

ASMS 2013

TP-605

Simultaneous Targeted
and Non-targeted
Screening for Pesticides in
Vegetables by Accurate
Mass GC/Q-TOF MS

¹Fang Zhang; ¹Yinlong Guo;
²Chongtian Yu; ²Zhe Cao; ²Zhixu
Zhang

¹Shanghai insititue of organic
chemistry, Shanghai, China;
²Agilent Technologies(China) Co.,
Ltd., Shanghai, CHINA

Introduction

The monitoring of pesticide residues in food can be done by targeted and non-targeted analysis. There are few analytical instruments can simultaneously perform both analyses well. GC/Q-TOF MS provides sensitivity, resolution and accurate mass capabilities in TOF mode, and can be used for both targeted and non-targeted analysis of pesticides in complex food matrices. In this work, low levels of pesticides were spiked into apple, spinach and scallion matrices. The samples were analyzed by GC/Q-TOF to demonstrate the capabilities of instrument.

The targeted pesticide screening workflow by GC/Q-TOF MS was performed in three steps. First, a total of 187 pesticides standards were measured by GC/Q-TOF MS to create an accurate mass database, which contained the retention time and accurate masses of MS1 spectrum for each pesticide. Second, the spiked samples were analyzed by the GC/Q-TOF, and the data was analyzed using the Find by Formula (FbF) algorithm and the accurate mass database that had been developed at the first step. Third, the screening results were confirmed either by Q/q ratio of MS1 spectra or by accurate masses of MS2 product ions.

The non-targeted pesticide screening workflow by GC/Q-TOF MS was also performed in three steps. Steps 1 and 2 were performed using the MassHunter Unknown Analysis software. To eliminate matrix interferences and separate co-eluting components, the acquired data were first deconvoluted and components spectra were extracted. The deconvoluted components were then searched by NIST11 library or user library with RI index, the latter can strongly improve the accuracy of identification. The third step involved Fragment Formula Annotation (FFA) of the ions present in component spectra according to both their accurate mass as well as calculated possible formula for molecular ion generated from the library search results.

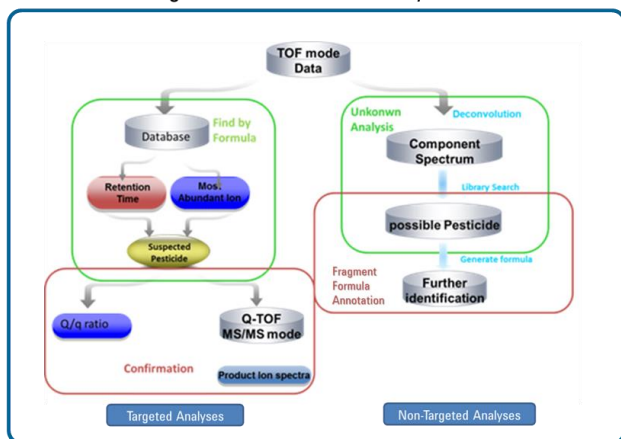


Figure 1. General workflow of targeted and non-targeted analyses

Experimental

Sample Preparation

20 g of each chopped vegetable or fruit was transferred into an 80 mL centrifuge tube and blended with 40 mL acetonitrile at a 15,000 rpm for 1 min. 5 g of sodium chloride was added during the blending. The homogenized sample was then centrifuged at 4,200 rpm for 5 min. The acetonitrile layer of 20 mL was transferred into a 100 mL rotary evaporator flask and concentrated to 1 mL at 40°C. The extract was cleaned up by Bond Elut Carbon/NH₂ cartridge (500 mg/500 mg, 6 mL), which was loaded with 2 cm anhydrous sodium sulfate and conditioned with 4 mL of acetonitrile/toluene (3:1). The cartridge was eluted with 25 mL of acetonitrile/toluene (3:1). The entire volume of effluent was collected and concentrated to 0.5 mL at 40°C. The residue was dissolved in n-hexane to make a 10 mL solution and different amount of pesticides (1 ng/mL to 100 ng/mL) was spiked into them.

Analytical Method

All experiment were performed using Agilent 7200 GC/Q-TOF MS shown in Figure 2. GC and MS condition are described in Table 1.



Figure 2. 7200 Series GC/Q-TOF system.

