

Determination of 200 Residual Pesticides in Food by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

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1. Introduction

Pesticides are widely used in crop planting and growth process. However, residual pesticides in food could enter human body and harm to human health. Therefore, the maximally allowed residual pesticides in food have strictly regulated in the world. In recent years, the China government continues to strengthen supervision and is developing quicker and highly sensitive analytical method. Usually, the qualitative LC/MS/MS is based on the ratio of intensities between targetions and reference ions.

But in some special occasions, especially for determination of low concentration samples, qualitative reliability is not high enough, which may cause false positive/negative results. So use of MS/MS library for screening and qualitative confirmation of the unknown pesticides is very meaningful. This paper describes UHPLC/MS/MS, a rapid and accurate screening method for determination of 200 residual pesticides in agricultural products.

2. Methods and Materials

Sample Preparation

The pesticides in food samples were extracted with acetonitrile. After centrifugation, solid-phase extraction with NH₂ sorbents was employed to purify the sample. After concentration and filtration, the final extract was injected to the LC-MS/MS instrument.

LC/MS/MS analysis

Analytical Conditions

UHPLC (Nexera, Shimadzu Corporation, Japan)

Column	: Shim-pack XR-ODSIII (150 mmL. × 2.0 mm i.d., 2.2 μm)
Mobile phase A	: 2 μmol/L ammonium acetate/0.1% formic acid water
Mobile phase B	: acetonitrile
Gradient program	: 10%B (0-1 min)-50%B (4 min)-75%B (20 min) -95%B (22-25 min)
Flow rate	: 0.4 μL/min
Oven temperature	: 40 °C
Injection volume	: 5 μL

MS/MS (LCMS-8040, Shimadzu Corporation, Japan)

Ionization	: ESI
Ionization voltage	: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 3.0 L/min
Drying gas pressure	: 20 L/min
DL temperature	: 250 °C
BH temperature	: 450 °C

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3. Results and Discussion

Analytes' specific MS/MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimized using automated flow injection analysis. So, each compound was optimized in only a few minutes using the automated

software function provided in Labsolutions. The compound information and MS/MS parameters for 200 pesticides are listed in an excel document for using conveniently, as shown in Table 1.

