MS
20	Atrazine	Herbicides	11.73	216.101	174.0543	0.9996	2	2	107.8	12.8	107.3	3.7	86.6	2	LC-Q-Orbitrap/MS
21	Azoxystrobin	Fungicides	15.39	404.1237	344.1031	0.9996	5	5	101.8	3.3	93.6	3.8	89.6	7	LC-Q-Orbitrap/MS
22	Benalaxyl	Fungicides	18.04	326.1747	148.1122	0.9998	2	2	98.2	5.1	98.7	3.1	94.9	4	LC-Q-Orbitrap/MS
23	Bendiocarb	Insecticides	8.38	224.0914	109.0285	0.9998	10	10	104.3	7.3	90.1	4.8	96.3	3	LC-Q-Orbitrap/MS
24	Benfluralin	Herbicides	13	292.0541	276.0589	0.9979	10	10	101.3	14	96.5	9	117.1	14	GC-Q-Orbitrap/MS
25	Benoxacor	Herbicides	13.66	260.0236	149.0836	0.9998	20	20	89.5	4.3	89.7	3.1	94.3	6	LC-Q-Orbitrap/MS
26	Benzovindiflupyr	/	32.37	159.0365	238.9715	0.9996	5	5	93.6	5.2	103.1	3.3	91.7	7	GC-Q-Orbitrap/MS
27	beta-HCH	Insecticides	14.52	180.9371	218.911	0.9975	10	10	108.8	4.9	93.1	5	113.3	11	GC-Q-Orbitrap/MS
28	Bifenox	Herbicides	27.67	340.985	173.0154	0.9991	20	20	84.6	17.8	93.6	12.9	92.6	1	GC-Q-Orbitrap/MS
29	Bifenthrin	Insecticides	27.12	181.1011	166.0776	0.9997	2	2	103.9	12.8	93	5.3	86.3	4	GC-Q-Orbitrap/MS
30	Bitertanol	Fungicides	30.1	170.0726	141.0699	0.9987	2	2	103.1	10.2	84	5.4	87	6	GC-Q-Orbitrap/MS
31	Boscalid	Fungicides	31.51	139.9898	111.9949	0.9996	5	5	94.3	8.8	104.4	2.2	93	2	GC-Q-Orbitrap/MS
32	Bromobutide	Herbicides	16.96	312.0951	119.0857	0.9993	20	20	90.8	6.3	92.9	3.5	94.1	5	LC-Q-Orbitrap/MS
33	Bromophos-methyl	Insecticides	19.46	328.8798	332.8746	0.9998	5	5	105.4	9.2	113.2	5.9	102.7	2	GC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
34	Bromopropylate	Insecticides	27.1	182.944	340.8993	0.9998	2	5	87.9	3.7	97.3	2.5	95	2	GC-Q-Orbitrap/MS
35	Bupirimate	Fungicides	17	317.1636	108.0114	0.9997	2	2	101.2	5	96.4	2.9	88.5	4	LC-Q-Orbitrap/MS
36	Buprofezin	Insecticides	18.93	306.1629	106.0652	0.9996	2	2	97.9	5.3	99.4	2.9	95.9	5	LC-Q-Orbitrap/MS
37	Butachlor	Herbicides	21.44	176.107	188.107	0.9997	5	5	90.7	2.8	99.8	2.3	97.1	3	GC-Q-Orbitrap/MS
38	Butamifos	Herbicides	18.33	333.1028	95.96676	0.9997	10	10	102.3	14.7	96.5	7	93	4	LC-Q-Orbitrap/MS
39	Butylate	Herbicides	18.99	218.157	156.1385	0.9995	1	2	73.1	15.5	75.3	9.4	81.4	6	LC-Q-Orbitrap/MS
40	Cadusafos	Insecticides	18.61	271.0946	158.9699	0.9999	2	2	90.7	9.8	114.9	11.4	94.4	11	LC-Q-Orbitrap/MS
41	Carbaryl	Insecticides	17.38	144.057	116.0621	0.9998	2	2	70.2	11.3	100	5.3	91.4	4	GC-Q-Orbitrap/MS
42	Carbendazim	Fungicides	4.04	192.0767	160.0506	0.9993	5	5	97.6	3.3	95.7	4.3	108.9	8	LC-Q-Orbitrap/MS
43	Carbofuran	Insecticides	8.47	222.1122	123.0441	0.9989	1	1	94.3	7.6	88.5	11	85.5	4	LC-Q-Orbitrap/MS
44	Carbofuran-3-hydroxy	Insecticides	4.94	238.107	163.0752	0.9973	0.5	10	95.1	0.9	100.5	2.4	99.6	4	LC-Q-Orbitrap/MS
45	Carfentrazone-ethyl	Herbicides	17.69	429.07	345.9957	0.9984	10	10	95.1	6.9	92.9	9	99.6	6	LC-Q-Orbitrap/MS
46	Chlorantraniliprole	Insecticides	14.15	481.9779	283.9221	0.9996	50	50	82.7	5.3	94.3	7.6	80.1	4	LC-Q-Orbitrap/MS
47	Chlorfenapyr	Insecticides	23.06	247.0479	363.9409	0.9988	50	50	94.3	13	104.7	12.2	89.6	2	GC-Q-Orbitrap/MS
48	Chlorfenvinphos	Insecticides	20.37	266.9379	323.0002	0.9998	5	5	92.3	9.6	94.5	2.8	96.3	9	GC-Q-Orbitrap/MS
49	Chloridazon	Herbicides	5.26	222.0427	104.0495	0.9997	5	5	108.8	2	107.1	2.1	87.3	5	LC-Q-Orbitrap/MS
50	Chlormequat	Plant growth regulators	0.91	122.073	62.9999	0.9984	50	50	76.6	11.3	75.9	5.2	79.6	6	LC-Q-Orbitrap/MS
51	Chloroneb	Fungicides	10.04	190.9663	205.9897	0.9996	2	2	78.6	10.6	93.1	7.2	84.6	2	GC-Q-Orbitrap/MS
52	Chlorotoluron	Herbicides	11.32	213.0788	72.04449	0.9999	5	20	88.4	3.2	85.5	4.4	82.6	2	LC-Q-Orbitrap/MS
53	Chlorpropham	Herbicides	12.76	213.055	152.9976	0.9996	20	20	70.9	11.6	96.3	6.3	94.6	4	GC-Q-Orbitrap/MS
54	Chlorpyrifos-methyl	Insecticides	16.91	285.9257	124.9822	0.9979	2	2	100.8	11.2	91	12.4	91.9	6	GC-Q-Orbitrap/MS
55	Chlozolinate	Fungicides	20.24	186.9586	258.9798	0.998	10	10	96.3	13.4	94.8	5.6	113.9	11	GC-Q-Orbitrap/MS
56	Cis-Chlordane (alpha)	Insecticides	21.49	370.8284	374.8226	0.9993	10	20	83.7	11.8	106.1	4.7	89.7	3	GC-Q-Orbitrap/MS
57	Clodinafop-propargyl	Herbicides	17.73	350.0586	266.038	0.9997	5	5	102.2	4.1	95	2.9	91.8	6	LC-Q-Orbitrap/MS
58	Clofentezine	Insecticides	18.61	303.0193	138.0106	0.9996	10	10	101.9	5.7	87.5	7.5	99	8	LC-Q-Orbitrap/MS
59	Clomazone	Herbicides	14.57	204.1019	127.0124	0.9998	5	5	105	3.3	100.1	0.9	87.8	4	GC-Q-Orbitrap/MS
60	Clothianidin	Insecticides	4.7	250.0157	131.967	0.9997	50	50	87.8	5	86.4	4.4	80.8	2	LC-Q-Orbitrap/MS
61	Cyanazine	Herbicides	7.63	241.096	214.0856	0.9998	5	5	110.5	2.2	100.9	1.6	86.3	5	LC-Q-Orbitrap/MS
62	Cyanofenphos	Insecticides	24.93	156.9872	169.0413	0.996	2	2	83.3	4.4	91.9	3.4	88.1	2	GC-Q-Orbitrap/MS
63	Cyanophos	Insecticides	14.94	243.0116	109.005	0.9997	5	10	88.4	7.6	96.6	2.5	114.9	11	GC-Q-Orbitrap/MS
64	Cycloate	Herbicides	18.6	216.1415	83.08556	0.9999	2	2	97.1	10.8	89.9	4.4	97.7	2	LC-Q-Orbitrap/MS
65	Cycloxydim	Herbicides	18.78	326.1779	180.1013	0.9981	10	10	83.3	7.4	75.2	8	72.5	7	LC-Q-Orbitrap/MS
66	Cyfluthrin	Insecticides	31.18	206.0601	163.0075	0.9993	20	20	98.9	13.6	91.9	2.3	93.7	1	GC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
67	Cypermethrin	Insecticides	31.69	181.0647	127.031	0.9973	50	50	84.5	9.5	96.6	13.9	83.6	3	GC-Q-Orbitrap/MS
68	Cyprodinil	Fungicides	20.03	224.1182	210.1027	0.9999	5	5	98.8	3.1	99.5	2.7	93	5	GC-Q-Orbitrap/MS
69	delta-HCH	Insecticides	15.82	180.9374	218.911	0.9993	5	5	92.5	11.2	104.6	4.6	95.2	4	GC-Q-Orbitrap/MS
70	Deltamethrin	Insecticides	33.53	181.0647	252.9047	0.9944	20	20	96.9	7	99.5	8.3	97.4	5	GC-Q-Orbitrap/MS
71	Desmetryn	Herbicides	9.51	214.1119	172.0653	0.9999	2	2	94.8	5.2	101	2.3	99.4	2	LC-Q-Orbitrap/MS
