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:

1. Weigh10g of mashed sample into a clean tube
2. Add 10mL of Acetonitrile with 1% acetic acid, votex for 1min
3. Add 1.5g of NaAC, 6g of MgSO4, votex, then Centrifuge for 5 mins
4. Precipitate 8mL of supernatant with Agela clean package
5. Centrifuge for 5 mins, transfer supernatant for analysis

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℃

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

IS Voltage: 5500V	*Air curtain gas CUR: 35psi*
Atomizing gas GS1: 55 psi	*Auxiliary gas GS2: 55 psi*
Source Temperature TEM: 550°C	*Collision Gas CAD: 7*
Collision energy CE ± CES: 35 ±15V	*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 second-order 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.

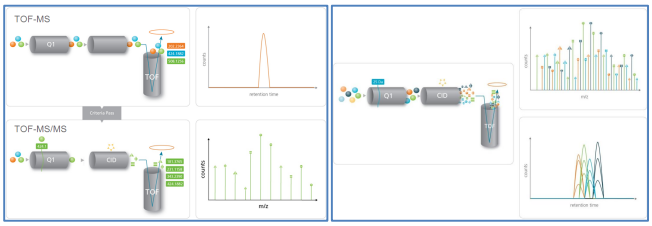


Figure 1 Left IDA schema; Right SWATH schema

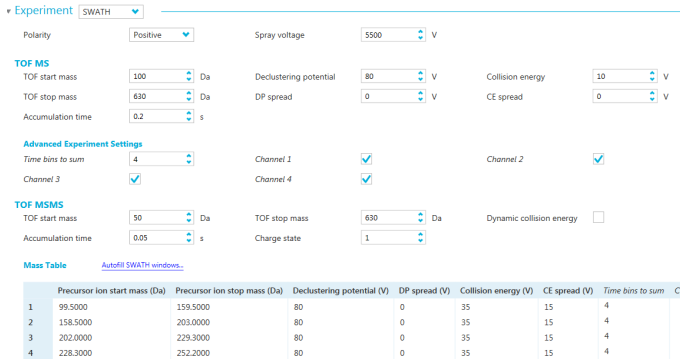


Figure 2 SWATH method settings

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.

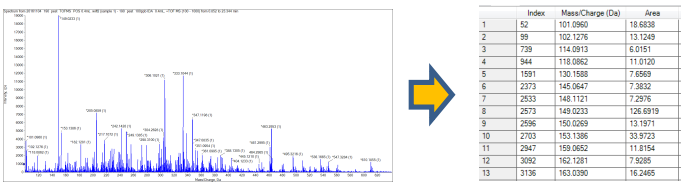


Figure 3 TOF/MS's parent ion

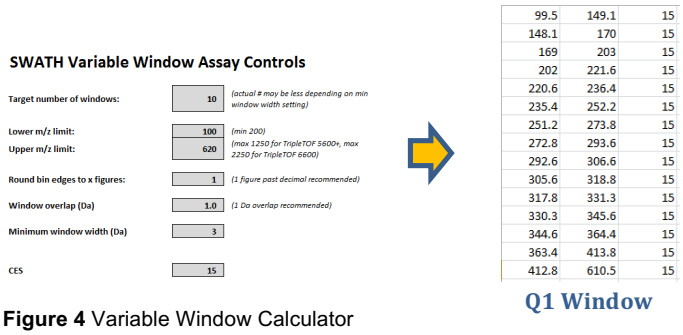
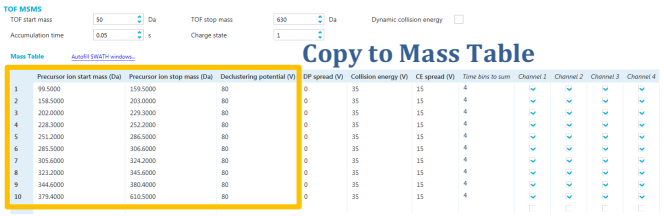


