Delivering the Right Results

Analysis of Fruit Commodities by GC-TOFMS and GCxGC-TOFMS Using QuEChERS Approach

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INTRODUCTION

The availability of fruit commodities from a wide variety of sources, ranging from local farm markets to large international suppliers, has increased the need for rapid and accurate screening for pesticides in fruit. Not all fruit commodities are brought to market under the strict guidelines of the United States Department of Agriculture. Determining whether or not the Environmental Protection Agency tolerances for various pesticides have been violated is important in the risk assessment for consumers in the United States. Unwashed fruit can be hazardous to those most vulnerable, such as children and infants. Rapid, sensitive, and accurate methods of pesticide detection are needed in order to meet the challenges presented by a multi-source supply of fruit commodities.

Fruit commodities contain thousands of analytes, making them an extremely difficult matrix for identification of pesticide residues. Current methodologies for pesticide detection incorporate complex, time-consuming sample cleanup techniques to eliminate much of the matrix interference prior to analysis by Gas Chromatography (GC). Selective GC detectors such as electron capture detector (ECD), nitrogen-phosphorous detector (NPD), and flame photometric detector (FPD) are often used. These detectors can provide good sensitivity and selectivity for some pesticide residues; however, they cannot provide unequivocal positive pesticide identifications. Interferences due to coelution with matrix components can cause quantitation bias. Mass spectrometers have become the detector of choice because they can provide positive identifications as well as good sensitivity. Due to the need for trace level analysis of pesticide residues, selected ion monitoring (SIM) modes are used instead of full scan mode. While this improves detection limits, the results are a significant loss of the data required for positive pesticide identification and analyses restricted to target pesticides. Even with SIM mode acquisition, interferences often cause uncertainty in quantitative pesticide determinations.

Time-of-Flight Mass Spectrometry (TOFMS) provides a valuable solution through its ability to acquire full range mass spectra without sacrificing speed or sensitivity. There is no need for SIM to enhance detection limits. TOFMS offers fast acquisition speeds (up to 500 full mass range spectra/second) and spectral continuity, allowing optimum performance of the mass spectral deconvolution algorithms incorporated into the LECO ChromaTOF® software, which add an extra dimension of qualitative analysis to complex samples.

The fast acquisition rates of TOFMS also support the use of comprehensive two-dimensional gas chromatography (GCxGC). GCxGC provides increased peak capacity and resolution, which is very beneficial when analyzing samples with complex matrices. In addition, GCxGC provides an increase in analyte detectability through the cryo-focusing effects of thermal modulation. The need for a fast detector is due to the extremely narrow peak widths typical of thermally modulated GCxGC peaks, usually on the order of 50 to 100 ms wide. When MS is combined with GCxGC, only TOF can achieve the necessary acquisition rates.

This application highlights the increased peak capacity of comprehensive two-dimensional gas chromatography, which can minimize the need for extensive sample cleanup methods. GC and GCxGC TOFMS analysis of fruit commodities is shown, with the emphasis being on GCxGC-TOFMS . QuEChERS sample extraction was used to facilitate pesticide screening.

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY OVERVIEW

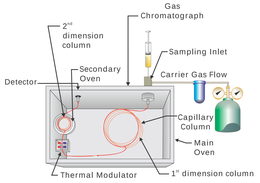
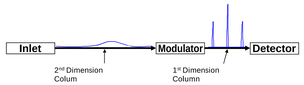
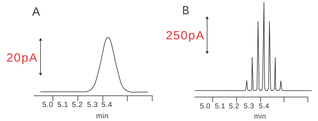


Diagram showing GCxGC-TOFMS Components.



Simplified example of modulated GCxGC flow. Notice the cryo-focusing effects of thermal modulation.



Example of the detectability enhancement provided by thermally modulated GCxGC. Through conservation of mass, the response of the wide and unfocussed peak on the left is improved by the cryo-focusing effects of thermal modulation.

STANDARDS/SAMPLES

Organochlorine (OC), organonitrogen (ON), and organophosphorous (OP) pesticide standards were purchased from Restek (Bellefonte, PA.) A complete list of these pesticides is shown below in Table I. These pesticide standard mixtures were spiked into various fruit commodities and the QuEChERS extraction approach was utilized. Extracts were then analyzed to evaluate the ability of the GCxGC-TOFMS method to detect and identify all standards within the complex fruit matrix.