72	Diallate	Herbicides	18.79	270.0477	86.06006	0.9983	10	10	94.3	4.8	88.7	5.3	89.6	4	LC-Q-Orbitrap/MS
73	Diazinon	Insecticides	15.26	179.1178	199.0631	0.9999	2	2	100.1	4.8	96.5	3.6	100.9	4	GC-Q-Orbitrap/MS
74	Dichlofenthion	Insecticides	16.71	222.938	224.935	0.9993	1	1	78.1	10.7	83.5	9.4	99.7	3	GC-Q-Orbitrap/MS
75	Dichlofluanid	Fungicides	18.3	123.0138	223.95	0.9983	20	20	85.5	4.7	89.9	5.1	93.9	7	GC-Q-Orbitrap/MS
76	Dichlorvos	Insecticides	6.41	184.9764	78.99435	0.9997	1	1	73.1	6.3	93.7	10.5	79.6	6	GC-Q-Orbitrap/MS
77	Dieldrin	Insecticides	22.47	260.8595	79.05431	0.9945	50	50	86.7	15.3	99.5	10.8	81.5	5	GC-Q-Orbitrap/MS
78	Difenoconazole	Fungicides	18.66	406.0715	251.0026	0.9992	10	10	94.5	4.4	94.8	6.7	93.8	7	LC-Q-Orbitrap/MS
79	Diflubenzuron	Insecticides	17.45	311.0389	141.0148	0.9998	10	10	99.6	5.1	87.9	6.3	91.9	6	LC-Q-Orbitrap/MS
80	Dimethenamid	Herbicides	15.03	276.0816	168.0842	0.9997	2	2	84.2	5.8	89.4	1.6	84.3	2	LC-Q-Orbitrap/MS
81	Dimethoate	Insecticides	14.03	124.9822	142.9927	0.9954	5	5	88.1	3.6	101.9	5.1	93.7	3	GC-Q-Orbitrap/MS
82	Dimethylvinphos (E)	Insecticides	18.29	294.9688	127.0155	0.9987	2	5	93.4	6.3	107.2	4.2	95.3	3	GC-Q-Orbitrap/MS
83	Dimethylvinphos (Z)	Insecticides	16.61	330.9452	127.0156	0.9996	10	10	96.9	4.5	92.5	5.1	91.3	3	LC-Q-Orbitrap/MS
84	Diniconazole	Fungicides	23.79	268.0041	232.0273	0.9974	10	10	88.7	12.5	91.3	4.8	111.6	8	GC-Q-Orbitrap/MS
85	Dinotefuran	Insecticides	3.23	203.1138	129.0897	0.9983	50	50	88.1	11.4	91.2	5.7	77.9	3	LC-Q-Orbitrap/MS
86	Dioxabenzofos	Insecticides	12.94	216.0005	200.977	0.9995	5	5	101.5	9.6	103.6	7.7	88.9	4	GC-Q-Orbitrap/MS
87	Dipropetryn	Herbicides	17.21	256.1586	186.0811	0.9996	2	2	94.1	4.4	100.7	2.2	98.9	3	LC-Q-Orbitrap/MS
88	Diuron	Herbicides	12.8	233.0241	72.04452	0.9996	5	50	85.5	5.1	92.7	5.9	81.9	1	LC-Q-Orbitrap/MS
89	Edifenphos	Fungicides	24.95	109.0108	172.9821	0.9996	2	2	98.1	3.2	99.9	2.6	101.4	3	GC-Q-Orbitrap/MS
90	Endosulfan-sulfate	Insecticides	25.03	269.8128	236.8407	0.9975	20	20	95.3	19.2	111.4	7	94.2	4	GC-Q-Orbitrap/MS
91	EPN	Insecticides	27.04	156.9872	169.0413	0.9998	2	2	87.3	16.7	79.1	8.2	86.4	4	GC-Q-Orbitrap/MS
92	EPTC	Herbicides	7.75	128.1071	104.0531	0.9997	5	5	86.4	11.1	80.1	8.2	76.1	4	GC-Q-Orbitrap/MS
93	Ethalfluralin	Herbicides	12.59	276.0595	292.0538	0.9998	20	50	114.2	16.5	93.4	11.4	81.1	1	GC-Q-Orbitrap/MS
94	Ethion	Insecticides	23.94	230.9732	96.95084	0.9998	2	2	79.2	17.2	91.5	4	91.3	3	GC-Q-Orbitrap/MS
95	Ethoprophos	Insecticides	17.05	243.0633	130.9386	0.9997	2	2	93.1	4	99.6	2.5	98.8	2	LC-Q-Orbitrap/MS
96	Etofenprox	Insecticides	31.87	163.1117	107.0492	0.9997	5	5	89.7	14.9	101.7	7.8	96.9	4	GC-Q-Orbitrap/MS
97	Etrimfos	Insecticides	17.79	293.0717	142.9928	0.9997	10	10	104.1	5.6	89.8	5.1	88.8	4	LC-Q-Orbitrap/MS
98	Fenamidone	Fungicides	27.44	268.0905	237.1023	0.9998	5	5	100.4	2	99.3	3	90.3	6	GC-Q-Orbitrap/MS
99	Fenamiphos	Insecticides	17.52	304.1125	217.0083	0.9989	2	2	92.7	9.1	86.7	4.9	87.7	6	LC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
100	Fenamiphos-sulfone	Insecticides	10.34	336.1026	139.0213	0.9998	5	5	95.8	4.8	104.4	2.7	86.9	5	LC-Q-Orbitrap/MS
101	Fenamiphos-sulfoxide	Insecticides	9.78	320.1076	171.0476	0.9973	10	10	119.7	4.1	112	11.8	99.9	4	LC-Q-Orbitrap/MS
102	Fenarimol	Fungicides	29.16	138.9946	251.0028	0.9999	5	5	75.9	6.4	95.5	4.7	90.2	3	GC-Q-Orbitrap/MS
103	Fenbuconazole	Fungicides	17.43	337.1209	125.0153	0.9998	5	5	103	5.6	94.3	2.4	88.3	5	LC-Q-Orbitrap/MS
104	Fenchlorphos	Insecticides	17.55	284.9304	124.9822	0.9974	2	2	102.2	8.5	102.4	3.7	88.3	3	GC-Q-Orbitrap/MS
105	Fenitrothion	Insecticides	18.08	277.017	124.9822	0.9987	20	20	73.5	13.1	115.4	7.8	90.2	4	GC-Q-Orbitrap/MS
106	Fenobucarb	Insecticides	11.79	121.0649	122.0682	0.9995	5	5	117.6	9.7	98.3	5.2	91.5	4	GC-Q-Orbitrap/MS
107	Fenpropathrin	Insecticides	27.45	181.0647	97.10129	0.9997	5	5	89.5	7.6	99.3	3.5	98.7	4	GC-Q-Orbitrap/MS
108	Fenpropimorph	Fungicides	19.13	128.1069	117.0697	0.9993	5	5	100.4	8.8	98.6	7.5	102	4	GC-Q-Orbitrap/MS
109	Fensulfothion	Insecticides	13.29	309.0375	157.0318	0.9996	5	5	99.7	2	98.4	3.1	83.9	5	LC-Q-Orbitrap/MS
110	Fenthion	Insecticides	18.84	278.0195	245.0398	0.9981	10	10	111.5	11.9	102.7	2.9	103.6	3	GC-Q-Orbitrap/MS
111	Fenthion-sulfone	Insecticides	10.93	311.0166	142.9928	0.9998	10	10	97.6	3.8	96.6	3.6	92.7	6	LC-Q-Orbitrap/MS
112	Fenthion-sulfoxide	Insecticides	10.21	295.022	127.0156	0.9997	5	5	106.1	3.3	102.7	3.6	107.2	11	LC-Q-Orbitrap/MS
113	Fipronil	Insecticides	20.24	366.9429	212.9481	0.9999	5	5	109.4	19.9	102.1	7.9	95.7	4	GC-Q-Orbitrap/MS
114	Fipronil Desulfinyl	Insecticides	17.2	332.9961	389.9683	0.9985	5	5	96.1	18.5	106.3	5.7	97.5	2	GC-Q-Orbitrap/MS
115	Fipronil-sulfide	Insecticides	19.86	350.9479	254.9699	0.9999	5	5	112.3	15	107.6	8.2	98.6	4	GC-Q-Orbitrap/MS
116	Fipronil-sulfone	Insecticides	22.51	382.9377	212.9481	0.9998	20	20	66.5	5.5	102.2	10.1	89.8	3	GC-Q-Orbitrap/MS
117	Fluacrypyrim	Insecticides	24.38	145.0649	204.0781	0.9998	10	10	79	4.4	90.7	8.5	102.4	10	GC-Q-Orbitrap/MS
118	Fluazifop-butyl	Herbicides	23.41	282.0736	268.0582	0.9993	2	2	94.1	8.9	87.1	1.5	92.6	3	GC-Q-Orbitrap/MS
119	Flucythrinate	Insecticides	31.7	157.046	181.0647	0.9999	5	5	103.2	14.8	99.5	7.8	90.3	4	GC-Q-Orbitrap/MS
120	Fluopicolide	Fungicides	15.97	382.9722	172.9555	0.9999	10	10	99	4.1	88.8	5.5	92.7	4	LC-Q-Orbitrap/MS
121	Fluquinconazole	Fungicides	30.38	340.0395	341.0428	0.9953	10	10	100.8	6.8	81.4	2.8	106.8	12	GC-Q-Orbitrap/MS
122	Fluridone	Herbicides	14.6	330.1095	259.0989	0.9999	2	2	95	2.6	100.3	2.3	94.9	2	LC-Q-Orbitrap/MS
123	Flusilazole	Fungicides	17.63	316.1072	165.0701	0.9998	2	10	97.7	4.1	85.9	5.4	94.9	6	LC-Q-Orbitrap/MS
