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Method of Test for Pesticide Residues in Foods - Multiresidue Analysis (6)

1. Scope

This method is applicable for the determination of 31 pesticide residues (bioresmethrin etc. listed in the attached tables) in fruits and vegetables, crops, dried beans, tea, spice plants and other herbs.

2. Method

After preparation of the sample solution by the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe), pesticides are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer

2.1.1.1. Ion source: electrospray ionization, ESI

2.1.1.2. Column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.1.3. Guard column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 5 mm, or an equivalent product.

2.1.2. Blender.

2.1.3. Grinder.

2.1.4. High speed dispersing device: SPEX SamplePrep P 2010 GenoGrinder®, > 1000 rpm, or an other mechanical shaker.

2.1.5. Centrifuge: centrifugal force > 3000 ×g, temperature control < 15°C.

2.1.6. Nitrogen evaporator.

2.2. Chemicals

Formic acid, reagent grade;
Ammonium aetate, reagent grade;
Acetonitrile, HPLC grade;
Methanol, HPLC grade;
Sodium chloride, AR grade;
Sodium citrate, AR grade;
Disodium hydrogen citrate, AR grade;
Magnesium sulfate anhydrous, AR grade;
Primary secondary amine (PSA), AR grade;
Octadecylsilane end-capped (C18 EC), AR grade;
Graphitized carbon black (GCB), AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);

Bioresmethrin and other pesticides listed in the attached tables, reference standards;

Triphenylphosphate, internal standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.2. Membrane filter: 0.22-μm, PTFE.

2.3.3. Volumetric flask: 25 mL and 50 mL.

2.3.4. Ceramic homogenizer(note 1): Bond Elut QuEChERS P/N 5982-9313,or an equivalent product.

2.3.5. Extraction powder(note 2): containing 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate, and 0.5 g of disodium hydrogen citrate.

2.3.6. Clean-up centrifuge tube I(note 2): containing 150 mg of PSA and 900 mg of magnesium sulfate anhydrous, 6 mL, used for type I samples (fresh fruits, vegetables, spice plants and other herbs with high water content).

2.3.7. Clean-up centrifuge tube II(note 2): containing 150 mg of PSA, 900 mg of magnesium sulfate anhydrous and 150 mg of C18, 6 mL, used for type II samples (crops and dried beans with high wax, fat and sugar content).

2.3.8. Clean-up centrifuge tube III(note 2): containing 150 mg of PSA, 855 mg of magnesium sulfate anhydrous and 45 mg of GCB, 6 mL, used for type III samples (dried tea, fruits, vegetables, spice plants and other herbs with high pigment content).

Note 1: Ceramic homogenizer can be used depending on the viscosity of the sample.

Note 2: Commercial extraction/clean-up kit can be used as needed.

2.4. Reagents

2.4.1. Acetonitrile: methanol (4:1, v/v)

Mix acetonitrile and methanol at the ratio of 4: 1 (v/v).

2.4.2. 1% Formic acid in acetonitrile: methanol (4:1, v/v)

Mix 10 mL of formic acid and 990 mL of acetonitrile: methanol (4:1, v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.39 g of ammonium acetate with deionized water to 1000 mL. Add 1 mL of formic acid, mix well, and filter with a membrane filter.

2.5.2. Solvent B

Dissolve and dilute 0.39 g of ammonium acetate with methanol to 1000 mL, and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer about 50 mg of triphenylphosphate internal standard accurately weighed into a 50-mL volumetric flask, dissolve and dilute with methanol to volume as the internal standard stock solutions. Store at -18℃ in the dark.

2.6.1. Dilute appropriate volume of the internal standard stock solution with methanol to 50 μg/mL as the internal standard solution for sample solution preparation in section 2.8.

2.6.2. Dilute appropriate volume of the internal standard stock solution with methanol to 5 μg/mL as the internal standard solution for the matrix-matched calibration curve preparation in section 2.9.