Figure 4 Variable Window Calculator



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

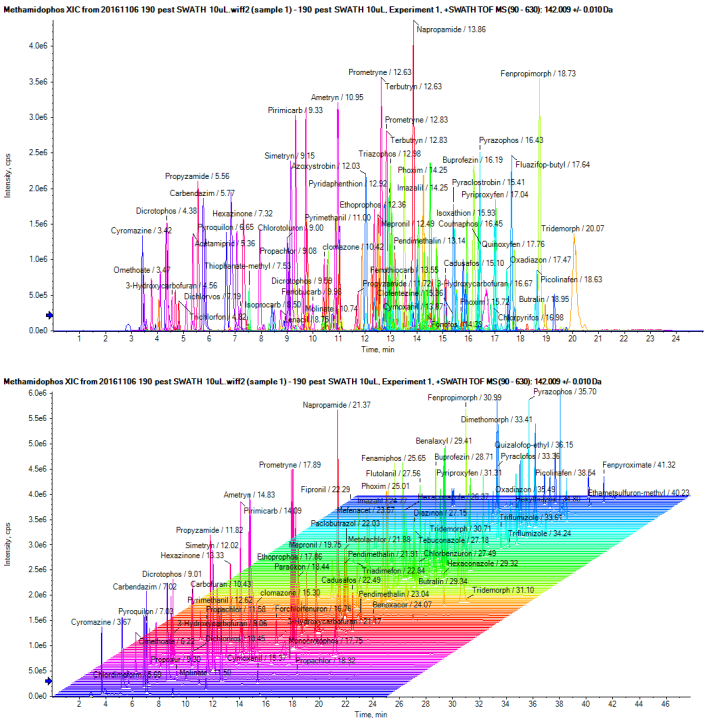


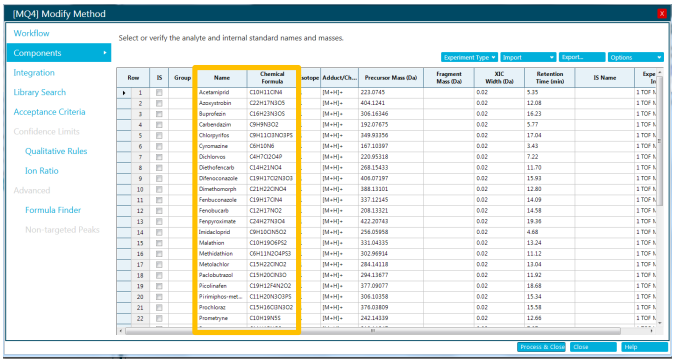
Figure 5 Chromatogram of 190 varieties of pesticide residue standard products collected by SWATH.

Data Analysis

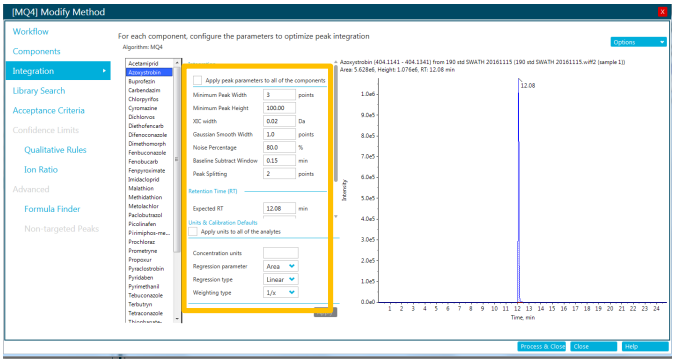
1. Qualitative screening

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.

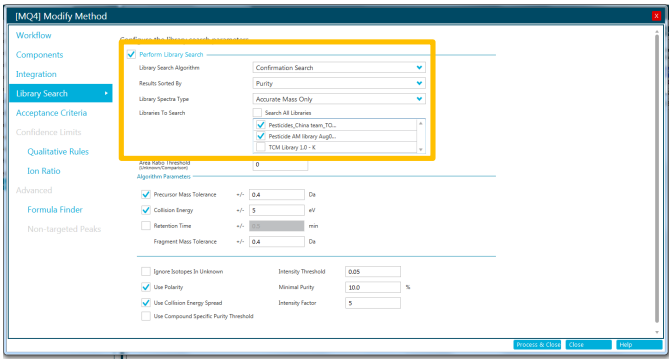
1. Select the standard product data to establish screening methods; import the screening list



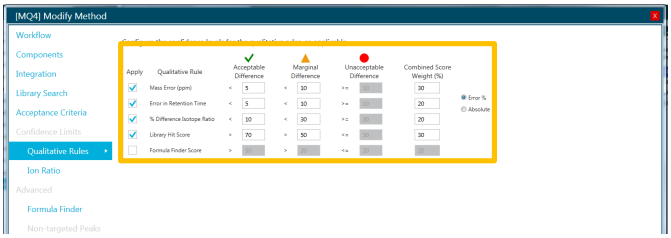
2. Set the quantitative integration parameters



3. Set the library search criteria

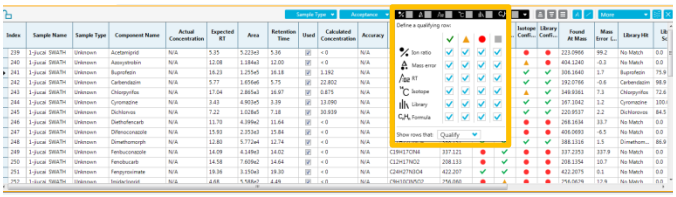


4. Set the screening confidence conditions

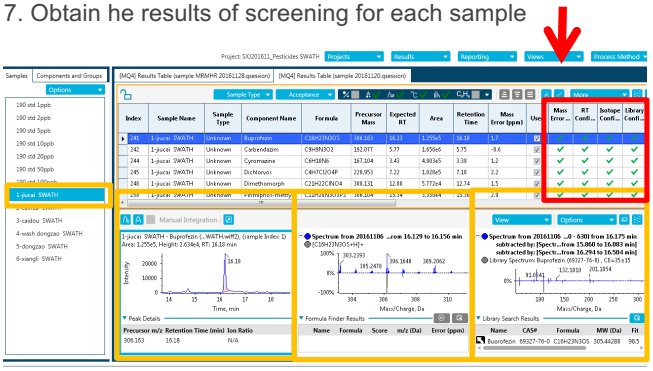


5. One time import of all standard product and samples' SWATH® data to perform screening

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



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.

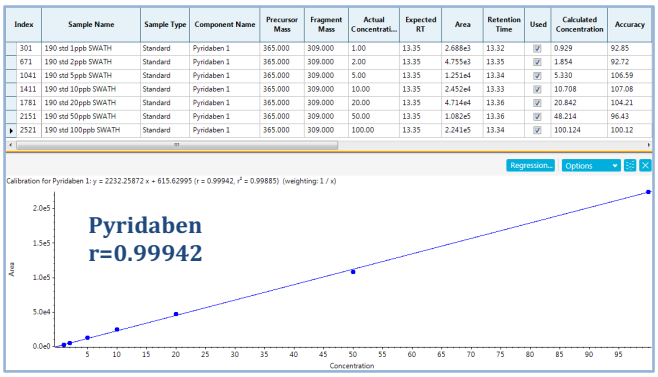


Figure 6 Quantitative linearity using pyridaben as an example

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	Kidney Bean	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-methyl	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 kresoxim-methyl 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

1. *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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