124	Flutriafol	Fungicides	12.4	302.1095	70.04012	0.9996	5	5	98.5	4.7	96.9	4	80.1	6	LC-Q-Orbitrap/MS
125	Fluxapyroxad	Fungicides	27.04	159.0364	139.0302	0.9998	1	2	91.8	7.3	91.1	3.6	84.4	5	GC-Q-Orbitrap/MS
126	Fonofos	Insecticides	15.05	137.0187	246.0297	0.9999	2	2	93.5	12.5	102.4	3	91	3	GC-Q-Orbitrap/MS
127	Fosthiazate	Insecticides	11.16	284.0536	104.0165	0.9999	10	10	102.2	7.5	80.4	7.5	90.2	6	LC-Q-Orbitrap/MS
128	Furathiocarb	Insecticides	27.97	163.0753	194.0396	0.9993	2	2	84.1	7.3	92.8	3.4	97.5	3	GC-Q-Orbitrap/MS
129	Haloxyfop	Herbicides	17.72	362.0396	91.05431	0.9981	20	20	78.3	6.4	97.8	4.4	97.7	6	LC-Q-Orbitrap/MS
130	Haloxyfop-2-ethoxyethyl	Herbicides	26.22	302.019	316.0345	0.9997	5	5	95.9	7	94.8	2.1	90.4	7	GC-Q-Orbitrap/MS
131	Haloxyfop-methyl	Herbicides	21.21	288.0035	375.0478	0.9999	5	5	95.4	4.1	90.3	3.7	92.8	7	GC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
132	Heptachlor	Insecticides	17.36	269.813	273.8069	0.9964	50	50	92.1	6.2	112.7	13.6	84.4	4	GC-Q-Orbitrap/MS
133	Hexachlorobenzene	Fungicides	13.67	281.8127	285.8068	0.9988	2	5	93.8	13.4	89.4	7.4	85.7	3	GC-Q-Orbitrap/MS
134	Hexaconazole	Fungicides	18.17	314.0817	70.04015	0.9995	5	10	96.1	3.1	84.5	5.2	90.3	7	LC-Q-Orbitrap/MS
135	Hexythiazox	Insecticides	19.53	353.1079	168.0576	0.9995	10	10	115.9	7.7	105.9	9.7	88.3	6	LC-Q-Orbitrap/MS
136	Imazalil	Fungicides	11.55	297.0553	158.9764	0.9991	10	10	99.3	1.1	86.1	3.8	86.9	6	LC-Q-Orbitrap/MS
137	Imazapyr	Herbicides	4.63	262.1184	217.0972	0.9995	5	5	102	4.9	99.1	2.9	88.1	4	LC-Q-Orbitrap/MS
138	Imidacloprid	Insecticides	4.76	256.0592	209.0589	0.9997	10	10	100.2	4.7	89.2	3.7	90.8	4	LC-Q-Orbitrap/MS
139	Imidaclothiz	Insecticides	5.06	262.0157	181.0542	0.9977	20	20	79.3	3.3	84.6	4.4	82.3	2	LC-Q-Orbitrap/MS
140	Ipconazole	Fungicides	29.05	125.0154	127.0124	0.9999	2	5	89.1	8.4	85.5	2.8	93.7	4	GC-Q-Orbitrap/MS
141	Iprobenfos	Fungicides	17.76	289.1018	91.0543	0.9993	20	20	85.5	7.3	93	3.7	93.9	3	LC-Q-Orbitrap/MS
142	Iprovalicarb	Fungicides	16.88	321.2167	119.0856	0.9996	10	10	73.2	8.3	89.5	4	100.4	5	LC-Q-Orbitrap/MS
143	Isazofos	Insecticides	15.66	118.9883	162.0429	0.9999	5	5	102.3	2.5	95.7	4.6	91.3	6	GC-Q-Orbitrap/MS
144	Isocarbophos	Insecticides	19.14	135.9976	120.0205	0.9998	2	5	72.4	10	87.9	9.8	95.7	4	GC-Q-Orbitrap/MS
145	Isofenphos	Insecticides	20.27	213.0311	121.0285	0.9998	2	2	96.5	19.4	97.9	7.1	86.3	2	GC-Q-Orbitrap/MS
146	Isoprocarb	Insecticides	11.76	194.1176	95.0492	0.9996	5	5	113.4	9.8	99.2	9.8	87.7	5	LC-Q-Orbitrap/MS
147	Isoproturon	Herbicides	12.5	207.149	72.0445	0.9994	2	10	97.2	2.4	89.3	3.8	93	5	LC-Q-Orbitrap/MS
148	Isopyrazam	/	18.64	360.1877	244.0882	0.9999	2	2	92.8	6.6	102.8	3.9	94.4	3	LC-Q-Orbitrap/MS
149	Kresoxim-methyl	Fungicides	22.79	116.0496	206.0812	0.9998	5	5	92.9	8.1	95.8	5.6	99.8	2	GC-Q-Orbitrap/MS
150	Lactofen	Herbicides	19.2	479.0826	222.977	0.9992	10	10	98.9	5.6	89.2	8.4	86.5	7	LC-Q-Orbitrap/MS
151	Lindane	Insecticides	14.52	180.9371	218.911	0.9933	10	20	90.4	7.7	94.7	6	92.8	6	GC-Q-Orbitrap/MS
152	Linuron	Herbicides	14.67	249.0189	159.9717	0.9996	10	10	102.1	2.7	97.5	5.4	97.3	5	LC-Q-Orbitrap/MS
153	Malaoxon	Insecticides	17.15	127.0156	194.9876	0.9994	5	5	98.1	6.3	109.6	4.4	86.4	4	GC-Q-Orbitrap/MS
154	Malathion	Insecticides	15.94	331.0428	99.00771	0.9999	10	10	99.9	6.7	91.6	3.9	94.4	3	LC-Q-Orbitrap/MS
155	Mepanipyrim	Fungicides	16.87	224.118	106.0652	0.9997	2	2	99.7	4.1	94.9	1.9	100.7	4	LC-Q-Orbitrap/MS
156	Metaflumizone	Insecticides	19.28	507.1238	178.0475	0.9994	50	50	83.4	9.1	89.7	6.4	80.7	6	LC-Q-Orbitrap/MS
157	Metalaxyl	Fungicides	17.22	160.1121	132.0809	0.9992	10	10	97.8	4.7	82.4	5.7	107.2	10	GC-Q-Orbitrap/MS
158	Metconazole	Fungicides	27.69	125.0154	138.0664	0.9997	5	5	97.7	6.6	100.9	4.5	95.3	7	GC-Q-Orbitrap/MS
159	Methidathion	Insecticides	20.98	145.0067	124.9822	0.9998	5	5	92.6	6	108.4	5.6	95.4	3	GC-Q-Orbitrap/MS
160	Methiocarb	Insecticides	14.95	226.0894	121.0648	0.9996	10	10	96.6	2.6	86.5	3.9	92.8	4	LC-Q-Orbitrap/MS
161	Methiocarb-sulfone	Insecticides	5.65	275.1055	122.0727	0.9995	50	50	90.4	4.1	100.5	4.4	83.1	2	LC-Q-Orbitrap/MS
162	Methiocarb-sulfoxide	Insecticides	5.16	242.0841	185.0631	0.9996	10	10	106.8	3.6	88.7	3.7	90.9	5	LC-Q-Orbitrap/MS
163	Metolachlor	Herbicides	17.15	284.1408	148.1121	0.9998	10	10	97.2	2.7	94.8	4.8	92	4	LC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
164	Metolcarb	Insecticides	9.31	108.057	79.05431	0.9997	5	5	112.9	9.1	105.9	9.6	90.7	12	GC-Q-Orbitrap/MS
165	Metrafenone	Fungicides	18.42	409.064	226.9703	0.9998	10	10	79.3	6.7	95.3	5.7	92.4	5	LC-Q-Orbitrap/MS
166	Metribuzin	Herbicides	16.93	198.0696	144.0465	0.9975	10	10	93	8.7	75.7	7.5	103.1	11	GC-Q-Orbitrap/MS
167	Mevinphos	Insecticides	6.23	225.052	127.0155	0.9995	2	2	75.8	5.9	89.6	3.7	84.8	3	LC-Q-Orbitrap/MS
168	Mirex	Insecticides	28.83	269.8128	273.8067	0.9991	10	10	81.7	10.6	75.3	5.4	111.7	9	GC-Q-Orbitrap/MS
169	Monocrotophos	Insecticides	4.24	224.068	127.0155	0.9997	2	10	95	5.6	78.4	5.2	101.1	2	LC-Q-Orbitrap/MS
170	Monolinuron	Herbicides	10.17	215.058	126.0107	0.9999	5	5	104.1	2.2	103	2.6	90.4	4	LC-Q-Orbitrap/MS
171	Myclobutanil	Fungicides	22.65	179.0244	181.0214	0.9993	5	5	104.4	2.7	96.8	4.3	86.8	5	GC-Q-Orbitrap/MS
172	Napropamide	Herbicides	17.17	272.1641	171.0806	0.9999	2	2	98.9	2.6	96.7	3.1	99	2	LC-Q-Orbitrap/MS
173	Norflurazon	Herbicides	13.21	304.0454	284.0396	0.9999	5	10	100.7	1.9	90.6	3.9	94.1	7	LC-Q-Orbitrap/MS
174	Omethoate	Insecticides	3.05	214.0296	142.9927	0.9996	10	10	97.8	4.9	91.4	10.6	113.7	8	LC-Q-Orbitrap/MS
175	Oxadiazon	Herbicides	22.5	174.9587	258.0323	0.9994	2	5	100.1	9.9	103.1	2.2	98	2	GC-Q-Orbitrap/MS
176	Oxadixyl	Fungicides	23.87	132.0809	233.0922	0.9966	1	1	86.6	8.7	76.8	12.2	81.6	10	GC-Q-Orbitrap/MS