2.7. Standard solution preparation

Transfer about 25 mg of bioresmethrin, chlormequat, cyromazine, dicamba, diclomezine, dodine, fluoroimide, fluroxypyr, imazapyr, MCPA, quinclorac, triclopyr and thiocyclam reference standards accurately weighed to each 25-mL volumetric flask, dissolve and dilute to volume with methanol; transfer about 25 mg of acifluorfen, bicyclopyrone, ethoxysulfuron, flusulfamide, gibberellic acid, imazosulfuron, mesotrione, metazosulfuron, MCPB, oxolinic acid, oxpoconazole fumarate and trinexapac-ethyl reference standards accurately weighed to each 25-mL volumetric flask, dissolve and dilute to volume with acetonitrile; transfer about 25 mg of dichlorprop, imazapic, naptalam and tecloftalam reference standards accurately weighed to each 25-mL volumetric flask, dissolve and dilute to volume with acetone; transfer about 25 mg of 2,4-D and dinoseb reference standards accurately weighed to each 25-mL volumetric flask, dissolve and dilute to volume with ethanol as the standard stock solutions. Store at -18℃ in the dark. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile to 1 μg/mL as the standard solution.

2.8. Sample solution preparation

2.8.1. Type I samples (fresh fruits, vegetables, spice plants and other herbs with high waters content)

Transfer about 10 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, and add 10 mL of 1% formic acid in acetonitrile: methanol (4:1, v/v) and 10 μL of 50 μg/mL internal standard solution after freezing. Add 1 granule of a ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 5 min at 15℃, and transfer 6 mL of the supernatant to a clean-up centrifuge tube I, shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15℃. Take 1 mL of the supernatant and evaporate to near dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 1 mL of methanol, mix well, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8.2. Type II samples (crops and dried beans with high wax, fat and sugar content)

Transfer about 5 g of the powdered sample accurately weighed into a 50-mL centrifuge tube, add 10 mL of pre-cooled deionized water, stand for 20 min, and add 10 mL of 1% formic acid in acetonitrile: methanol (4:1, v/v) and 10 μL of 50 μg/mL internal standard solution. Add 1 granule of a ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 5 min at 15℃, and transfer 6 mL of the supernatant to a clean-up centrifuge tube II, shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15℃. Take 1 mL of the supernatant and evaporate to near dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 1 mL of methanol, mix well, and filter with a membrane filter. Take the filtrate as the sample

solution.

2.8.3. Type III samples (dried tea, fruits, vegetables, spice plants and other herbs with high pigment content)

Transfer about 2 g of the powdered sample accurately weighed into a 50-mL centrifuge tube, and add 10 mL of pre-cooled deionized water, stand for 20 min, add 10 mL of 1% formic acid in acetonitrile: methanol (4:1, v/v) and 10 µL of 50 µg/mL internal standard solution. Add 1 granule of a ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 5 min at 15℃, and transfer 6 mL of the supernatant to a clean-up centrifuge tube III, shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15℃. Take 1 mL of the supernatant and evaporate to near dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 1 mL of methanol, mix well, and filter with a membrane filter. Take the filtrate as the sample solution.

2.9. Matrix-matched calibration curve

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.8 to obtain the supernatant after the clean-up procedure. Take several 1 mL of the supernatant, and evaporate to near dryness by gently flushing with a stream of nitrogen. Separately add 2 - 200 µL of 1 µg/mL the standard solution, 10 µL of 5 µg/mL the internal standard solution and methanol to achieve a final volume of 1 mL, and mix well as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each pesticide by the ratios of the peak area of each pesticide to that of the internal standard vs. the added concentrations (0.002 - 0.2 µg/mL).

LC-MS/MS operating conditions(note 3)

Column: CORTECS UPLC, C18, 1.6 µm, 2.1 mm i.d. × 10 cm.