OC Pesticides	ON Pesticides	OP Pesticides
Alpha-BHC	EPTC	Dichlorvos
gamma-BHC (Lindane)	Butylate	Mevinphos
Beta-BHC	Vernolate	Demeton O
Delta-BHC	Refulate	Demeton S
Heptachlor	Eptam	Ethoprophos
Aldrin	Tebuthiuron	Naled
Heptachlor epoxide	Molinate	Phorate
Trans-Chlordane	Propachlor	Diazinon
alpha-Chlordane	Cycloate	Disulfoton
4,4'DDE	Chlorpropham	Methyl parathion
Endosulfan I	Trifluralin	Ronnel
Dieldrin	Atraton	Fenthion
Endrin	Prometon	Chlorpyrifos
4,4'-DDD	Simazine	Trichloronate
Endosulfan II	Atrazine	Merphos
4,4'-DDT	Propazine	Tetrachlorvinphos
Endrin aldehyde	Propyzamide	Prothiofos
Methoxychlor	Terbacil	Fensulfothion
Endosulfan sulfate	Simetryn	Sulprofos
Endrin ketone	Metribuzin	Azinphos methyl
	Ametryn	Coumaphos
	Alachlor	TIPP
	Prometryne	Sulfotepp
	Terbutryn	Monocrotophos
	Bromacil	Dimethoate
	Metolchlor	Malathion
	Cyanazine	Parathion
	Triadimefon	EPN
	MGK-264	
	Diphenamid	
	Butachlor	
	Napropamide	
	Tricyclazole	
	Norflurazon	
	Hexazinone	
	Fenarimol	
	Flurridone	

Table 1. The QuEChERS extraction approach was used to prepare fruits for both GC and GCxGC-TOFMS analysis. This extraction was performed by weighing 10 g of blended fruit into a 50 mL centrifuge tube, adding 10 mL acetonitrile (shake for 1 minute), adding buffer salts (4 g magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate), shaking for additional minute, centrifuging to separate solids, and removing portion for analysis. Prior to extraction, the fruits were spiked with the above pesticides to yield a concentration representing approximately 3 ng/gram (3 ppb) of commodity. Resulting extracts were analyzed by GCxGC-TOFMS under the conditions shown below.

EXPERIMENTAL CONDITIONS

GCxGC Gas Chromatograph
Agilent 7890 w/LECO thermal modulator and secondary oven
Injection: 1 µL (GERSTEL MPS2), splitless at 250°C
Carrier: Helium at 1.5 ml/min, corrected constant flow
Primary Oven: 85°C hold 0.2 min, 6°C/min to 335°C
Secondary Column: BPX-50, 1.4 m x 0.10 mm x 0.1 µm
Secondary Oven: +5°C offset from main oven
Modulator Offset: 20°C (relative to the secondary oven)
Modulator Period: 5 s
Transfer Line Temp: 320°C

MS: LECO Pegasus 4D
Saved Mass Range: 35 to 400 m/z
Acquisition Rate: 20 spectra/s
Source Temperature: 200°C

GC Gas Chromatograph
Agilent 6890
Injection: 1 µL (GERSTEL MPS2), splitless at 250°C
Carrier: Helium at 1.5 ml/min, corrected constant flow
Column: Rxi-5 Sil MS 15 m x 0.25 mm x 0.25 µm
Oven: 90°C Hold 1 min, 25°C/min to 340°C
Transfer Line Temp: 320°C

MS: LECO Pegasus HT
Saved Mass Range: 45 to 450 m/z
Acquisition Rate: 20 spectra/s
Source Temperature: 225°C

RESULTS

The GCxGC-TOFMS total ion contour plot for the pesticide spiked QuEChERS extract of apple commodity is shown below in Figure 1. There were over 1,800 analytes detected in this extract, including 85 spiked pesticides. The black dots represent peak markers for the pesticide and matrix analytes, which were placed by the Automated Peak Find algorithm of the ChromaTOF software. Figure 2 illustrates the same total ion contour plot as Figure 1, but only pesticide library hits are indicated. Figure 3 is a GCxGC total ion contour plot of another fruit commodity: pesticide spiked QuEChERS blueberry jam extract. Over 1,300 analytes were detected in the blueberry jam extract, including 85 spiked pesticides. Figure 4 is the same total ion contour plot as in Figure 3, but as in Figure 2, only pesticide library hits in the blueberry jam are indicated.

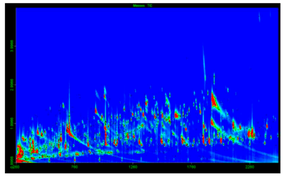


Figure 1. The total ion contour plot for the pesticide spiked QuEChERS extract of apple commodity including matrix analytes.

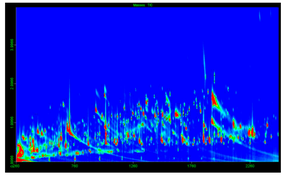


Figure 2. The same total ion contour plot as in Figure 1 with only pesticide library hits indicated.