177	Oxyfluorfen	Herbicides	22.72	252.0395	317.006	0.9993	10	10	92.7	14.8	92.6	4	112.4	13	GC-Q-Orbitrap/MS
178	Paclobutrazol	Plant growth regulators	15.83	294.1364	70.04013	0.9994	5	10	98.3	2.7	87.1	4.3	90.5	5	LC-Q-Orbitrap/MS
179	Parathion	Insecticides	18.98	291.0326	155.0036	0.9987	50	50	84.1	9.1	82.7	10.2	84	5	GC-Q-Orbitrap/MS
180	Pendimethalin	Herbicides	19.92	252.098	191.0688	0.9991	5	5	78.7	9.3	92.1	6.8	88.9	3	GC-Q-Orbitrap/MS
181	Pentachloroaniline	/	16.36	262.8627	266.8568	0.9975	2	2	107.5	14.2	91	9.5	85.4	6	GC-Q-Orbitrap/MS
182	Pentachloroanisole	/	13.87	262.8389	236.8409	0.9993	5	5	85	10.9	99.8	9.9	90.7	3	GC-Q-Orbitrap/MS
183	Penthiopyrad	Fungicides	17.95	360.1346	177.0271	0.9993	2	2	90	8.8	90.9	2.3	90.2	4	LC-Q-Orbitrap/MS
184	Phenothrin	Insecticides	28.19	183.0804	81.06996	0.9998	5	5	94.3	9.3	102	13.9	94.4	3	GC-Q-Orbitrap/MS
185	Phenthoate	Insecticides	20.48	273.9883	245.9933	0.9997	5	5	114.6	12.8	100.4	9.2	91.6	4	GC-Q-Orbitrap/MS
186	Phorate	Insecticides	18.23	261.0201	75.02644	0.9918	10	20	116.2	9.8	97.3	5.9	95.4	2	LC-Q-Orbitrap/MS
187	Phorate-Sulfone	Insecticides	11.8	293.0096	171.024	0.9995	20	20	90.8	2.3	87.3	4.6	94.4	5	LC-Q-Orbitrap/MS
188	Phorate-Sulfoxide	Insecticides	11.48	277.0147	114.9614	0.9998	10	10	106.2	2.2	105.4	10.4	98.7	5	LC-Q-Orbitrap/MS
189	Phosalone	Insecticides	28.22	182.0003	121.0414	0.9996	5	5	104.8	11.6	97.8	6.7	97.6	6	GC-Q-Orbitrap/MS
190	Phosmet	Insecticides	14.19	318.0018	160.0394	0.9994	20	20	78	15.2	95.6	10.2	95.3	2	LC-Q-Orbitrap/MS
191	Phosphamidon	Insecticides	7.68	300.076	127.0156	0.999	10	10	71.9	2.7	81.3	13.5	89	11	LC-Q-Orbitrap/MS
192	Phoxim	Insecticides	18.28	299.061	129.0448	0.9997	10	10	105.6	5.8	90.8	4.4	90	5	LC-Q-Orbitrap/MS
193	Picoxystrobin	Fungicides	17.68	368.11	145.065	0.9969	20	20	80	3.3	99	6.3	101.2	3	LC-Q-Orbitrap/MS
194	Piperonyl Butoxide	Insecticides	26.16	176.0832	119.0856	0.9997	5	5	96.3	7.3	112.6	7.2	88.1	5	GC-Q-Orbitrap/MS
195	Pirimicarb	Insecticides	7.76	239.1493	72.04455	0.9999	5	5	102.2	2.6	95.6	1	87.1	4	LC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
196	Pirimiphos-methyl	Insecticides	18.02	306.1032	108.0557	0.9999	2	2	103.2	6	96.2	2.9	94.6	4	LC-Q-Orbitrap/MS
197	Pretilachlor	Herbicides	18.84	312.1719	252.115	0.9994	5	5	113	5.4	96.6	3.7	89.5	3	LC-Q-Orbitrap/MS
198	Prochloraz	Fungicides	18.09	376.0377	70.02892	0.9997	10	10	105.1	3.7	90.6	6.2	92.3	6	LC-Q-Orbitrap/MS
199	Procymidone	Fungicides	20.64	283.0162	285.0132	0.9989	5	10	110.3	7.1	81.7	3.4	119.8	11	GC-Q-Orbitrap/MS
200	Profenofos	Insecticides	19.07	372.9418	302.8643	0.9997	10	10	98.7	2.1	95.3	5.3	88	6	LC-Q-Orbitrap/MS
201	Prometryn	Herbicides	17.85	241.1357	226.1122	0.9983	5	5	93	7.8	93.8	9	96.1	3	GC-Q-Orbitrap/MS
202	Propamocarb	Fungicides	3	189.1596	102.055	0.9999	10	10	79.8	3.9	98.9	13.5	86.8	12	LC-Q-Orbitrap/MS
203	Propanil	Herbicides	16.85	160.9793	217.0055	0.9996	1	2	83.4	6.3	90.7	3.1	85.6	5	GC-Q-Orbitrap/MS
204	Propaphos	Insecticides	17.98	305.0968	221.0033	0.9996	2	2	81.6	8.6	88.8	4.7	71	19	LC-Q-Orbitrap/MS
205	Propargite	Insecticides	19.69	368.1884	81.06996	0.9997	5	5	117.5	5.7	98.9	4	92.5	4	LC-Q-Orbitrap/MS
206	Propazine	Herbicides	14.54	230.1164	146.0229	0.9999	1	1	106.2	2.1	102	1.8	99.7	2	LC-Q-Orbitrap/MS
207	Propham	Herbicides	9.26	179.094	93.0574	0.9928	2	5	91.6	8.7	96.2	6.2	94.4	2	GC-Q-Orbitrap/MS
208	Propiconazole	Fungicides	25.27	172.9556	174.9526	0.9992	5	5	90.7	8.5	92.8	9.5	91.8	2	GC-Q-Orbitrap/MS
209	Propyzamide	Herbicides	15.14	172.9556	254.0135	0.999	2	2	78.4	11.9	98.8	4.5	91.5	2	GC-Q-Orbitrap/MS
210	Prothioconazole-desthio	Fungicides	17.14	312.066	70.04014	0.9991	5	5	103.4	2	102.8	2.9	93.5	6	LC-Q-Orbitrap/MS
211	Prothiofos	Insecticides	20.39	344.9699	258.9149	0.9993	50	50	84.9	10.6	72.9	4.5	80.2	4	LC-Q-Orbitrap/MS
212	Pymetrozine	Insecticides	2.76	218.1034	105.0448	0.9963	50	50	71.4	10.6	93.4	11.4	89	10	LC-Q-Orbitrap/MS
213	Pyraclostrobin	Fungicides	18.31	388.1054	163.0629	0.9995	2	5	93.8	7.5	103.6	5.6	99.7	5	LC-Q-Orbitrap/MS
214	Pyridaben	Insecticides	20.22	365.1444	147.1169	0.9998	5	5	101.9	3.8	99.3	4.3	91.1	5	LC-Q-Orbitrap/MS
215	Pyridaphenthion	Insecticides	16.58	341.0715	189.066	0.9997	2	2	99.2	4	101.8	3.3	89.9	5	LC-Q-Orbitrap/MS
216	Pyrimethanil	Fungicides	15.51	198.1026	183.0791	0.9975	2	2	72.5	8.8	97.7	7.2	79.4	5	GC-Q-Orbitrap/MS
217	Pyriproxyfen	Insecticides	19.44	322.1433	96.0444	0.9998	1	1	90.9	5.4	102	4.1	97.9	4	LC-Q-Orbitrap/MS
218	Quinalphos	Insecticides	20.51	146.0475	157.076	0.9998	2	2	104.1	6.7	101.8	2.9	95.3	4	GC-Q-Orbitrap/MS
219	Quinoxyfen	Fungicides	25.06	237.0585	306.9964	0.9999	2	2	85.5	6.1	100.8	3.2	99.7	3	GC-Q-Orbitrap/MS
220	Quintozene	Fungicides	14.57	234.8438	238.8379	0.9995	10	10	74.6	10.6	74.2	11.9	112.3	14	GC-Q-Orbitrap/MS
221	Quizalofop-ethyl	Herbicides	19.03	373.0945	299.0581	0.9999	5	5	104.8	4	105.3	2	92.7	7	LC-Q-Orbitrap/MS
222	Resmethrin	Insecticides	20.38	339.1951	128.0621	0.9985	5	5	74.1	3.5	72.8	5.6	80.9	4	LC-Q-Orbitrap/MS
223	Sedaxane	Fungicides	28.88	159.0365	130.0652	0.9998	10	10	87	6.2	85.7	5.9	104.7	12	GC-Q-Orbitrap/MS
224	Simazine	Herbicides	8.35	202.0851	132.0324	0.9998	10	10	98.8	1.6	88.4	2.5	92.4	5	LC-Q-Orbitrap/MS
225	Spinosyn A	Insecticides	18.35	732.4695	142.1228	0.9997	20	50	94.3	6.9	86	7.7	85.5	4	LC-Q-Orbitrap/MS
226	Spinosyn D	Insecticides	18.73	746.4852	142.1228	0.9955	50	50	80.7	17.7	97.5	6	80.7	3	LC-Q-Orbitrap/MS
227	Spirodiclofen	Insecticides	19.93	411.1121	71.08568	0.9995	10	10	92.4	4.7	91	6.4	96.4	5	LC-Q-Orbitrap/MS
228	Spirotetramat	Insecticides	17	374.1957	216.102	0.9997	20	20	87.7	9.4	91.5	9.4	89.4	3	LC-Q-Orbitrap/MS