Guard column: CORTECS UPLC, C18, 1.6 µm, 2.1mm i.d. × 5 mm.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 2.0	99 → 50	1 → 50
2.0 → 8.0	50 → 30	50 → 70
8.0 → 15.0	30 → 1	70 → 99
15.0 → 18.0	1 → 1	99 → 99
18.0 → 18.5	1 → 99	99 → 1
18.5 → 20.5	99 → 99	1 → 1

Flow rate: 0.3 mL/min.

Injection volume: 5 μL.

Interface voltage: ESI^+, 4 kV; ESI^-, 3 kV.

Interface temperature: 270℃.

Nebulizing gas flow: 3.0 L/min.

Heating gas flow: 15.0 L/min.

Desolvent line temperature: 200℃.

Heating block temperature: 350℃.

Drying gas flow: 5.0 L/min.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, Q1/Q3 Pre Bias and collision voltage are shown in the attached tables.

Note 3: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.10. Identification and quantification

Accurately inject 5 μL of the sample solution and the matrix-matched standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.9. Identify each pesticide based on the retention time and the relative ion intensities(note 4). Calculate the amount of each pesticide in the sample by the following formula:

$$\text{The amount of each pesticide in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each pesticide in the sample solution calculated by the matrix-matched calibration curve (μg/mL)

V: the volume of 1% formic acid in acetonitrile: methanol (4:1, v/v) for sample extraction (10 mL)

M: the weight of the sample (g)

Note 4: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤100%). Maximum permitted tolerances of relative ion intensities are as the following:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Remark

1. Limit of quantification (LOQ) for each pesticide is listed in the attached tables.
2. Because the added internal standard, triphenylphosphate (TPP), used in the procedure may not represent the physicochemical properties of all items of pesticides, it is optional for applying it in the formula to calculate the amount of pesticides in the sample. The TPP is recommended to serve as a quality control factor to confirm the operating procedure.
3. This method is not applicable for bioresmethrin in type I samples, fluoroimide in type II samples, and dicamba, dodine, fluoroimide and thiocyclam in type III samples.
4. Further validation should be performed when interfering compounds are found in the samples.

Reference

1. European Committee for Standardization. 2018. Foods of plant origin– Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE– Modular QuEChERS-method. NF EN 15662:2018 (English version).
2. Yu, C. Y., Chen, C. Y., Peng, G. J., Liao, C. D., Kao, Y. M., Wang, D. Y., Cheng, H. F., Lin, S. K., Chuang, W. C., Huang, C. H. and Shyu, T. H. 2018. Development of a multi-residue method for 17 pesticides analysis in agricultural products. Ann. Rept. Food Drug Res. 9: 39-51.