Table 1 Representative compound information and MS/MS parameters of pesticides

1	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Serial#	Cas#	Target Flag	Compound Name (CHN)	Compound Name (ENC)	Fomular	Mw	Monitor Ion	Mode	Precursor Ion1	Product Ion 1	Dwell Time (msec)	Q1 Pre Bias(V) 1		
173	172	24017-47-8	Target	三唑磷	Triazophos	C ₁₂ H ₁₆ N ₂ O ₃ PS	313.3	[M-H] ⁺	ESI-	Quan	314.05	162.15	30	-23
174	173	52-68-6	Target	敌百虫	Trichlorophon	C ₄ H ₃ Cl ₃ O ₂ P	257.4	[M-H] ⁺	ESI-	Quan	256.90	108.95	30	-29
175	174	41814-78-2	Target	三环唑	Tricyclazole	C ₉ H ₇ N ₃ S	189.2	[M-H] ⁺	ESI+	Quan	190.00	163.00	30	-21
176	175	24602-86-6	Target	十三吗啉	Tridemorph	C ₁₂ H ₁₂ NO	297.5	[M-H] ⁺	ESI+	Quan	298.30	130.10	30	-30
177	176	141517-21-7	Target	肟菌酯	Trifloxystrobin	C ₂₂ H ₁₉ F ₃ N ₂ O ₄	408.4	[M-H] ⁺	ESI-	Quan	409.10	186.05	30	-20
178	177	99387-89-0	Target	氟菌唑	Triflumazole	C ₁₂ H ₁₀ ClF ₂ N ₂ O	345.8	[M-H] ⁺	ESI+	Quan	346.05	278.05	30	-17
179	178	64628-44-0	Target	苄嘧磺隆	Trifluralin	C ₁₂ H ₁₀ ClF ₂ N ₂ O ₂	358.7	[M-H] ⁺	ESI+	Quan	359.10	156.00	30	-17
180	179	131983-72-7	Target	灭菌唑	Triticonazole	C ₁₇ H ₂₀ ClN ₂ O	317.8	[M-H] ⁺	ESI+	Quan	318.10	70.05	30	-16
181	180	81-81-2	Target	华法林	Warfarin	C ₁₉ H ₁₆ O ₄	308.3	[M-H] ⁺	ESI-	Quan	309.10	163.05	30	-15
182	181	202480-67-9	Target	2,4-二氯苯氧基-3,5,6-三氯-乙醚	2,4-D D3	C ₉ H ₂ Cl ₅ O ₂	224.1	[M-H] ⁺	ESI-	Quan	222.00	164.10	30	15
183	182	94-82-6	Target	2,4-滴丁酸	2,4-DB	C ₁₀ H ₁₀ Cl ₂ O ₂	249.1	[M-H] ⁺	ESI-	Quan	247.00	161.10	30	17
184	183	94-75-7	Target	2,4-滴	Dichlorophenoxyacetic	C ₆ H ₂ Cl ₂ O ₂	221.0	[M-H] ⁺	ESI-	Quan	219.00	161.10	30	15
185	184	50594-66-6	Target	三氟拉草酮	Acifluorfen	C ₁₄ H ₈ ClF ₃ N ₂ O ₂	361.7	[M-H] ⁺	ESI-	Quan	360.00	316.05	30	26
186	185	28772-56-7	Target	溴敌隆	Bromadiolone	C ₂₀ H ₂₂ BrO ₄	527.4	[M-H] ⁺	ESI-	Quan	527.10	250.10	30	38
187	186	1689-84-5	Target	溴苯腈	Bromoxynil	C ₈ H ₇ BrNO	276.9	[M-H] ⁺	ESI-	Quan	275.90	81.05	30	19
188	187	94-74-6	Target	二甲四氯	Chlortox	C ₉ H ₂ ClO ₂	200.6	[M-H] ⁺	ESI-	Quan	199.10	141.10	30	14
189	188	56-75-7	Target	氯霉素	Chloramphenicol	C ₁₁ H ₁₂ Cl ₂ N ₂ O ₂	323.1	[M-H] ⁺	ESI-	Quan	321.00	152.15	30	22
190	189	196791-54-5	Target	灭幼磷	Chlorobenzuron	C ₁₄ H ₁₀ Cl ₂ N ₂ O ₂	309.2	[M-H] ⁺	ESI-	Quan	307.00	154.15	30	21
191	190	1918-00-9	Target	莠草畏	Dicamba	C ₈ H ₈ Cl ₂ O ₂	221.0	[M-H] ⁺	ESI-	Quan	219.00	175.10	30	25
192	191	120-36-5	Target	2,4-D丙酸	Dichloroprop	C ₆ H ₂ Cl ₂ O ₂	235.1	[M-H] ⁺	ESI-	Quan	233.00	161.00	30	16
193	192	83164-33-4	Target	吡氟醚草胺	Diflufenican	C ₁₉ H ₁₁ F ₂ N ₂ O ₂	394.3	[M-H] ⁺	ESI-	Quan	393.10	272.10	30	28
194	193	131341-86-1	Target	啶菌脲	Fludioxonil	C ₁₂ H ₈ F ₂ N ₂ O ₂	248.2	[M-H] ⁺	ESI-	Quan	247.10	180.20	30	17
195	194	101463-69-8	Target	氟虫脲	Flufenoxuron	C ₂₁ H ₁₁ ClF ₃ N ₂ O ₂	488.8	[M-H] ⁺	ESI-	Quan	487.10	156.10	30	17

For UHPLC/MS/MS analysis of residual pesticides which give positive and negative ions, the mobile phase was optimized. 200 pesticides were analyzed and the MRM chromatograms were obtained as shown in Fig. 1 The calibration curves, limits of quantitation (LOQs), repeatability, and recoveries were investigated. The calibration curves were given with good correlation coefficients greater than 0.999. LOQs for 93% of pesticides were better than 1 µg/kg.

The repeatability (%RSD) of standard pesticides at 10 µg/L was less than 5% for 95% of the target pesticides. Recoveries of pesticides spiked in a cucumber sample at 1 µg/kg were between 57.2% and 125.3%. Recoveries for 84% of all pesticides ranged from 80% to 120%. Representative quantitative results are shown in Table 2. The results indicate that this method is rapid and reliable.

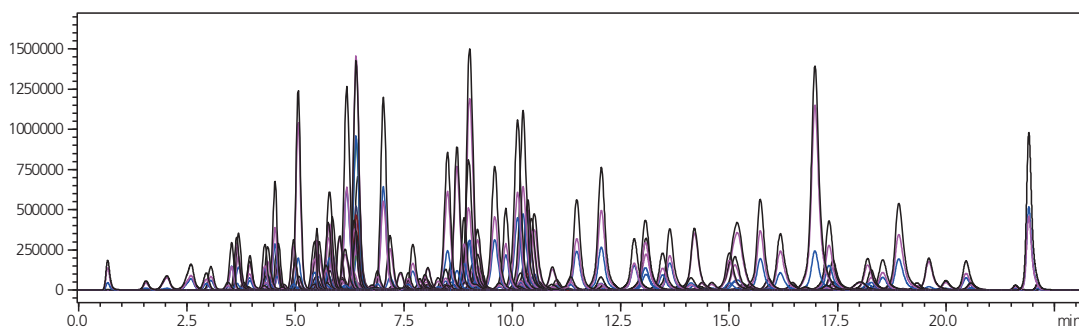


Fig. 1 MRM chromatograms of 200 standard pesticides (50 µg/L each)