Table 1. *Cont.*

No.	Compound	Category	RT/Min	MS_1 ion (*m*/*z*)	MS_2 ion (*m*/*z*)	R^2	SDL ($\mu g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)	1-LOQ		2-LOQ		10-LOQ		Instrumentation
									REC/%	RSD/%	REC/%	RSD/%	REC/%	RSD/%	
229	Spirotetramat-enol	Insecticides	11.3	302.1748	216.102	0.9996	5	5	102.1	3	100.6	2.2	86.6	5	LC-Q-Orbitrap/MS
230	Spiroxamine	Fungicides	15.41	298.2737	144.1384	0.9997	2	2	87.6	8.5	99.5	5.3	87.7	5	LC-Q-Orbitrap/MS
231	Sulfotep	Insecticides	17.84	323.0296	114.9614	0.9999	2	2	97.6	6.6	96.1	3	95.3	5	LC-Q-Orbitrap/MS
232	Sulprofos	Insecticides	19.63	323.035	218.9701	0.9967	10	10	79.9	9.8	82	5.5	81.4	10	LC-Q-Orbitrap/MS
233	Tebuconazole	Fungicides	17.87	308.152	125.0153	0.9995	5	5	76.2	8.5	95.8	5	96	2	LC-Q-Orbitrap/MS
234	Terbufos	Insecticides	14.94	230.9733	174.9106	0.992	10	10	91.6	8.5	117.6	4.6	104	14	GC-Q-Orbitrap/MS
235	Terbufos-sulfone	Insecticides	20.08	199.001	170.9698	0.9999	5	5	117.6	7.8	116.8	7.2	93.2	3	GC-Q-Orbitrap/MS
236	Terbufos-Sulfoxide	Insecticides	14.77	305.0459	130.9386	0.9981	20	50	98.9	7.6	93.6	2.7	84.6	3	LC-Q-Orbitrap/MS
237	Terbumeton	Herbicides	11.1	226.1661	170.1039	0.9952	20	20	78.1	8	95.3	2.5	83.5	7	LC-Q-Orbitrap/MS
238	Terbuthylazine	Herbicides	15.23	230.1164	174.0543	0.9997	2	2	97.9	4.7	97.2	1.4	102.2	2	LC-Q-Orbitrap/MS
239	Tetradifon	Insecticides	28	158.9666	226.8887	0.9997	5	5	90.2	3.7	93.3	3.8	93.9	3	GC-Q-Orbitrap/MS
240	Tetramethrin	Insecticides	19.12	332.1852	164.0707	0.9998	1	1	96.1	13.7	93.6	5.8	96.4	4	LC-Q-Orbitrap/MS
241	Thiabendazole	Fungicides	4.78	202.0432	175.0325	0.9995	5	5	92.9	3.8	95.5	4.2	82.8	6	LC-Q-Orbitrap/MS
242	Thiacloprid	Insecticides	6.22	253.0307	126.0106	0.9997	5	5	106.6	4.6	98.6	2.6	83.9	5	LC-Q-Orbitrap/MS
243	Thiamethoxam	Insecticides	4.04	292.0263	131.967	0.9984	20	20	89.1	5.5	94.8	4.7	91.9	5	LC-Q-Orbitrap/MS
244	Thiobencarb	Herbicides	18.47	258.0711	125.0154	0.9997	5	5	98.5	3.4	98.2	6.4	92.3	5	LC-Q-Orbitrap/MS
245	Thiophanate-methyl	Fungicides	8.05	343.0526	151.0326	0.9904	50	50	71.8	8.3	71.5	6.2	79.8	9	LC-Q-Orbitrap/MS
246	Tolfenpyrad	Insecticides	19.34	384.147	197.0962	0.9996	5	5	96.8	6.2	97	4.7	89.5	6	LC-Q-Orbitrap/MS
247	Tolylfluanid	Fungicides	17.88	346.9848	137.0295	0.9982	50	50	100	8.1	93.1	7.3	82.4	3	LC-Q-Orbitrap/MS
248	Triadimefon	Fungicides	16.2	294.1	197.0729	0.9993	5	5	101.8	4	97.3	2.8	90.9	7	LC-Q-Orbitrap/MS
249	Triadimenol	Fungicides	16.5	296.1156	70.04016	0.9999	5	5	84	13.1	90.7	2.6	92.8	2	LC-Q-Orbitrap/MS
250	Triazophos	Insecticides	16.73	314.0718	162.0662	0.9995	2	2	95.6	1.9	105.8	4.3	96.4	5	LC-Q-Orbitrap/MS
251	Trichlorfon	Insecticides	4.93	256.9295	127.0155	0.9987	20	20	88.3	5.1	93.8	3.8	92.5	3	LC-Q-Orbitrap/MS
252	Trifloxystrobin	Fungicides	18.77	409.1364	186.0525	0.9984	5	5	98.2	5.5	102	2.6	92.2	5	LC-Q-Orbitrap/MS
253	Triflumizole	Fungicides	18.8	346.0923	278.0554	0.9999	10	10	76.7	12.2	81.7	8.3	85.1	7	LC-Q-Orbitrap/MS
254	Trinexapac-ethyl	Plant growth regulators	12.72	253.1067	69.03366	0.9964	20	20	85.2	9.5	96.2	11.9	114	6	LC-Q-Orbitrap/MS
255	Uniconazole	Fungicides	22.49	234.0429	165.0102	0.9997	5	5	106.2	3.5	97	2.5	89.5	6	GC-Q-Orbitrap/MS
256	Vinclozolin	Fungicides	17.09	212.0029	197.9872	0.9994	5	5	97.8	7.3	105.8	3.5	90.8	6	GC-Q-Orbitrap/MS
257	Warfarin	/	15.51	309.1118	163.0391	0.9999	2	2	100.8	5.7	102.7	3.9	91.1	3	LC-Q-Orbitrap/MS
258	Zoxamide	Fungicides	17.89	336.0315	186.971	0.9999	5	5	91.3	6.2	103.8	3.1	97	7	LC-Q-Orbitrap/MS

2.2. Reagents and Materials

Silage samples were collected from local dairy farms and consisted of corn stover (Inner Mongolia; Shaanxi; Hebei; Heilongjiang; Shandong Province, China). All pesticide standards (purity grade, >98%) were obtained from Alta Company (Tianjin, China); formic acid, ammonium acetate, acetonitrile, methanol (all LC-MS grade), and ethyl acetate (HPLC grade) were obtained from Fisher Scientific Co. (Cranbury, NJ, USA); analytical grade forms of acetic acid, sodium chloride, anhydrous Na_2SO_4, trisodium citrate, disodium citrate, and anhydrous $MgSO_4$ were obtained from Shanghai Anpu Experimental Technology (Shanghai, China). The clean-up absorbents as octadecylsilane (C_{18}) and primary secondary amine (PSA) were obtained from Tianjin Agela Technology (Tianjin, China).

2.3. Preparation of Standard Solutions

The purchased standard solution should be stored at −18 °C or 4 °C under dark conditions. When mixing the standard configuration, a 10 μg mL^{-1} group of mixed standards should be prepared according to different categories, then diluted to a concentration of 1 μg mL^{-1} for the large group mixed standard. The standard should be stored at 4 °C under dark conditions for one month.

2.4. Sample Preparation

2.4.1. Extraction

A sample of 2.0 g silage was weighed into a 50 mL tube. A 2 mL volume of water was added, and then 20 mL of 1% acetic acid acetonitrile (*v*/*v*) was added and homogenized at 12,000 rpm for 2 min. After that, EN salt (4 g $MgSO_4$, 1 g NaCl, 0.5 g disodium citrate, and 1 g trisodium citrate) was added. The tube was shaken for 10 min, followed by centrifugation for 5 min at 4500 r min^{-1}.

2.4.2. Clean-up

Five milliliters of supernatant was pipetted into a 15 mL clean-up tube (containing 500 mg $MgSO_4$, 30 mg PSA, and 50 mg C_{18}). The clean-up tube was shaken for 10 min, followed by centrifugation at 4200 rpm for 5 min. Subsequently, 2 mL of the supernatant from the clean-up tube was pipetted into a 10 mL glass tube and evaporated to dryness in a 40 °C water bath with a gentle stream of nitrogen. Finally, 1 mL of acetonitrile/water (3:2, *v*/*v*) solution was used to re-dissolve the solution and pass it over the membrane for UHPLC-Q-Orbitrap/MS analysis, or 1 mL of ethyl acetate was used to re-dissolve the solution and pass it over the membrane for GC-Q-Orbitrap/MS analysis. The flow chart of the silage sample analysis program is shown in Figure 1.