Reference chromatograms

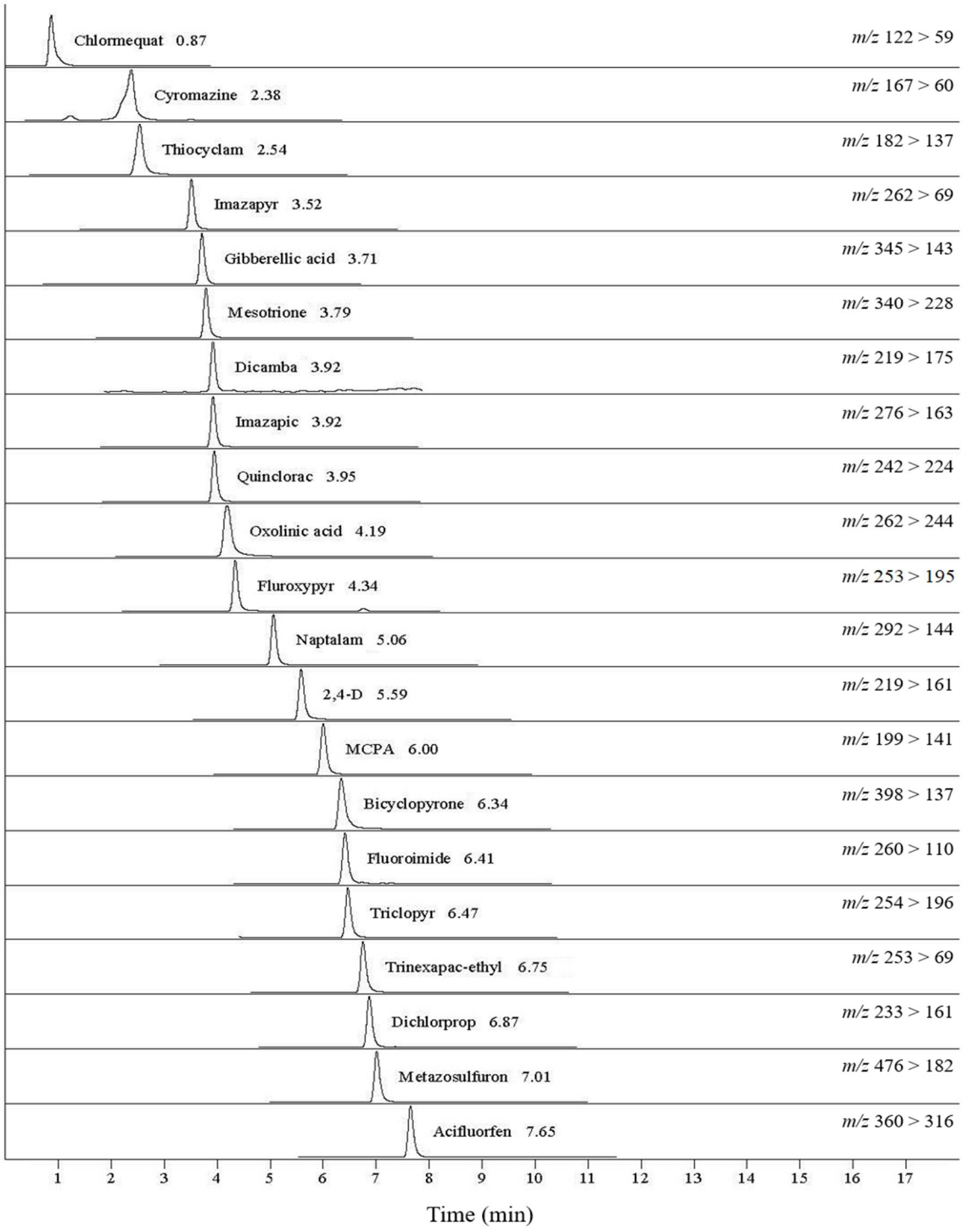

Figure. MRM chromatograms of 31 pesticide standards and the internal standard (TPP) analyzed by LC-MS/MS.