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Table 2 Representative quantitative results of pesticides

No.	Name	Y=aX+b	Rinearity Lange (µg/L)	r	LQQ (µg/kg)	%RSD (1 µg/L)	Recovery (1 µg/kg)
52	Diethofencarb	Y=(11607.9)X+(11387.8)	0.5~200	0.9997	0.22	3.21	94.20
53	Diethyltoluamide	Y=(79943.4)X+(63810.1)	0.5~200	0.9993	0.01	2.79	92.70
54	Dimepiperate	Y=(39447.4)X+(3718.42)	0.5~200	0.9997	0.02	2.67	89.60
55	Dimethirimol	Y=(30050.7)X+(21635.8)	0.5~200	0.9993	0.02	2.13	93.80
56	Dimethoate	Y=(11607.9)X+(26206.1)	0.5~200	0.9992	0.02	1.87	93.10
57	Dimethomorph	Y=(13116.6)X+(-2309.89)	0.5~200	0.9998	0.07	3.10	93.40
58	Diniconazole	Y=(4273.37)X+(-1549.44)	0.5~200	0.9991	0.23	3.39	110.70
59	Dinotefuran	Y=(14619.3)X+(3801.66)	0.5~200	0.9993	0.02	3.42	95.40
60	Diuron	Y=(7835.16)X+(1847.00)	0.5~100	0.9997	0.07	4.23	104.00
61	Dymron	Y=(246705)X+(158759)	0.5~200	0.9991	0.00	2.44	87.90
62	Epoxiconazole	Y=(8801.47)X+(4813.78)	0.5~200	0.9996	0.05	4.58	77.10
63	Esprocarb	Y=(34385.0)X+(-9941.46)	0.5~200	0.9999	0.07	3.68	79.00
64	Ethametsulfuron-methyl	Y=(13881.3)X+(-1589.33)	0.5~200	0.9994	0.02	4.46	102.10
65	Ethofencarb	Y=(41267.8)X+(15848.2)	0.5~100	0.9998	0.04	2.95	99.50
66	Ethion	Y=(6519.60)X+(-5245.53)	0.5~200	0.9995	0.06	1.90	90.70
67	Ethoprophos	Y=(14174.9)X+(15071.9)	0.5~200	0.9993	0.05	3.46	85.20
68	Fenamiphos	Y=(23409.3)X+(10202.8)	0.5~200	0.9993	0.02	2.65	99.50
69	Fenarimol	Y=(303.283)X+(728.986)	2~200	0.9997	2.64	8.84	57.20
70	Fenobucarb	Y=(4791.08)X+(6489.96)	0.5~200	0.9990	0.09	4.52	87.80
71	Fenoxycarb	Y=(2134.24)X+(-225.561)	0.5~200	0.9999	0.83	4.48	105.30

Nowadays, laboratories are expanding the number of target pesticides. Rapid and reliable method for determination of trace amount of residual pesticides is necessary. Usually, the qualitative LC/MS/MS is based on the ratio of intensities between target ions and reference ions.

But in some cases, especially for determination of pesticides at low concentration, qualitative reliability is not high enough, which may cause false positive/negative results. So use of the MS/MS spectra library for searching and qualitative confirmation of the pesticides is very meaningful.

Type	Event#	+/-	Compound Name	m/z	Time (0.350 min - 22.413 min)
Product Ion Scan	12	+	氧乐果	100.00 > 50.00:250	
MRM	13	+	涕灭威亚砷	229.00>166.10	
Product Ion Scan	14	+	涕灭威亚砷	100.00 > 50.00	
MRM	15	+	咪虫胺	203.10>129.10, 20	
Product Ion Scan	16	+	咪虫胺	100.00 > 50.00:250	
MRM	17	+	涕灭威砷	245.10>166.10,	
Product Ion Scan	18	+	涕灭威砷	100.00 > 50.00:3	
MRM	19	+	灭多威	224.10>127.10 ??	

