Food and Environmental



X500R QTOF System with SWATH[®] Acquisition for Pesticide Residue Screening in Fruits and Vegetables

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Introduction

It is widely accepted that modern Chinese agriculture has a long history of excessive chemical fertilizer, pesticide, and herbicide use, and this has not only resulted in reduced nutrient content in food, but also in a variety of chemical residues that have harmed human health. It has been confirmed that pesticide residues may interfere with the body's endocrine effects on the immune system and hematopoietic system, and can even cause in-vivo fetal visceral hypoplasia or deformity in pregnant women. Weighed against the benefits of consuming more fruits and vegetables, people have recently become more heavily concerned about the widespread existence of pesticide residues and the excessive damage they can cause to the human body.

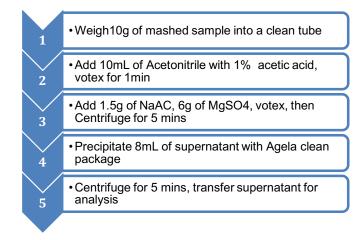
With the continued development and popularization of liquid chromatography / mass spectrometry, more and more pesticide residue detection technologies are being developed based on the LC-MS/MS system. The most commonly used pesticide residue screening method includes the MRM-IDA-EPI system, which is based on QTRAP[®] system and high resolution TOF-IDA-MSMS technology. While the SWATH technology is based on high-resolution systems, it also combines the advantages of IDA and MRM by dividing the mass range of the parent ion into multiple mass windows and allowing all ions in each window to collide with each other and fragment, resulting in fragmentation information for all ions in the entire mass range. SWATH® technology's measurement of second-order fragmentation differs from the IDA, in which only the selected ions are triggered, ensuring the continuity of all ion debris and achieving SWATH's second degree of quantification. By customizing the unique variable window settings, the size of the mass window is automatically adjusted according to the quantity of ions, ensuring the collection of high-quality data.

The SCIEX High Resolution Mass Spectrometry X500R QTOF system provides high resolution, high accuracy, high sensitivity and high linearity range scan speeds, making SCIEX SWATH technology not only popular for protein macromolecules but also for small molecule pesticide residue screening. The X500R QTOF system uses newly designed SCIEX OS software to achieve an all-in-one whole process analysis with instrument control, data acquisition and data processing. The software has the built-in SWATH method of setup and powerful automatic deconvolution capabilities. This simple and convenient design meets food safety field use requirements.

Experimental considerations:

- 1. Collect and process samples of fruits and vegetables, and measure the actual SWATH data
- 2. Prepare Standard Curve, Test 190 Pesticide Standard SWATH data
- 3. Screening of Pesticide Residues in Vegetables and Fruits
- 4. The pesticide residue was quantified at two levels

Sample treatment:



The QuEChERS method was used to pretreat received samples: 1 leek, 2 cauliflower, 3 bean, 4 jujube (after washing), 5 jujube (not cleaned), 6 pear.



Chromatographic Methods

Chromatography column: Phenomenex Kinetex C18, 100*2.1 mm, 2.6µm

Mobile phase: A: Contains 5mM ammonium acetate in water; B: Contains 5mM ammonium acetate in methanol gradient elution

Flow rate: 0.4mL/min

Column temperature: 40°C

Input volume: 10µL

Time (min)	B%
0	3
1	3
2	45
19	95
22	95
22.1	5
25	5

Mass Spectrometry Method

Scanning method: SWATH[®] Acquisition methods

Ion source: ESI+source CDS automatic calibration

Table 1: Ion source parameters

Air curtain gas CUR: 35psi
Auxiliary gas GS2: 55 psi
Collision Gas CAD: 7
Air curtain gas CUR: 35psi

Data acquisition and SWATH setup process

IDA (Information Dependent Acquisition) uses TOF/MS Survey Scan to pre-scan. When a peak ion is successfully triggered and detected, the scan mode is switched to Q1 and the parent ion is selected to acquire a high sensitivity MS/MS secondary spectrum of the target ion. SWATH distributes all the ions into successive windows, and all the ions in each window are transferred to the collision chamber and broken into secondorder MS/MS debris and then traced back to the parent ion through the software's powerful de-convolution function. Thus all of the second-order fragments of all abundant ions can be obtained through this technique, which ensures that the secondary information of the low-content target is included, allowing the trace residue screening to become more complete and accurate.

Unique intelligent variable windows, according to the distribution of ions in the sample, set narrow windows in the high density distribution areas and set up wider windows in the regions with fewer ions to ensure high-quality secondary mass spectra are collected for all ions.

