

## Detection of 10 kind of pesticides multi-residue in fruits and vegetables by LC-MS/MS

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**Abstract.** This paper established a multi-residue detection method of amides, neonicotinoids, triazoles, heterocyclic, and other pesticides in fruits and vegetables samples by liquid chromatography - tandem mass spectrometry. Using QuEChERS as sample pretreatment method, then extracted by acetonitrile, cleaned up by graphitized carbon black using dispersed solid phase extraction. After the separation of chromatographic column, the extraction was analyzed under the LC-MS/MS multiple reaction monitoring mode. Ten kind of pesticides detection is limited between 0.006 ~ 0.133 µg/kg, and the quantitative is limited between 0.018 ~ 0.415 µg/kg. On the three adding levels between 1~10 µg/kg, the average recovery is 76.2 % ~ 96.3 %, RSD is less than 2.4 %. This method can be used in the pesticides multi-residue detection of fruits and vegetables samples.

This article selected amides<sup>[1-2]</sup>, nicotine, carbamate, triazole and methoxyl acrylic ester and other 5 kind of pesticides, boscalid, fluopicolide, flonicamid, desflurane bacteria amide, dinotefuran, epoxiconazole, imidacloprid, thiacloprid, azoxystrobin, isopropyl Granville, and boscalid fungicide belong to amides, mainly applied for controlling gray mold, sclerotinia rot and various other fungi of fruits and vegetables<sup>[3-7]</sup>. Neonicotinoid pesticides dinotefuran are for aphids, planthoppers and other pests<sup>[8-9]</sup>. Carbamate pesticides are widely applied in agriculture, forestry, and animal husbandry<sup>[12]</sup>. Isopropyl Granville are for planthoppers, rice leafhopper, aphids, bugs, etc. <sup>[11-12]</sup> Azoxystrobin is one strobilurin fungicide first listing, with broad-spectrum, long persistence, and high selection<sup>[13-14]</sup>. The pesticides are featured about high efficiency and broad spectrum, which has been widely applied in the fruits and vegetables.

Studies have shown that these pesticides cause some harm to human health, so our country developed its maximum levels for fruits and vegetables in the standard (MRLs), the imidacloprid is 1 mg/kg, organism bacteria acyl amine is 5 mg/kg, fluorine pyrazole bacterium amine is 0.5 mg/kg, desflurane bacteria amide is 0.9 mg/kg, fluorine organism worm amide is 1 mg/kg, fluorine ringazole to 3 mg/kg, its ester is 5 mg/kg, thiamethoxam moiety to 1 mg/kg, isopropyl is 0.5 mg/kg, cefuroxime worm amine is 0.8 mg/kg <sup>[15-16]</sup>.

Liquid tandem mass spectrometry, featuring of high sensitivity, high accuracy and high selectivity, has been widely used in multi-component analysis and detection of pesticide multi-residues and become an important means of measuring small molecule compounds in complex matrices<sup>[20-21]</sup>. This article selected apples, grapes, tomatoes, cucumbers, using QuChERS technology for pretreatment, multiple reaction monitoring mode (MRM) of liquid chromatography-tandem mass spectrometry (LC/MS/MS) for multi-residues detection.

By studying test conditions, the linear range, detection limit, and precision of these five classes of 10 kind of pesticides, this article established a multi-residues detection method by liquid chromatography tandem mass spectrometry, to provide faster, more accurate and more practical method for further study.

## 1 Experiment

### 1.1 Instruments and reagents

Liquid chromatography-mass spectrometry(Agilent 1200-ESI 6410, the United States)

H-1850R centrifuge (Changsha Xiang Yi centrifuge machines Ltd.)

AWL-1002-U Echo Pu Ultrapure water system (Chongqing Shun Yang Enterprise Development Co., Ltd.)

BF-2000 nitrogen blow drying apparatus eight square technology co., LTD. (Beijing)

50 ml plastic centrifuge tube (BD Biosciences company)

HPLC grade acetonitrile (Burdick&Jackson, Honeywell)

Analysis of pure formic acid, chemical reagent company (Beijing)

Bacteria acyl amine (97.0% purity), fluorine pyrazole bacteria amine (95.0%), desflurane bacteria amide standard (98.0%), were purchased from Shenzhen Rowe agrochemical co., LTD. Thiamethoxam moiety (99.5%, CHEM SERVICE), fluorine organism worm amide (96.0%, shaanxi road on grid biological science co., LTD.), its bacteria ester (99.0%), imidacloprid (98.0%), were purchased from Hebei ChengYue chemical co., LTD. cefuroxime worm amine (98.0%, shandong joint), fluorine ringazole (97.0%), isopropyl(98.0%) were purchased from Beijing and shun technology co., LTD.