Results and Discussion

GC and MS conditions	
Column	DB-35MS UI, 30meter,0.25mm ID,0.25µm film
Injection volumn	1µl
Injection Mode	Splitless
Injector Temperature	250°C
Oven temperature program	80°C for 1min
	25°C/min to 170°C
Carrier gas	6°C/min to 300°C, hold 10min
	Helium at 1.2ml/min constant flow
transfer line temperature	300°C
Ionization mode	EI
Ion source temperature	250°C
Quadrupole temperature	150°C
TOF mass range	50 to 600 M/Z
Spectral acquisition rate	5Hz,collecting both in centroid and profile modes
collision gas	Nitrogen at 1.5ml/min
QTOF precursor ion resolution	medium at 1.4amu
QTOF product ion scan range	50 to 600 M/Z

Table 1. GC-MS conditions used in the study

Accurate Mass Database Creation

The accurate mass database creation is essential for target pesticide analysis workflow, and included several steps. First, multiple pesticides standards at relatively high concentration were divided into several groups, each contained pesticides well separated under the GC condition described in Table 1. Each group of pesticides was injected onto GC/Q-TOF to acquire the data in TOF mode. A total of 187 pesticides, such as organophosphate, organochlorine, organfluorine and pyrethroid insecticides, glyphosate herbicide, and azole fungicides, were collected and divided into 11 groups at 200 ppb. Before injection, TOF MS was calibrated by using PFTBA to achieve a mass accuracy of < 5 ppm. Second, few ions from the mass spectrum of each pesticide were selected to calculate their ion formula. The ion formulas were confirmed for the most abundant or characteristic ions and their exact mass (calculated) values were entered into a database. Third, the retention time (RT) of the pesticides was also entered into the database. Table 2 provides few examples of pesticides with accurate mass and RT.

###	Formula	Retention Time RT	Mass	Compound name	CAS number
C5	H10 N O	4.622	100.0762	Ethiolate	2941-55-1
C2	H6 O3 P	5.056	109.0055	Dichlorvos	62-73-7
C	H5 N O2 P	5.433	94.0058	Methamidophos	10265-92-6
C5	H10 N	5.79	84.0813	Nicotine	54-11-5
C3	H8 N	5.919	58.0657	Propamocarb	24579-73-5
C12	H10	6.164	154.0783	Biphenyl	92-52-4
C7	H3 Cl2 N	6.213	170.9643	Dichlobenil	1194-85-6
C2	H8 O4 P	6.637	127.016	Mevinphos	7786-34-7
C5	H5 Cl2 N2 O S	6.739	210.95	Etridiazole	2593-15-9
C3	H7 N O3 P	7.308	136.0164	Acephate	30560-19-1
C6	H Cl5	7.411	247.8521	Pentachlorobenzene	608-93-5
C12	H10 O	7.659	170.0732	o-Phenylphenol	90-43-7
C8	H9 O	7.814	121.0653	Isoprocarb	2631-40-5
C8	H5 F3 N3 O4	7.963	264.0232	Trifluralin	1582-09-8
C6	H7	8.023	79.0548	cis-1,2,3,6-Tetrahydrophthalimide	27813-21-4
C10	H9 F3 N3 O4	8.053	292.0545	Benfluralin	1861-40-1
C6	H11	8.516	83.0861	Cycloate	1134-23-2
C8	H9 O	8.535	121.0653	Fenobucarb	3766-81-2
C5	H Cl4	8.592	200.8832	Tecnazene	117-18-0

Table 2. Examples of pesticides in the accurate mass database

Targeted pesticides were screened using Find by Formula algorithm and the accurate mass database. Parameter optimization and results filtering should also be considered to avoid false negatives, and will be discussed later.

Confirmation

Figure 3 shows how to confirm tentatively identified pesticide using MassHunter Quantitative Analysis software by comparing Q/q ratio with that of the standard. High resolution mass spectra help to confirm the identity of the compound because of low matrix interference when using narrow extraction mass window (± 100 ppm). Figure 4 shows an example of the other confirmation procedure, that utilizes mass accuracy of each product ion generated in MS/MS mode.