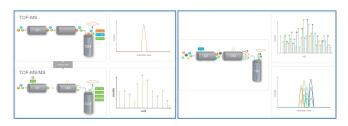


Figure 1 Left IDA schema; Right SWATH schema

• Experim	nent SWATH	•											
Polarity		Positive	٠		Spray vo	Itage	5500	\$	v				
TOF MS													
TOF star	t mass	100	\$	Da	Decluste	ring potential	80	\$	v	Collision energ	v	10 🗘	v
TOF stop	p mass	630	\$	Da	DP sprea	d	0	\$	v	CE spread		0 🗘	v
Accumu	lation time	0.2	\$	s									
Advanc	ed Experiment Sett	inas											
	is to sum	4	\$		Channel	1	✓			Channel 2		 Image: A start of the start of	
Channel	13	 Image: A start of the start of			Channel	4	~						
TOF MSM	NS												
TOF star	t mass	50	\$	Da	TOF stop	mass	630	\$	Da	Dynamic collisi	on energy		
Accumu	lation time	0.05	\$	s	Charge s	tate	1	\$					
Mass Ta	able <u>Autofil</u>	SWATH windows	<u>.</u>										
	Precursor ion start	mass (Da) F	recu	irsor ion stop n	nass (Da)	Declustering pote	ntial (V)	DP sprea	d (V)	Collision energy (V)	CE spread (V)	Time bins to sur	m C
1	99.5000		159.5	000		80		0	3	35	15	4	
2	158.5000		203.0	000		80		0	3	35	15	4	
3	202.0000		229.3	000		80		0	3	35	15	4	
4	228.3000	1	252.2	000		80		0		35	15	4	

Figure 2 SWATH method settings

In the SCIEX OS Software, choose "Experiment" and then pick the SWATH Acquisition mode. The software then automatically lists the required parameters for the SWATH mode. Mass Table is for the Q1 window.

The Variable Window Calculator can be based on TOF/MS's parent ion to automatically calculate the SWATH smart variable window. The mode can be established by copying and pasting to the Mass Table, which is a method that is simple, rapid, and easy to use.

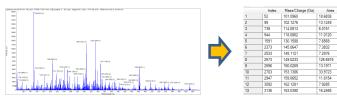


Figure 3 TOF/MS's parent ion



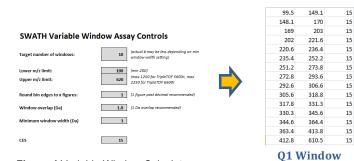
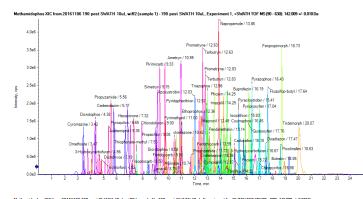


Figure 4 Variable Window Calculator



Establish SWATH[®] Acquisition method and initial test of 190 varieties of standard pesticide products.



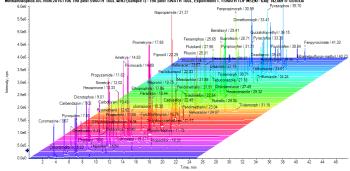


Figure 5 Chromatogram of 190 varieties of pesticide residue standard products collected by SWATH.

Data Analysis

1. Qualitative screening

Test SWATH data of 1 (leek), 2(cauliflower), 3 (kidney beans), 4 (jujube, washed), 5 (winter jujube, unwashed), and 6 (pear). Use X500R SCIEX OS Software to perform data analysis by passing four confidence conditions: mass accuracy, retention time,

isotope distribution and secondary library matching to screen pesticide residues in the 6 samples.