### 1.2 The preparation of the standard solution

#### 1.2.1 Standard stock solution

Respectively put 0.0101g bacteria acyl amine and 0.0103g desflurane bacteria amide pesticide standard into 100 ml volumetric flask and dissolved by acetone and constant volume; Respectively put 0.0103g furbenicillin, 0.0100g isopropyl amine , 0.0102g fluorine organism worm amide pesticide standard into 100ml volumetric flask and dissolved by methanol and constant volume; Respectively put 0.0102g fluorine pyrazole bacteria amine, 0.0102 g fluorine ringazole pesticide standard into 100 ml volumetric flask, dissolved by methylene chloride and constant volume; Respectively put 0.0104g its bacteria ester, 0.0102g thiamethoxam moiety pesticide imidacloprid,0.0103g standard into 100ml volumetric flask, dissolved by acetonitrile and constant volume, and stored at -18 °C.

#### 1.2.2 Mixed standard solution

Respectively add 5 mL single pesticide standard stock solution into 100 mL volumetric flask, then use acetonitrile solution constant volume, configurate 5 ug/mL mixed standard solution, 4 °C freezing away from light.

### 1.3 Sample treatment

Weigh 10.00g samples into 50 mL stoppered centrifuge tube, 20 mL acetonitrile and 5g sodium chloride were added, after high-speed homogenization 1 min and centrifuge 5 min 7 000 r/min, take 1.5 mL supernatant into 2 mL centrifuge tube, 50 mg PSA and 2 g anhydrous magnesium sulfate was added, shock centrifugal and take 1 mL supernatant, filtrated through 0.22 um microporous membrane, dried by nitrogen blow, use 1 mL acetonitrile solution to constant volume and stored..

### 1.4 Conditions of chromatography and mass spectrometry

Conditions of Chromatographic column : Pursuit C18 (250 mm x 4.6 mm, 5 um, Agilent companies in the United States); Mobile phase: A is 0.1% formic acid aqueous solution, B is acetonitrile; Flow rate: 0.4 mL/min; column temperature: 30 °C. Injection volume is 5uL.

Conditions of mass spectrometry: electrospray ionization sourcepositive ion mode (ESI +) , the reactive ion detection mode (MRM), the MRM parameter table shown in table 1. The dry nitrogen gas temperature is 300 °C, velocity of 6 L/min; Spray atomization gas pressure 137931 pa (20 psi) capillary voltage 4000v.

Table 1 LC-MS/MS parameters of 10 pesticide

type	Parent ion	Quantitative ion	Qualitative ion	CE (eV)	FV (V)	Peak time (min)
Boscalid	343.2	307.0	139.9	15	130	20.464
Fluopicolide	383.1	172.7	364.6	15	130	21.291
Flonicamid	230.1	202.7	173.8	15	130	8.596
Fluoride ether Bacteria amide	415.4	206.8	398.7	15	90	25.531
Thiacloprid	253.1	125.8	185.7	15	135	9.744
Dinotefuran	202.9	129.0	113.1	10	105	7.739
Epoxiconazole	330.1	120.9	140.9	15	135	19.376
Isoprocarb	194.1	95.1	137.1	10	105	14.574
Imidacloprid	256.1	175.1	209.1	15	90	9.017

## 2 Results and discussion

### 2.1 Optimization of extraction conditions

Acetonitrile is commonly used in the laboratory extraction agent, easy to volatile and water soluble, after adding the amount of sodium chloride can be separated with water, fat, protein, sugar compounds are not easy to extract, high recovery rate of pesticides. In the analysis of pesticide residue, in order to improve the recovery rate of the easily degradable pesticide in the basic medium, the samples were extracted with acetonitrile.

PSA contains primary amine and secondary amine groups, with weak anion exchange capacity, commonly used in the extraction of liquid dispersed solid phase extraction purification. In the experiment, PSA was used to purify the acetonitrile extract, and PSA was used to preserve the 10 target compounds, so the extraction solution was not needed to be concentrated and purified.