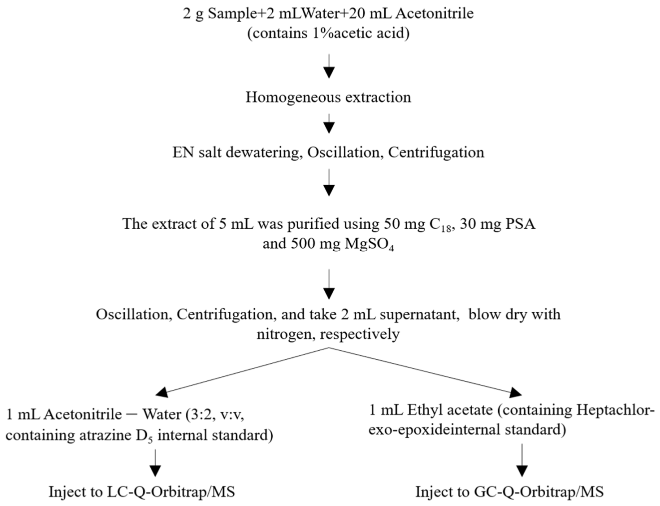

Figure 1. Flow chart of silage analysis program.

2.5. Validation of the Method

The method was validated in the silage matrix by evaluating the following parameters: matrix effect, linearity, screening detection limit (SDL), the limit of quantification (LOQ), recovery, and precision. To define the SDL, according to SANTE/11312/2021 [26], this was the lowest level at which pesticide had been screened in at least 95% of the samples. Calibration curves were investigated by determining the results of a series of standard addition recovery experiments (1, 2, 5, 10, 20, 50, 100, 200, 300, and 500 $\mu g\ kg^{-1}$) of blank matrix extract solutions before injection. Matrix effects were calculated using the following formula: matrix effect (ME, %) = [(slope of matrix matching standard curve—the slope of solvent standard curve)/slope of solvent standard curve] × 100. To validate the accuracy and precision of the established method, recovery was performed for each compound in six replicates for three spiked levels at 1-, 2-, and 10 times the LOQ.

Thermo Fisher Scientific ^{TM} Tracefinder ^{TM} (version 4.1) software was used to analyze the data based on the self-built database. To ensure the accuracy of target compounds identification, the specific settings of the corresponding screening parameters included the retention time offset threshold ($\leq$0.15 min) and mass deviation ($\leq$5 ppm). The data results were analyzed using Excel (Version 2016) software, and an analysis of graphs was drawn using Origin 2018 software.

3. Results and Discussion

3.1. Sample Extraction and Clean-Up

Considering silage's high pigment and fiber-rich characteristics, the sample weight should be appropriately reduced when using QuEChERS for pretreatment. Combined with the research of the previous group on forage matrix pretreatment [27], the sample weight was fully considered for instrument detection sensitivity and matrix interference, and so 2 g of substance was selected for further research in this work. The QuEChERS procedure was evaluated due to the potential for matrix interference, one of the most challenging situations in high-throughput screening, and essential for validating quantitative determination. Therefore, different parameters based on the QuEChERS method have been evaluated.

3.1.1. Optimization of Extraction Solvent Volume

During the pretreatment process, the extraction solution significantly influences the extraction efficiency of the method. According to the reported literature, acidified acetonitrile was chosen as the extraction solution for pesticide residue detection from relatively simple matrices such as fruits and vegetables, to complex matrices such as tea and wolfberries [28,29]. Therefore, 1% acetate acetonitrile (v/v) was chosen as the extraction solution, and the volume was optimized.

The volume of the extraction solution affects the extraction efficiency of the target compounds and the strength of the matrix background. This paper optimized the extraction solution volumes of 10 mL, 20 mL, and 40 mL. As shown in Figure 2, when the extract volume is 10 mL, the percentage of pesticides that can be screened out of the total pesticides was 92.8%, while the percentages for 20 mL and 40 mL were 91.5% and 89.3%, respectively. There was no significant difference between 10 mL and 20 mL. On the other hand, considering the number of compounds that met the recovery criteria (REC: 70–120%, RSD $\leq$ 20%), 88.6% of the compounds satisfying the recovery criteria were effectively extracted under 20 mL. In comparison, the percentages for 10 mL and 40 mL were 87.0% and 83.7%, respectively. The results showed differences in pesticides meeting the recovery in silage with different extraction solution volumes. As the extraction volume increased, the sample matrix interference weakened, but the instrument detection sensitivity decreased. Therefore, using 20 mL of 1% acetate acetonitrile as the extraction solution could improve the screening capability and extraction efficiency of the target compounds. The volume of extraction solution was eventually chosen to be 20 mL.

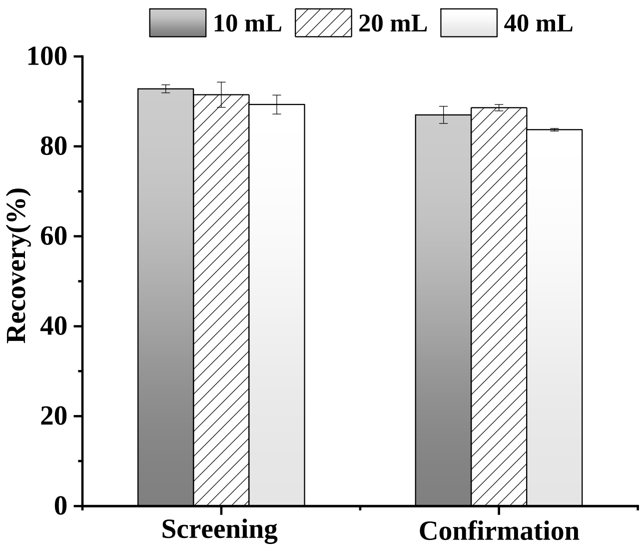

Figure 2. The effect of extract volume on compounds.

3.1.2. Optimization of Hydration Volume

Silage has a high fiber content and a low water content (<70%). Homogenizing the extraction without water will lead to uneven samples and will affect the extraction efficiency of the target compounds [30]. Under a spiking level of 100 μg kg^{-1}, this work investigated hydration volumes of 0 mL, 2 mL, and 3 mL. The optimal conditions were selected according to the percentage of recoveries of target compounds in silage. As shown in Figure 3, 90% of the target compounds could be screened out if the extraction was carried out without water. While there were relatively few compounds that met the hydration recovery criteria, the percentage of quantification was 93%. The above results indicate that the target compounds could not be effectively transferred from the matrix to the extraction solution. Therefore, water was added to the sample for hydration before extraction, to improve sample dispersion and to increase solute transfer efficiency. When the hydration volume was 2 mL or 3 mL, the screening rate of pesticides was 97.7%, and the quantification was 95.3% and 94.6%, respectively. Both the screening and the quantification were higher than those without hydration.

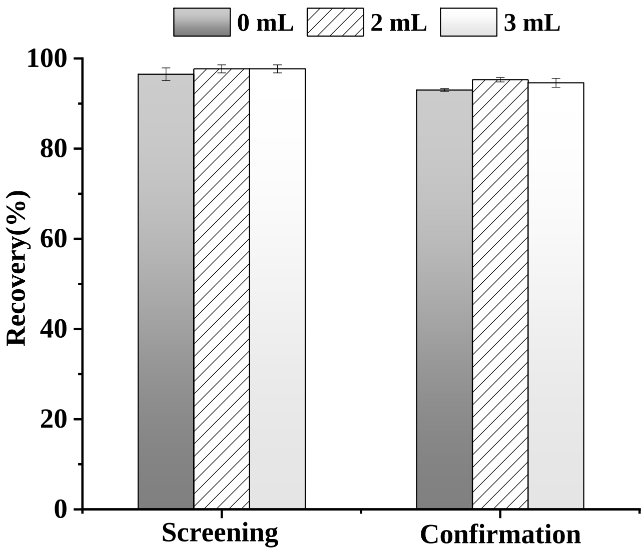

Figure 3. The effects of different hydration volumes on 258 compounds in silage.

In contrast, hydration significantly improved the extraction efficiency of some organonitrogen pesticides (containing nitro functional groups or triazine ring compounds). Ethalfluralin, for example, has a dinitro functional group, which is a conjugated system with a unique bond between the single and double bonds. It can form O-H-O hydrogen bonds with water molecules, and its coordination number varies depending on the spatial configuration, exhibiting a certain degree of hydrophilicity. Its hydrophilic functional group forms a solvent shell around the compound as the number of water molecules increases, thus increasing the extraction efficiency of the compound during the subsequent extraction [31]. The results found that the recovery of ethalfluralin was less than 60% when unhydrated, and this increased to 119.8% and 108.4% with 2 mL and 3 mL of water, respectively. Terbutamol was not screened when it was not hydrated. After hydration, it cannot only be screened, but the recovery can be increased to 93.9% and 92.0%, respectively. Although hydration can improve the screening of some compounds, the amount of added water should not be excessive. Otherwise, this will increase the amount of anhydrous magnesium sulfate, which can affect some heat-, acid-, and base-sensitive pesticides. As a result, 2 mL of water for hydration was selected for further research.