1. Select the standard product data to establish screening methods; import the screening list

Workflow	Sele	ect or	verify	the anal	yte and interna	I standard name	is and i	nasses.						
										Experime	nt Type 👻 Impor	• Export.	Optio	15
integration	F	low	IS	Group	Name	Chemical Formula	sotope	Adduct/Ch	Precursor Mass (Da)	Fragment Mass (Da)	XIC Width (Da)	Retention Time (min)	IS Name	Expe b
Library Search		1	B	_	Acetamiprid	C10H11CIM	_	[M+H]+	223.0745		0.02	5.35		1 TOF
		2	10		Azoxystrobin	C22H17N3O5		[M+H]+	404.1241		0.02	12.08		1 TOF
Acceptance Criteria		3	13		8uprofesin	C16H23N3O5		[M+H]+	305.16346		0.02	16.23		1 TOF
		- 4	13		Carbendazim	C9H9NBO2		[M+H]+	192.07675		0.02	5.77		1 TOF
		5	8		Chlorpyrifes	C9H11CI3NCI3PS		[M+H]+	349.93356		0.02	17.04		1 TOP
Oualitative Rules		6	83		Cyromatine	C6H10N6		[M+H]+	167.10397		0.02	3.43		1 TOF
Qualitative Rules		7	13		Dichlorvos	C4H7Cl2O4P		[M+H]+	220.95318		0.02	7.22		1 TOP
Ion Ratio		8	13		Diethofencarb	C14H21N04		[M+H]+	268.15433		0.02	11.70		1 TOF
		9	8		Difenoconazole	C19H17CI2N3O3		[M+H]+	405.07197		0.02	15.93		1 TOP
		10	13		Dimethomorph	C21H22CIN04		[M+H]+	388.13101		0.02	12.80		1 TOF
		11	15		Fenbuconazole	C19H17CIN4		[M+H]+	337.12145		0.02	14.09		1 TOF
Formula Finder		12	13		Fenobucarb	C12H17NO2		[M+H]+	208.13321		0.02	14.58		1 TOF
		13	8		Fenpyroximate	C24H27N3O4		[M+H]+	422-20743		0.02	19.35		1 TOP
		14	13		Inidacloprid	C9H10CN502		[M+H]+	256.05958		0.02	4.68		1 TOF
		15	15		Malathion	C10H19O6P52		[M+H]+	331.04335		0.02	13.24		1 TOP
		16	13		Methidathion	C6H11N2O4P53		[M+H]+	302.96914		0.02	11.12		1 TOF
		17	8		Metolachior	C15H22CIN02		[M+H]+	284.14118		0.02	13.04		1 TOP
		18	13		Paclobutracol	C15H20CN30		[M+H]+	294.13677		0.02	11.92		1 TOF
		19	13		Picolinafen	C19H12F4N2O2		[M+H]+	377.09077		0.02	18.68		1 TOP
		20	13		Pirimiphos-met	C11H20N3O3P5		[M+H]+	305.10358		0.02	15.34		1 TOP
		21	13		Prochloraz	C15H16CBN3O2		[M+H]+	376.03809		0.02	15.58		1 TOP
		22	13		Prometryne	C10H19N55		[M+H]+	242.14339		0.02	12.65		1 TOF
	20													

2. Set the quantitative integration parameters

[MQ4] Modify Method							×
Workflow	For each compone	ent, configure the parame	ters to opti	mize peak	integration		
Components	Algorithm: MQ4						Options •
Integration +	Acetamiprid * Azorystrobin					34.1141 - 404.1341) from 190 etd SWATH 20161115 leight: 1.076e6, RT: 12.08 min	
Library Search	Buprofezin Carbendazim	Apply peak parameter		onponents points	1.0+6		12.08
Acceptance Criteria	Chlorpyrifos Cyromazine	Minimum Peak Height	100.00		9,045		
	Dichlorvos Diethofencarb	XIC width		Da			
Oualitative Rules	Difenoconazole Dimethomorph	Gaussian Smooth Width Noise Percentage		points %	8.0e5 -		
Ion Ratio	Fenbuconazole Fenobucarb	Baseline Subtract Window	0.15	nin	7.0e5		
	Fenpyroximate Imidacloprid	Peak Spitting	2	points	2 6.045-		
	Malathion Methidathion	Retention Time (RT)			freedy 5.045		
Formula Finder	Metolachior Paclobutrazol	Expected RT	12.08	nin	* 4.0e5		
	Picolinafen Pirimiphos-me	Apply units to all of the	analytes		3.045		
	Prochloraz Prometryne	Concentration units					
	Proposur Pyraclostrobin	Regression parameter	Area 👻		2.0e5 -		
	Pyridaben Pyrimethanil	Regression type Weighting type	Linear 💙		1.045		
	Tebuconazole Terbutryn	weighting type	1/X •			1 2 3 4 5 6 7 8 9 10 11	12 13 14 15 16 17 18 19 20 21 22 23 24
	Tetraconadole Thissharate			BARRA DO			me nin
							Process & Close Help