MRM Acq. Time: 3.117 - 3.717 min Compound Name: 涕灭威砷

Ch	Precursor m/z	Product m/z	Pause Time (msec)	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bi
Ch1	245.10	166.10	1.0	1.0	-12.0	-15.0	-17.0
Ch2	245.10	109.10	1.0	1.0	-12.0	-20.0	-19.0
Ch3							

Fig. 2 Setting of product ion scan event triggered by MRM

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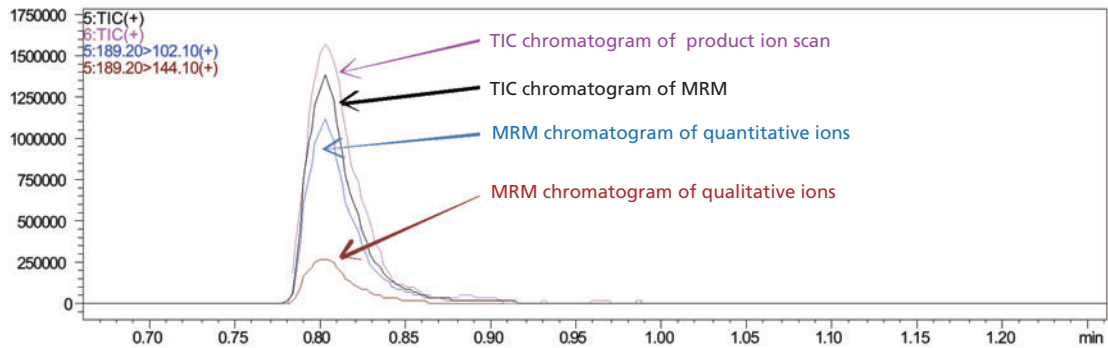


Fig. 3 Mass chromatograms of a target pesticide

A simultaneous quantitative and qualitative method file with a function of product ion scan event triggered by MRM is shown in Fig. 2 Both the TIC chromatogram of MRM and the TIC chromatogram of product ion scan were obtained as shown in Fig. 3 By searching the spectrum of product ion

scan using the pesticide spectra library, a pesticide (isoprocarb) was found (Fig. 4). Here, 9 pesticides were detected, and their similarity indexes were over 70 when the concentrations of pesticides were larger than 1 µg/kg (Table 3).

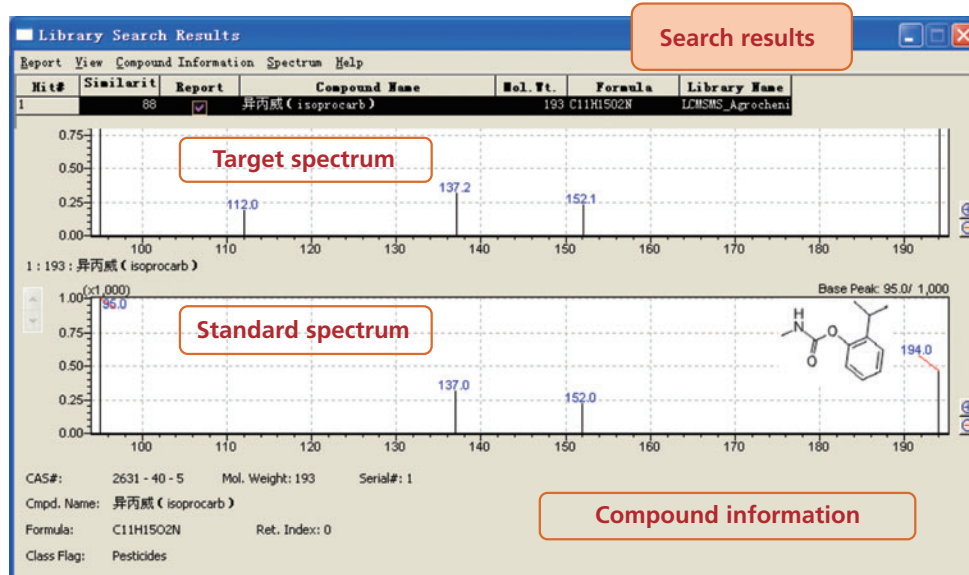


Fig. 4 Library search result of isoprocarb detected in cucumber sample

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Table 3 The results of detected pesticides and their similarity score of library searching

No.	Compound	Concentration (µg/kg)	Similarity index
1	Aldicarb-sulfone	3.26	72
2	Carbaryl	2.54	91
3	Carbendazim	3.87	100
4	Carbofuran-3-hydroxy	11.90	89
5	Dimethomorph	0.19	67
6	Isoprocarb	3.31	88
7	Methomyl	4.95	99
8	Metolcarb	4.02	82
9	Propamocarb	0.77	88

4. Conclusions

An LC/MS/MS method has been developed for quantitative and qualitative analysis of 200 pesticides in food sample. Method validation was demonstrated to evaluate instrument capability. The results showed that this method

is rapid and reliable. For qualitative confirmation, the MS/MS spectra library search was performed, which made the qualitative results more reliable.