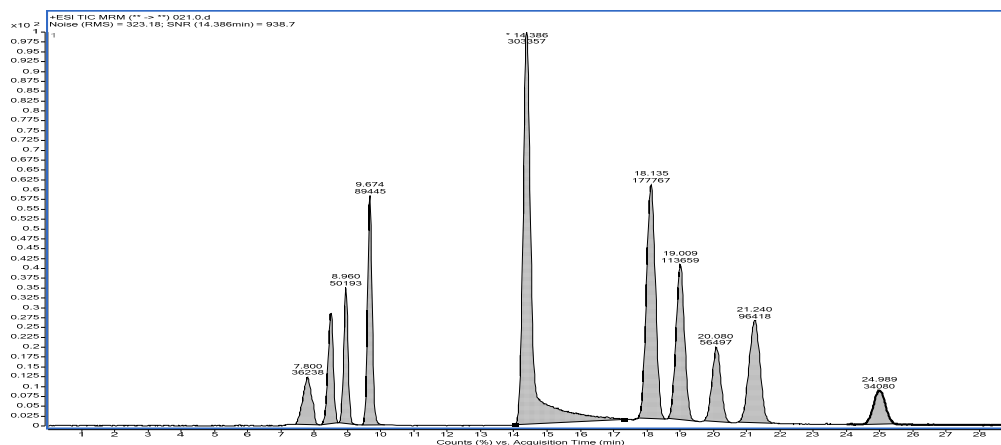
### 2.2 Optimization of conditions for liquid chromatography tandem mass spectrometry

#### 2.2.1 Selection of mobile phase

To the best separation effect were investigated. Ammonium acetate - methanol, ammonium acetate acetonitrile and formic acid methanol and formic acid in acetonitrile solution as mobile phase separation. The results showed that in acetonitrile under the condition of income spectrum baseline fluctuations are smaller than those of the fluctuation of methanol and imidacloprid, Fluopicolide and boscalid failed to completely separated under the conditions of methanol, extend the detection time, so the choice of separation effect better acetonitrile as the organic phase. In the water adding formic acid or ammonium acetate is conducive to the ionization of target compounds, due to ammonium acetate than formic acid ionization effect is poor, so formate as inorganic phase, and investigated the effects of the concentration of formic acid solution on the separation effect. It was found that when the acid was 0.1%, the 10 pesticides were completely separated. To sum up, in the 0.1% formic acid acetonitrile separation effect is the best, so the choice of 0.1% formic acid acetonitrile as mobile phase. Under optimized conditions, the total ion flow diagram of the 10 pesticides in the standard solution is shown in figure 1.

#### 2.2.2 Effect of flow rate on separation time

Experiments were presented to investigate the flow rate on the separation time of impact, within the pressure range of the column, When the flow rate is /min 0.3mL, the separation and detection of the 10 pesticides need to be 40min, When the flow rate is transferred to /min 0.4mL, the flow time of the target compound is ahead of time, and the complete separation can be achieved in the 30min.



Picture 1 MRM chromatogram under optimized condition

### 2.3 linear range and detection limit

Take a certain amount of mixed standard solution and acetonitrile diluted into standard solution 1,2,5,10,20,50,100 g / L, in HPLC-MS / MS analysis, the peak area (y) on the quality of compound concentration (x) linear regression, draw the standard curve, 10 kinds of pesticide standard curve linear correlation coefficient (R<sup>2</sup>) between the 0.9981~0.9997, show that in 1 ~ 100 g / l range with good linearity.

The detection limit of the method with the lowest dilution standard curve of blank sample matrix on the peak when SNR = 3 R/N calculated. Quantitation limit is in blank sample matrix dilution standard curve, the lowest concentration peak, to win the trust of noise than R/N for 10 calculated, pesticide detection and quantitation limits were 0.15 to 0.2 g / kg and 0.5 ~ 0.6 u g / kg.

Table 2 Linear equations and correlation coefficients of 10 pesticides in the sample solution

Type	Linear equation	r <sup>2</sup>
Boscalid	y = 48920x - 180.25	r <sup>2</sup> =0.9992
fluopicolide	y = 88075x + 1261.8	r <sup>2</sup> =0.9989
flonicamid	y = 29584x - 202.34	r <sup>2</sup> =0.9992
Fluoride ether bacteria amide	y = 28019x + 85.438	r <sup>2</sup> =0.9997
Thiacloprid	y = 85809x + 90.41	r <sup>2</sup> =0.9998
dinotefuran	y=17534x + 62.718	r <sup>2</sup> = 0.9989
epoxiconazole	y = 103805x + 696.47	r <sup>2</sup> =0.9995
Isoprocorb	y = 182397x + 2523.3	r <sup>2</sup> =0.9990
Imidacloprid	y = 25145x - 6.1854	r <sup>2</sup> =0.9997
Azoxystrobin	y = 177501x + 1705.3	r <sup>2</sup> =0.9981

Table 3 10 kinds of pesticides in apple, grape, tomato, cucumber in the added recovery rate, LOD, LOQ