3. Set the library search criteria

[MQ4] Modify Method					×
Workflow	Configure the Diversion of the second				î
Components	Perform Library Search				
Integration	Library Search Algorithm	Confirmation Search	•		
Library Search •	Results Sorted By Library Spectra Type	Purity Accurate Mass Only	*		
Acceptance Criteria	Libraries To Search	Search All Libraries			
Confidence Limits		 Pesticides_China team_TO Pesticide AM library Aug0 	^		
Qualitative Rules		TCM Library 1.0 - K	v		
Ion Ratio	Area Kabo Threshold (Unknown/Comparison) Algorithm Parameters	0			
Advanced		/- 0.4 De			
Formula Finder	Collision Energy	/- 5 eV			
Non-targeted Peaks		/- 0.5 min			
	Fragment Mass Tolerance	/* 0.4 De			
	Ignore Isotopes In Unknown	Intensity Threshold	0.05		
	Use Polarity	Minimal Parity	10.0 %		
	Use Collision Energy Spread	Intensity Factor	5		
	Use Compound Specific Purity The	shold			
				Process & Close Close	Help

4. Set the screening confidence conditions

[MQ4] Modify Method										
Workflow							e			
Components				~				•		
Integration	Apply	Qualitative Rule		cceptable Xifference		Marginal Difference		Unacceptable Difference	Weight (%)	
Library Search	~	Mass Error (ppm)	*	5	*	30		10	30	@ Error %
Acceptance Criteria	~	Error in Retention Time	۰	5	<	10		10	20	© Absolute
	~	% Difference Isotope Ratio	*	10	*	30		a 30	20	
Confidence Limits	~	Library Hit Score	*	70		50		- 50	30	
Qualitative Rules 🔹 🔸		Formula Finder Score	>	50	>	20		• 20	20	
Ion Ratio										
Formula Finder										



5. One time import of all standard product and samples' SWATH[®] data to perform screening



6. Filter results through the Mass error, RT, Isotope, Library

Ъ								Sample 1	iyye 💌 🗛	ceptance -	X A A		10	ık.	L C)		8		More		
Index	Sample Name	Sample Type	Component Name	Actual Concentration	Expected RT	Area	Retention Time	Used	Calculated Concentration	Асситису	Define a qualifying			•		. Isotope		Found At Mass	Mass Error L.	Library Hit	Lib Sc
239	1-juce SWATH	Unknown	Acetamiprid	N/A	5.35	5.223e3	5.36	12	<0	NA	🖌 Ion ratio	~	~	~	\mathbf{v}	•	•	223.0966	99.2	No Match	0.0 :
240	1-jucai SWATH	Unknown	Assystebin	N/A	12.08	1.18443	12.00	10	< 0	N/A	A Mass error						•	404.1240	-0.3	No Match	0.0
241	1-jucai SWATH	Unknown	Buprofesin	N/A	16.23	1.255e5	36.18	12	1.192	N/A		-			÷.	×	×	306-1640	1.7	Buprofesin	75.9
242	1-jucai SWATH	Unknown	Carbendazim	N/A	5.77	1.656e6	5.75	58	22.802	N/A	Ase RT	~	~	~	~	×	×	192.0766	-0.6	Carbendazim	98.9
243	1-jucai SWATH	Unknown	Chlorpyrifes	N/A	17.04	2.865#3	16.97	1	0.875	N/A	"C instage	\checkmark	\checkmark	~	~		×	349.9361	7.3	Chlorpyrifes	72.6
244	1-juce SWATH	Unknown	Cyromatine	N/A	3.43	4.903e5	3.39	50	13.090	N/A	I Library				\mathbf{v}	×	×	167.1042	1.2	Cyromatine	100.0
245	1-jucal SWATH	Unknown	Dichloryos	N/A	7.22	1.028e5	7.18	100	33.939	N/A	G.H. Formula		~			×	×	220.9537	2.2	Dichlorovos	84.5
246	1-jucai SWATH	Unknown	Diethofencarb	N/A	11.70	4.399+2	11.64	50	< 0	N/A	Cyfly Hormuta	~	~	~	×	•	٠	268.1634	33.7	No Match	0.0
247	1-Jucal SWATH	Unknown	Difenoconacole	N/A	15.93	2.353e3	15.84	10	< 0	N/A	Show rows that	Dualif				•	•	435.0693	-6.5	No Match	0.0
248	1-jucai SWATH	Unknown	Dimethomorph	N/A	12.80	5.772e4	12.74	121	<0	N/A				-		×	~	388.1316	1.5	Dimethom	85.9
249	1-Juce SWATH	Unknown	Fenbuconacole	N/A	14.09	4.349e3	34.02	10	< 0	N/A	C19H17CN4	337.13	12	٠	×	•	•	337.2353	337.9	No Match	0.0
250	1-jucai SWATH	Unknown	Fenobucarb	N/A	14.58	7.609e2	14.64	121	< 0	N/A	C12H17NO2	208.13	13	٠	~	•	•	208.1354	10.7	No Match	0.0
251	1-juce SMATH	Unknown	Fenpyroximate	N/A	19.36	3.150e3	29.30	10	< 0	N/A	C24H27N3O4	422.20	97	~	~	•	•	422.2075	0.1	No Match	0.0
14.1	A Doord Charlenger	Status and	Resident and A	4114		a ana	4.40	1.00	- 0		001120000000	244.04						144 0410	12.0		