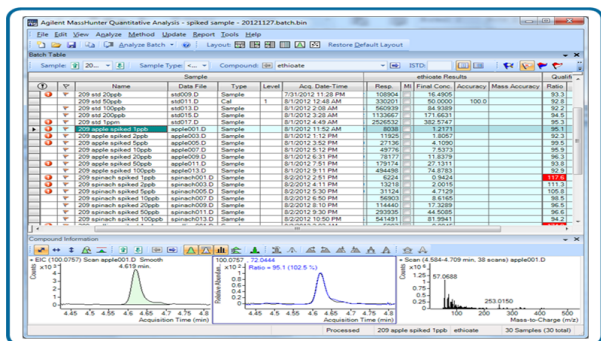


Figure 3. Confirmation using MassHunter Quantitative Analysis software

Results and Discussion

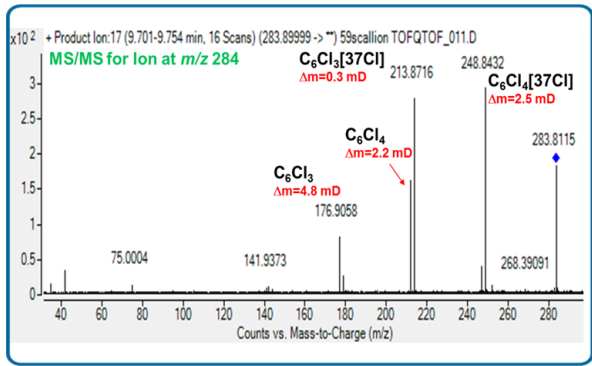


Figure 4. Product ion spectrum of hexachlorbenzene spiked in scallion at 10 ppb

Unknowns Analysis

Non-targeted analysis was performed in Unknowns Analysis where both chromatographic deconvolution and library search were simultaneously performed (Figure 5). Example of Fragment Formula Annotation workflow is shown on Figure 6.

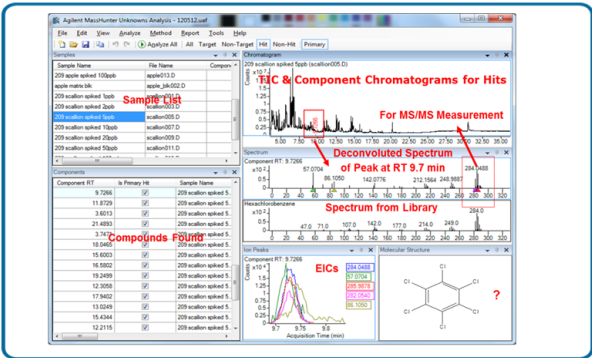


Figure 5. Unknowns Analysis software tool

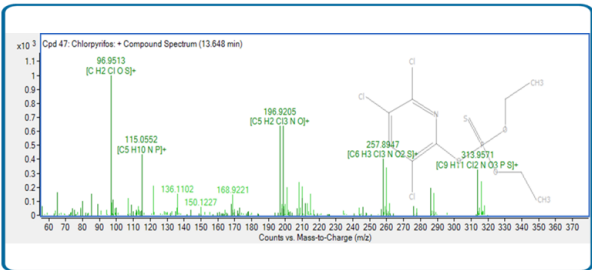


Figure 6. Fragment Formula Annotation of the deconvoluted spectrum of chlorpyrifos in scallion at 10ppb concentration

Sensitivity of Targeted Analysis

Figure 7 shows the number of detected pesticides spiked at three food matrices at different concentrations, that were screened using Find by Formula. False negatives can be avoided by setting up the retention time window to 0.25 min and mass deviation to 5 mDa. 155, 164 and 154 out of 187 pesticides have been confirmed in apple, spinach and scallion respectively at 5ng/ml spiked concentration.

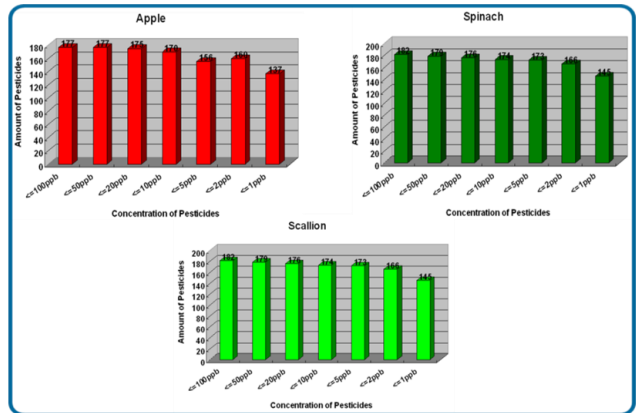


Figure 7. Screening results for three different matrices

Sensitivity of Non-targeted Analysis

Based on preliminary data, about a half of total pesticides used in this study could be identified with library search score higher than 50 at 20 ng/ml spiked concentration. The sensitivity of non-targeted analysis appears to be lower than that of targeted analysis. However, the sensitivity in full spectrum mode was higher than that of most other mass spectrometers in this mode.

Conclusions

- GC/Q-TOF demonstrated the simultaneous capability to perform targeted and non-targeted analyses of pesticides in complex matrices with high sensitivity and selectivity in TOF mode.
- Easy confirmation of tentatively identified pesticides by either Q/q ratio in TOF mode or mass accuracy of product ion in QTOF MS/MS mode was also demonstrated.
- Unknowns Analysis software and Fragment Formula Annotation are powerful tools for identification unknown compounds present in complex matrix.