3.1.3. Optimization of the Type of Extraction Salt

This work compared the effects of several extraction salts to make the target compounds easier to extract. The extraction salts contain AOAC with sodium acetate (6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate), EN with citrate as a buffer salt (0.5 g disodium hydrogen citrate, 1 g sodium citrate, 4 g anhydrous $MgSO_4$, and 1 g sodium chloride), and traditional QuEChERS with sodium chloride (4 g anhydrous $MgSO_4$ and 1 g sodium chloride) [32]. There was no significant difference in the number of pesticides that the three salting agents could screen at a spiked level of 100 $\mu g\ kg^{-1}$. However, the recovery of carbamate pesticides (e.g., pirimicarb) is significantly higher with EN salts than with the other two extraction salts. The result indicated that the recoveries of some acid- and base- sensitive compounds are more stable under the buffer salt system. Otherwise, this work also compared the responses of compounds after using different extraction salts. Certain compounds have reduced responses after utilizing AOAC, whereas the responses of compounds using EN and QuEChERS increased. As shown in Figure 4A, the response changes of dichlofluanid and desmetryn were EN > QuEChERS > AOAC and EN > AOAC > QuEChERS, respectively. Therefore, EN was chosen as an extraction salt to extract the target compounds more effectively.

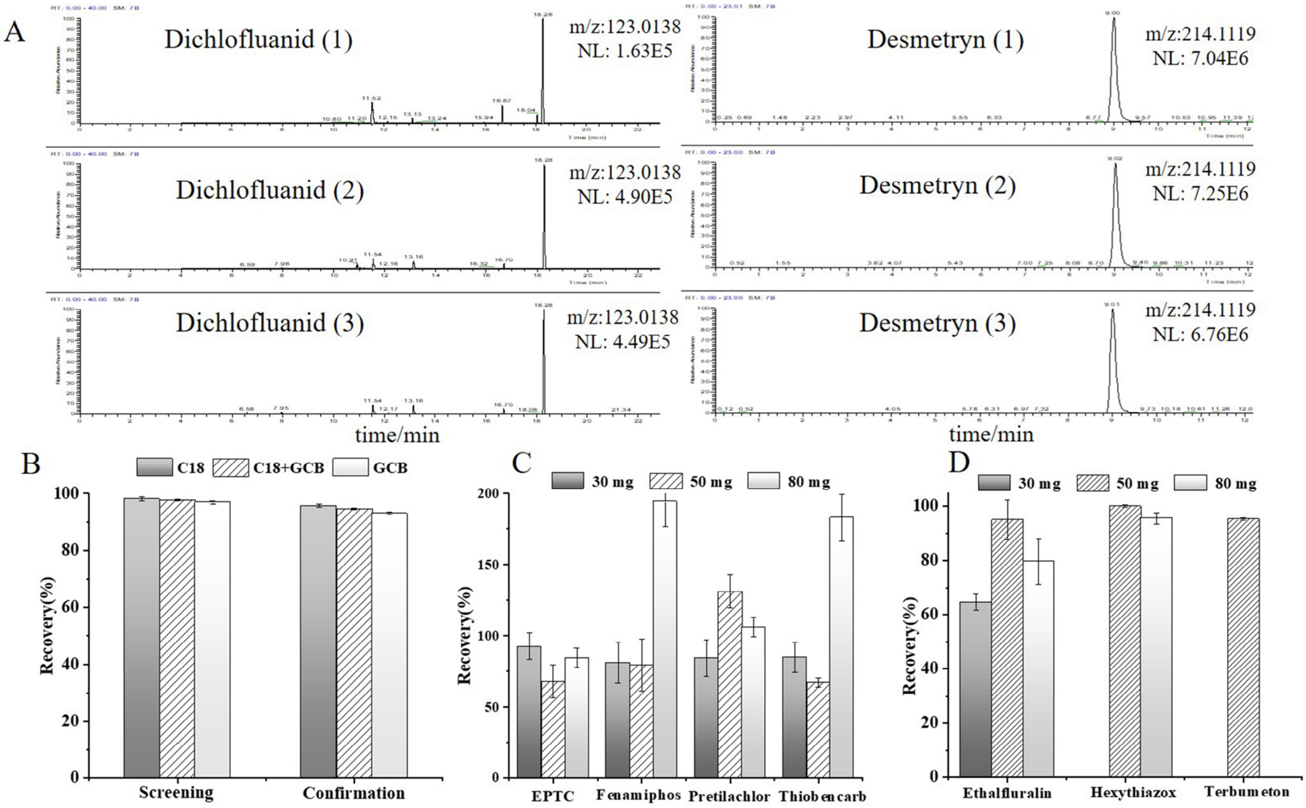

Figure 4. The effects of salting-out agent and purifying agent on compounds. (**A**): The effects of salting-out on the response of compounds Dichlofluanid and Desmetryn; (1): AOAC, (2): EN, (3): QuEChERs; (**B**): The effect of purifiers on screening; (**C**): The effect of C_{18} on recovery; (**D**): The effect of PSA on recovery.

3.1.4. Optimization of the Adsorbent

Silage is mainly formed by fermenting fresh straw, and it contains a large amount of crude fiber, crude protein, organic acids, etc. It is dark in color and has a high pigment content. To effectively reduce the influence of interferents and pigments on the target compounds, different purification fillers, including anhydrous $MgSO_4$, PSA, C_{18}, and GCB were commonly applied to the purification. Anhydrous $MgSO_4$ is used to remove residual water in the extraction. PSA could eliminate the interference of organic acids and pigments. C_{18} has a strong adsorption capacity and can be used to remove non-polar impurities such as lipids. GCB is used to remove pigments from the extraction [32]. Anhydrous $MgSO_4$ and PSA were chosen as the main clean-up adsorbents, as the samples contained a large amount of organic acid and had been hydrated during the pretreatment process. Otherwise, considering other interferences in silage, C_{18} and GCB were also introduced. The following experimental conditions were compared: (1) anhydrous $MgSO_4$ + PSA + C_{18} + GCB, (2) anhydrous $MgSO_4$ + PSA + C_{18}, and (3) anhydrous $MgSO_4$ + PSA + GCB. The comparison shows that although GCB is effective in removing pigments, the use of GCB reduces the number of pesticides screened and the recovery of some compounds, especially planar compounds. For example, the recovery rate for thiabendazole using GCB is 49.5–59.1%, while the recovery increased to 83.5% without GCB. As shown in Figure 4B, 98.1% of compounds could be screened without GCB, of which 95.7% could be quantified for target compounds. In contrast, the screening capacity decreased to 97.7% and 96.9%, and the quantification capacity decreased to 94.6% and 93.0% with GCB. Therefore, anhydrous $MgSO_4$ + PSA + C_{18} was selected for the purification step.

This work optimized the dosages of PSA and C_{18} to remove interfering substances from the silage effectively. To eliminate residual water, 500 mg anhydrous $MgSO_4$ was applied. The dosage of C_{18} and PSA had little effect on the screening capacity, but had a more significant impact on the recovery of some compounds. For EPTC, fenamiphos, pretilachlor, and thiobencarb, the dosage of C_{18} significantly affects the recovery. As shown in Figure 4C, the recoveries of the four compounds range over 68.2–92.7%, 81.1–194.3%, 84.3–131.5%, and 67.4–183%, respectively, and the dosages of C_{18} were optimized as 30 mg, 50 mg, and 80 mg. As shown in Figure 4D, the dosage of PSA has a more significant impact on the organic nitrogen compounds, such as ethalfluralin, hexythiazox, and terbumeton. Hexythiazox and terbumeton were not screened out when the dosage was 30 mg, and the recovery of ethalfluralin was 64.6%. The recoveries of ethalfluralin, hexythiazox, and terbumeton ranged from 93% to 100.1% at 50 mg, while tebutone was not detected at 80 mg. The study selected 500 mg anhydrous $MgSO_4$, 30 mg C_{18}, and 50 mg PSA for further research.

3.1.5. Optimization of Purification Volume

The volume for purification is also one of the factors affecting the responses of target compounds. The interferents have a more significant effect on the target compounds as the volume for purification increases, and conversely, the interference effect is smaller. However, a small purification volume will result in lower mass spectrometry detection concentrations, reducing the sensitivity for some compounds. Different purification volumes of 3 mL, 5 mL, and 10 mL were optimized. By comparison, the lower volume significantly reduced the recovery of compounds. As Figure 5 shows, for buprofezin, when the volume for purification increases, the recovery increases from 60.6% to 73.9%, while phorate and terbufos increase from 54.9% to 79.6%, and from 53.4% to 79.2%, respectively. However, the target pesticides were affected by interferents with a lower response as the volume for purification increased. In the case of organic nitrogen compounds (such as fluazifop-butyl and buprofezin), the highest response of buprofezin was achieved at a purification volume of 5 mL, and the response was essentially the same at 3 mL and 10 mL. For fluazifop-butyl, the response decreased when the purification volume increased. The purification volume selected was 5 mL, to reduce the influence of interferents on compounds, and to improve the recovery of compounds.

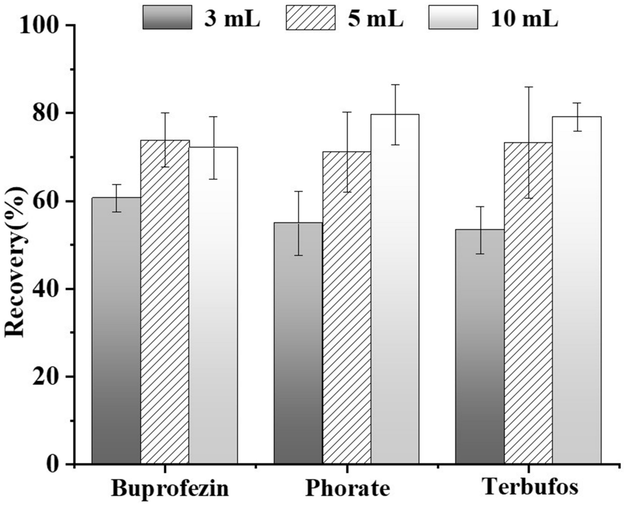

Figure 5. The effect of purification volume on recovery of compounds.