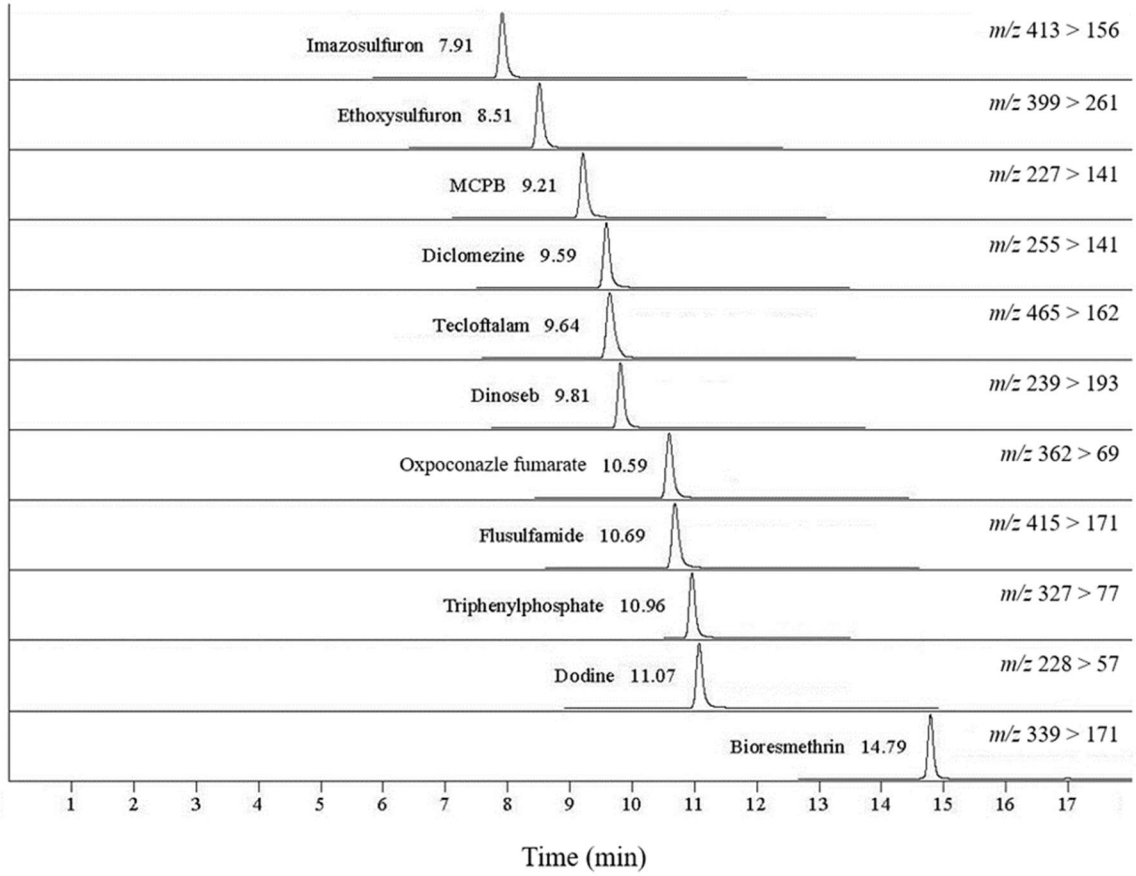

Figure. MRM chromatograms of 31 pesticide standards and the internal standard (TPP) analyzed by LC-MS/MS (continued).

Table 1. MRM parameters and LOQs of 19 pesticides including bioresmethrin etc. and the internal standard (LC-MS/MS positive ion mode)

No.	Analyte	Ion pair			LOQ (ppm)		
		Precursor ion (*m/z*) > product ion (*m/z*)	Q1/Q3 Pre Bias (V)	Collision voltage (V)	Type I[a]	Type II[b]	Type III[c]
1	Bioremethrin	339 > 171* 339 > 143	28/30 27/26	15 21	—	0.02	0.05
2	Chlormequat	122 > 59* 122 > 65 122 > 58 122 > 63	28/22 16/23 27/25 12/10	22 23 26 22	0.01	0.02	0.05
3	Cyromazine	167 > 60* 167 > 125 167 > 108 167 > 85	17/10 16/12 16/10 17/14	21 21 19 20	0.01	0.02	0.05
4	Diclomezine	255 > 141* 255 > 80	13/24 13/13	30 29	0.01	0.02	0.05
5	Dodine	228 > 57* 228 > 60 228 > 186	20/20 21/18 20/20	25 20 24	0.01	0.02	—
6	Ethoxysulfuron	399 > 261* 399 > 218	19/17 19/14	11 20	0.01	0.02	0.05
7	Fluroimide	260 > 110* 260 > 168 262 > 110	13/10 14/16 13/18	33 23 32	0.05	—	—
8	Imazapic	276 > 163* 276 > 145	25/15 25/25	27 35	0.01	0.02	0.05
9	Imazapyr	262 > 69* 262 > 86	24/11 23/14	28 27	0.01	0.02	0.05
10	Imazosulfuron	413 > 156* 413 > 258 415 > 260	20/15 12/17 13/26	19 26 27	0.01	0.02	0.05
11	Mesotrione	340 > 228* 340 > 104	16/14 17/17	16 30	0.01	0.01	0.05
12	Metazosulfuron	476 > 182* 476 > 295	17/18 11/30	20 17	0.01	0.02	0.05
13	Naptalam	292 > 144* 292 > 149 292 > 127	30/13 14/14 15/25	11 21 41	0.01	0.02	0.05