Type	Adding concentration (mg*kg <sup>-1</sup> )	Apple		Grape		Tomatoes		Cucumber		LOD (μg*k g <sup>-1</sup> )	LOQ (μg*k g <sup>-1</sup> )
		rate of recovery %	RSD %	rate of recovery %	RSD %	rate of recovery %	RSD %	rate of recovery %	RSD %		
Boscalid	0.01	77.6	2.3	89.6	1.6	83.6	2.9	82.1	3.1	0.15	0.5
	0.05	80.3	1.9	88.1	1.9	81.6	1.6	84.3	1.2		
	0.5	76.2	2.8	88.9	2.3	83.9	1.4	81.1	1.5		
fluopicolide	0.01	79.3	1.7	83.6	0.9	78.7	3.9	79.8	1.8	0.15	0.5
	0.05	78.8	3.3	86.4	1.5	80.3	2.5	83.4	2.8		
	0.5	85.1	2.1	88.2	1.2	83.4	1.3	83.3	1.4		
flonicamid	0.01	89.4	2.8	90.6	1.8	87.6	4.3	89.5	1.6	0.2	0.6
	0.05	87.1	2.1	89.3	4.4	84.5	2.1	88.6	7.2		
	0.5	86.4	3.9	92.7	2.3	88.9	1.7	93.1	1.8		
Fluoride ether bacteria amide	0.01	85.6	1.3	83.1	0.8	79.2	1.4	81.4	0.9	0.15	0.5
	0.05	86.9	0.8	85.6	1.6	78.4	2.4	82.8	2.1		
	0.5	85.3	1.5	88.1	3.6	80.3	1.2	80.7	3.2		
Thiacloprid	0.01	80.6	1.6	79.6	1.8	80.9	3.5	82.6	1.6	0.2	0.6
	0.05	86.1	2.5	82.3	1.5	81.8	1.1	84.1	1.2		
	0.5	88.3	1.4	81.2	2.9	77.6	4.1	80.8	4.6		
dinotefuran	0.01	87.3	4.1	87.7	1.3	86.8	0.9	90.8	1.4	0.2	0.6
	0.05	89.6	1.6	90.3	1.6	88.4	2.1	89.6	1.9		
	0.5	92.1	1.2	91.2	5.2	85.2	1.3	89.9	1.1		
epoxiconazole	0.01	79.8	2.2	87.2	1.9	80.1	3.7	80.2	4.1	0.2	0.6
	0.05	82.4	1.4	85.1	1.6	78.6	1.3	79.8	2.4		
	0.5	83.3	1.9	88.4	3.5	79.4	6.3	78.1	1.9		
Isoprocarb	0.01	80.3	0.8	82.5	1.3	80.6	1.4	82.5	2.6	0.15	0.5
	0.05	82.6	1.6	85.4	2.1	82.4	2.1	84.4	0.7		
	0.5	83.5	6.4	86.6	1.8	79.1	1.4	81.2	1.1		
Imidacloprid	0.01	79.6	2.3	79.6	6.4	83.4	1.9	86.1	1.3	0.15	0.5
	0.05	87.5	1.2	83.5	1.3	79.6	1.2	89.9	3.3		
	0.5	83.6	2.2	84.8	2.1	81.5	4.2	85.3	1.5		
Azoxystrobin	0.01	86.5	3.1	96.3	0.8	85.4	1.2	89.7	1.9	0.15	0.5
	0.05	88.9	1.6	92.1	2.8	86.9	1.9	86.5	2.2		
	0.5	89.7	2.1	94.4	1.3	82.2	2.3	91.2	1.8		

#### 2.4 recovery and precision of the method

This method uses tomatoes, grapes, apples and cucumbers 4 kinds of fruit and vegetable as samples, respectively with the addition of 0.001,0.05 and 0.1 mg / kg three concentration levels of mixed standard solution, according to the pretreatment method, rate of recovery experiments, each repeated 3 times measured precision. The results are shown in the table.

As we can seen from table ,10 kinds of pesticides in the three levels, The average recovery rate was between 76.2%~96.3%, the relative standard deviation (RSD) between 0.8%~2.4%, the accuracy and precision of the method are in line with the requirements of residue analysis. According to this method to detect the local supermarket fruits and vegetables samples, including

apples, grapes, tomatoes and cucumbers in the sample did not exceed the maximum pesticide residue limits standard.

### 3 Summary

In this article, the rapid detection methods of 10 pesticides in 4 fruits and vegetables were successfully established by QuEChERS pretreatment, liquid chromatography separation and tandem quadrupole mass spectrometry. The results show that the method has a simple sample processing, high sensitivity, good reproducibility, qualitative and quantitative results are reliable for apple, grapes, simultaneous determination of 10 kinds of pesticide residues in tomatoes and cucumbers, but also for other fruits and vegetables pesticides in simultaneous determination of residues of reference.

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