3.2. Matrix Effect

The matrix effect was measured by comparing the slopes of the two standard curves, which were 1, 2, 5, 10, 20, 50, 100, 200, 300, and 500 μg kg^{-1} for the matrix standard curve and the solvent standard curve. The matrix effect in silage was calculated using the following formula: matrix effect (ME, %) = [(slope of matrix matching standard curve—the slope of solvent standard curve)/slope of solvent standard curve] × 100. Matrix effects can be classified into three categories based on the results of the calculated data:

$|ME|$, lower than 20% shows a weak matrix effect;
$|ME|$, between 20% and 50% shows a medium matrix effect;
$|ME|$, higher than 50% shows a strong matrix effect.

As shown in Figure 6, 27.1% of the compounds showed a weak matrix effect, 52% showed a medium matrix effect, and 20.9% showed a strong matrix effect. As a result, the matrix-matched calibration standard was used for quantitative analysis to reduce the matrix effect.

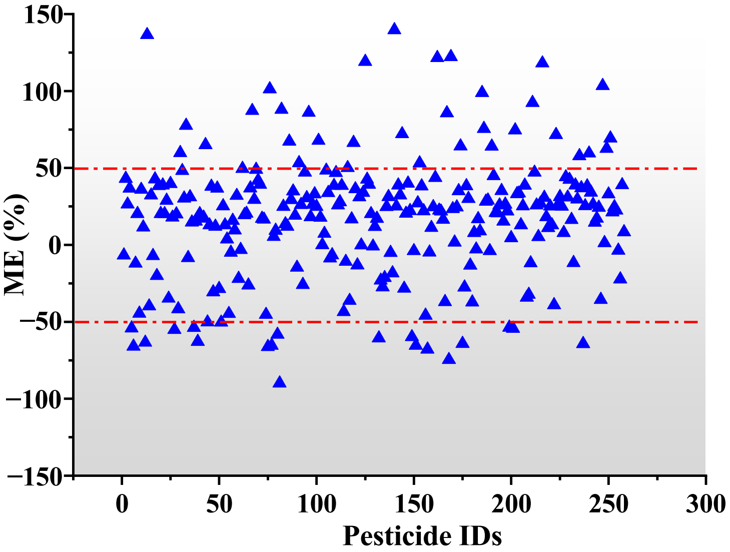

Figure 6. Matrix effect distribution of 258 compounds.

3.3. Method Validation

3.3.1. Linear Range, SDL, and LOQ

The mixed standard working solution of different concentrations was added into the blank silage sample, the sample was extracted and purified according to 2.4, and the matrix matching standard curve was established. High-resolution mass spectrometry (HRMS) was used to identify the target compounds. The ordinate of the standard curve was the peak area of the quantitative ions of each compound, and the abscissa was the matching mass concentration. There was good linearity in the range of 0.1–50 µg L^{-1}, and the coefficients of determination (R^2) were higher than 0.99 for 258 compounds in different linear ranges (see Table 1).

According to SANTE/12682/2021 [26], the method's sensitivity was assessed using SDL. SDLs were determined by spiking a series of mixed standard solutions in 20 blank samples, and using the lowest level at which pesticides were screened in at least 95% of the samples. As shown in Table 1, the percentage of pesticides with SDLs of less than 10 µg kg^{-1} was 82.9%, indicating that the method has high sensitivity.

LOQs were determined as the lowest validated spike level, based on the recovery results by spiking a series of mixed standard solutions in blank samples. For silage, the LOQs were 1–50 µg kg^{-1}, with 209 compounds that were less than or equal to 10 µg kg^{-1}, as shown in Table 1. The method was proven to have a high sensitivity, and it could be routinely used for high-throughput screening and the quantitative analysis of multi-pesticide residues in silage at low concentration.

3.3.2. Recovery and Precision

This work evaluated accuracy and precision at three spiked levels. LOQs mixed standard working solution at 1-, 2-, and 10 times were used as the spiked levels, extraction and purification were performed according to Section 2.4, and six replicates were determined for each spiked level. The blank was run simultaneously, and the recovery and RSD were calculated after deducting the background. The method results are shown in Table 1. The recoveries of 258 pesticides in silage at three spiked levels were 66.5–119.7%, 71.5–117.6%, and 71.0–119.8%, respectively, with RSD being in the range of 0.93–20%, 0.88–14%, and 1.4–19%, respectively. This shows that the accuracy and precision of the method met the criteria of accurate quantification.

3.4. Comparison of LC/GC-Q-Orbitrap/MS

LC-Q-Orbitrap/MS and GC-Q-Orbitrap/MS were used to analyze the extracts of the same silage, to identify 258 pesticide residues (including 143 pesticides identified via LC or GC, 63 pesticides detected via GC, and 52 pesticides detected via LC). Because of the differences in the physico-chemical characteristics of some compounds, the monitoring could only be performed with one type of instrument. Neonicotinoid insecticides, for example, can only be identified using LC-Q-Orbitrap/MS. Nevertheless, some organochlorine pesticides can only be detected with high sensitivity via GC-Q-Orbitrap/MS. On the other hand, although some compounds were established using both LC-Q-Orbitrap/MS and GC-Q-Orbitrap/MS, there was a significant difference in detection sensitivity in the actual detection. For example, some polar pesticides were identified via GC-Q-Orbitrap/MS, but the SDL was so high that it was impossible to apply to sample detection. Some organophosphorus and pyrethroid insecticides such as fenthion, isocarbophos, fenpropathrin, and flucythrinate were often detected via GC-, which had a better detection sensitivity than LC-. However, GC- has been more widely used than LC- in traditional pesticide residue analysis methods. Some organophosphorus and pyrethroid insecticides (e.g., fenthion, isocarbophos, fenpropathrin, and flucythrinate) had a better detection sensitivity when using GC, than LC. The combination of the two techniques increased the detection capacity by 124.3% (GC-Q-Orbitrap/MS, 115 species) and 80.4% (LC-Q-Orbitrap/MS, 143 species) compared to the single technology. The percentage of pesticides with SDLs of less than 10 μg kg^{-1} was 82.9% when the two technologies were combined, meeting the supervision requirement for silage safety.

3.5. Analysis of Real Samples

The validated method was applied to screen and quantify pesticides in 37 silages from different provinces and farms. According to the test results, pyraclostrobin was found in one batch of silage, with a content of 3.2 μg kg^{-1}, as shown in Figure 7. The actual sample testing showed a low risk of pesticide residue contamination in the silage. At the same time, pyraclostrobin residues in silage may be caused by the control of fungal diseases during corn planting.

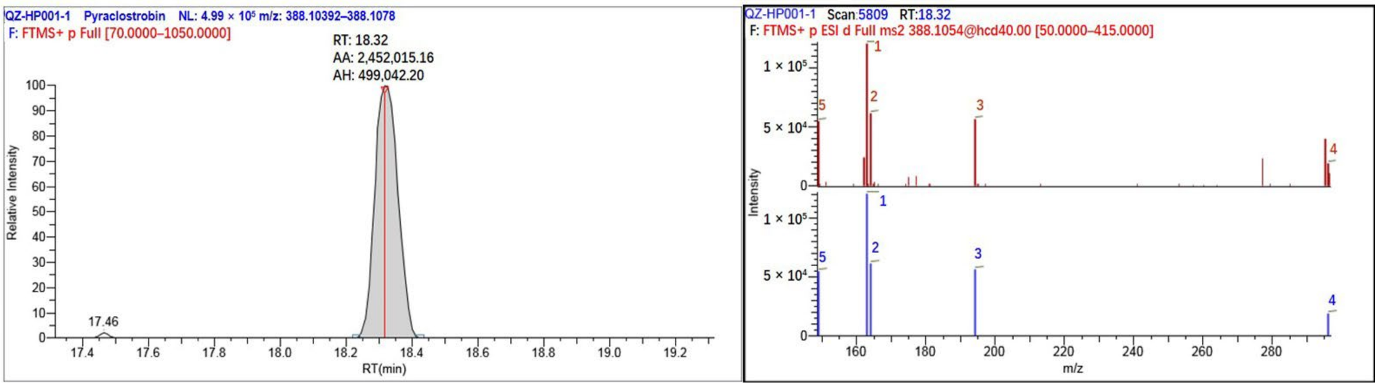

Figure 7. Chromatographic and mass spectra of pyraclostrobin in real samples.

4. Conclusions

This work differs from conventional detection methods, as it focuses on the screening and quantitation of compounds in silage, based on QuEChERS with LC/GC-Q-Orbitrap/MS. A rapid screening and quantification method of 258 pesticides in silage was established. Combining the two HRMS technologies allowed for the detection of several pesticide residues in silage with excellent sensitivity. The SDLs of pesticides that LC-Q-Orbitrap/MS or GC-Q-Orbitrap/MS may detect at less than 5 μg kg^{-1} accounted for 51.7 and 73.9% of the total, respectively. The method was applied to the rapid screening of 37 batches of silage from different farms. Pyaclostrobin was discovered in one of the silage samples. The technique was simple, quick, and sensitive. It could be used to screen and quantify several pesticide residues in forage represented by silage, according to the validation of the methodology and the determination of actual samples.

Author Contributions: Conceptualization, Y.X.; Data curation, Y.S. (Yanling Song); Funding acquisition, C.F.; Methodology, X.W.; Project administration, H.C.; Resources, Y.S. (Yini Sun); Software, K.T. and X.Y.; Validation, X.W.; Writing—original draft, Y.X.; Writing—review & editing, H.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the science and technology project of the State Administration for Market Regulation (2021MK165).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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