14	Oxolinic acid	262 > 244* 262 > 216	25/15 25/21	15 23	0.01	0.02	0.05
15	Oxpoconazole fumarate	362 > 69* 362 > 179	17/11 18/16	24 25	0.01	0.01	0.05
16	Quinclorac	242 > 224* 242 > 161	29/21 27/15	15 36	0.01	0.02	0.05
17	Tecloftlam	465 > 162* 463 > 162 467 > 450	14/15 10/16 12/15	14 16 8	0.01	0.02	0.05
18	Thiocyclam	182 > 137* 182 > 73 182 > 104	18/13 22/12 22/17	16 24 27	0.01	0.02	—
19	Trinexapac-ethyl	253 > 69* 253 > 185 253 > 207	26/11 26/12 12/20	22 12 12	0.01	0.02	0.05
I.S.	Triphenylphosphate	327 > 77	26/13	45	—	—	—

[a] Applicable for fresh fruits, vegetables, spice plants and other herbs with high water content.

[b] Applicable for crops and dried beans with high wax, fat and sugar content.

[c] Applicable for dried tea, fruits, vegetables, spice plants and other herbs with high pigment content.

*The quantitative ion, and a qualitative ion can be selected based on the matrix condition.

Table 2. MRM parameters and LOQs of 12 pesticides including 2,4-D etc. (LC-MS/MS negative ion mode)

No.	Analyte	Ion pair			LOQ (ppm)		
		Precursor ion (*m/z*) > product ion (*m/z*)	Q1/Q3 Pre Bias (V)	Collision voltage (V)	Type I[a]	Type II[b]	Type III[c]
1	2,4-D	219 > 161*	10/15	12	0.01	0.02	0.05
		219 > 125	14/11	26			
		221 > 163	22/30	14			
2	Acifluorfen	360 > 316*	19/14	12	0.01	0.02	0.05
		360 > 286	19/18	17			
		362 > 318	11/15	12			
3	Bicyclopyrone	398 > 137*	19/13	33	0.01	0.02	0.05
		398 > 175	19/10	28			
4	Dicamba	219 > 175*	23/23	8	0.05	0.04	—
		221 > 177	25/22	8			
5	Dichlorprop	233 > 161*	11/15	13	0.01	0.02	0.05
		233 > 125	11/12	26			
		235 > 163	11/10	14			
6	Dinoseb	239 > 193*	11/18	25	0.01	0.02	0.05
		239 > 163	11/16	32			
7	Fluroxypyr	253 > 195*	12/30	13	0.01	0.02	0.2
		253 > 233	12/10	9			
8	Flusulfamide	415 > 171*	21/16	36	0.01	0.02	0.05
		413 > 171	21/11	28			
		413 > 349	21/16	40			
		413 > 179	21/15	37			
9	Gibberellic acid	345 > 143*	16/21	28	0.01	0.02	0.2
		345 > 239	16/10	15			
10	MCPA	199 > 141*	21/13	14	0.01	0.02	0.05
		201 > 143	21/13	14			
11	MCPB	227 > 141*	23/13	14	0.01	0.02	0.05
		229 > 143	24/16	12			
12	Triclopyr	254 > 196*	13/20	13	0.01	0.02	0.05
		256 > 198	28/22	12			

[a] Applicable for fresh fruits, vegetables, spice plants and other herbs with high water content.

[b] Applicable for crops and dried beans with high wax, fat and sugar content.

[c] Applicable for dried tea, fruits, vegetables, spice plants and other herbs with high pigment content.

*The quantitative ion, and a qualitative ion can be selected based on the matrix condition.