7. Obtain he results of screening for each sample



Chromatogram isotope pattern MS/MS FIT

2. SWATH second degree quantification

Using TOF/MS's first degree quantitative data in complex matrix samples has disadvantages such as high baseline noise and a narrow linear range, etc. The SCIEX OS Software in the X500R QTOF system can be used to directly copy and paste the ion pairs of compounds when a quantitative method is established, obtaining the results of the second quantification by using the MRM^{HR} method to process SWATH data.

Preparation of 190 kinds of pesticide on the standard curve1ng/mL~100ng/mL established the second degree quantitative SWATH[®] method to obtain second degree quantitative linear relationships, see Fig. 6.

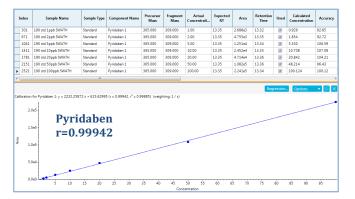


Figure 6 Quantitative linearity using pyridaben as an example

For use of the high sensitivity and high selectivity SWATH second degree quantitative method to quantify the pesticide residues contained in leek, cauliflower, kidney bean, winter jujube (washed), winter jujube (unwashed), and pear, please see the table below for the pesticide residues contained in the above samples.

Detected pesticides (unit: ng / mL)

	Leek	Cauliflower		Winter Jujube (washed)	Winter Jujube (unwashed)	Pear
Carbendazim	8.7		1		1.4	
Insecticide	4.5					
Methylpyrimidine	3.5					
Prometryn	33					
Pyrimethanil	23		270			
Thiophanate-methy	/1 2.1					
Imidacloprid		1.2			580	3.4
Propoxur		50	13.7			
Tebuconazole		1.1	4.5			3.4
Acetamiprid			3.7		5.1	86
Kresoxim			8.2	15	22	
Streptozotocin			35	18	74	
Buprofezin					160	
Fenpyroximate					61	
Paclobutrazol					140	
Triadimefon					23	

Summary

The experiment used the SCIEX X500R QTOF system's SWATH technology to screen pesticide residues in six varieties of vegetables and fruits, among which leeks, kidney beans and



jujube contained 6 or more types of pesticide residue. In particular, in the jujube, the imidacloprid content reached 580 ppb, far exceeding the limits of pesticide residue standards; buprofezin and paclobutrazol content also exceeded100ppb. Through the analysis of the washed jujube samples we found that although the pesticide residue is extremely high, it is fortunately possible to be washed off with detergent. Even so, the washed jujube still contains more than 10ppb of kresoximmethyl and pyraclostrobin.

This experiment established the SWATH[®] screening and quantitative methods for residues of the190 most commonly used types of pesticides for the Ministry of Agriculture risk assessment. SWATH technology obtained the primary and secondary data of all pesticide residues by entering the samples only a single time. The highly sensitive secondary spectra were still able to identify each compound in the spectral library and obtained the secondary spectra even when the pesticide content in the sample was very low, using four confidence conditions: mass accuracy, isotope distribution, retention time, and secondary library matching to corroborate.

Any ion's continuous chromatographic peak data in the SWATH secondary spectrum can be used as the basis for quantitative data analysis. When the sample in the first class chromatographic peak has matrix interference, SWATH second degree quantification can effectively reduce the background noise and eliminate interfering ions so that the quantitative results are more accurate and reliable. 190 kinds of standard curve pesticide residues were profiled, and qualitative and quantitative analysis of the 6 samples was performed.

Setting up the SWATH method is very easy. One can directly establish methods by going to SCIEX OS software's built-in SWATH options, and through the Variable Windows, the settings allow the user to intelligently partition the ions' Q1 mass window, to ensure that each ion can receive high quality SWATH data, in order to meet the qualitative and quantitative needs.

References

- André Schreiber, SCIEX, Concord, Ontario (Canada), X500R and SWATH for pesticide screening RUO-MKT-11-4711-A
- 2. *SCIEX*, European Union Reference Laboratory (EURL-FV) Almeria, Spain and the EMEA team, Analysis of Regulated Pesticides in Baby Food Using SCIEX X500R QTOF

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