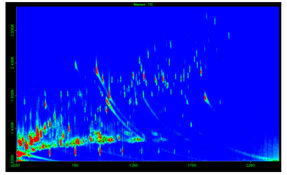


Figure 3. The total ion contour plot for the pesticide spiked QuEChERS extract of blueberry jam commodity.

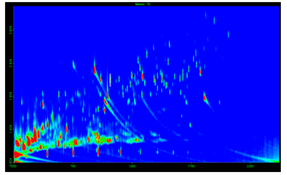


Figure 4. The same total ion contour plot as in Figure 3 with only pesticide library hits indicated.

A portion of a GC-TOFMS total ion chromatogram, showing a mixture of the pesticide standards listed in Table I, is shown in Figure 5. Although it is a difficult separation on a one-dimensional system, the Pegasus HT with ChromaTOF software effectively separated Demeton-S and Dimethoate. In Figure 6 the GCxGC contour plot displays the chromatographic separation of Demeton-S and Dimethoate on a LECO Pegasus 4D. This is a two-dimensional plane with the x-axis representing the chromatographic separation on the Rtx-5 Sil MS column and the y-axis representing the chromatographic separation on the BPX-50 column. The intensity of the peaks is shown using a color scale, with blue representing baseline and red representing the most intense peaks. The black peak markers indicate pesticide standards identified by the mass spectral Deconvolution algorithms incorporated into the LECO ChromaTOF software. It is important to note that analytes that are vertically aligned have a high probability of coeluting in a one-dimensional GC separation. In Figure 6 the benefit of second-dimension separation of Demeton-S and Dimethoate is demonstrated.

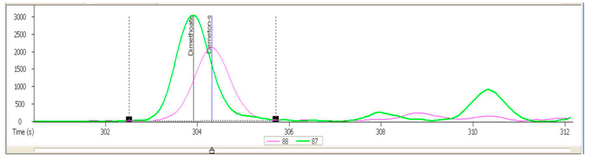


Figure 5. GC first-dimension linear extracted Ion chromatogram of the Quant Masses contour plot showing effective separation of Demeton-S (88) and Dimethoate (87) pesticide residues.

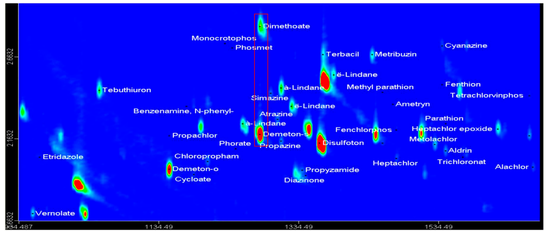


Figure 6. A portion of the LECO Pegasus 4D GCxGC total ion contour plot highlighting the region in which pesticides would have coeluted in a traditional one-dimensional GC analysis. The value of second-dimension GCxGC separation is evident in this figure.

Figure 7 and Figure 8 below illustrate another useful way of displaying GCxGC-TOFMS data; the 3D surface plot. Figure 7 shows a view of the 3D surface plot tilted to show only the separation along the x-axis (primary column separation). Notice how Demeton-S and Dimethoate coelute on the primary column. Figure 8 on the right shows a rotated view of the 3D surface plot displaying the secondary column separation. In this view, it is apparent that these two analytes are baseline resolved on the secondary column separation.

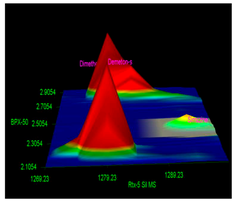


Figure 7. 3D surface plot of Demeton-S, Dimethoate and Simazine. Plot orientation along x-axis.

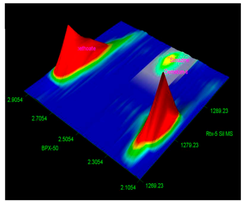


Figure 8. Plot rotated to show secondary column separation.

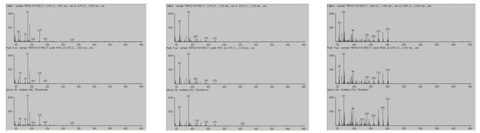


Figure 9. Caliper (raw), Peak True (Deconvoluted), and Pesticide Library matched spectra for Demeton-S, Dimethoate, and Simazine.

CONCLUSIONS

GCxGC-TOFMS was shown to be a valuable tool for determining pesticide residues in the complex matrix of fruit commodities. GCxGC separation provided the necessary peak capacity and resolution to eliminate matrix interference from the pesticide residues of interest. The full mass range acquisition capability of the TOFMS provides positive confirmation of not only targeted pesticides, but also adds the ability to determine new and emerging contaminants. Unlike scanning instruments, TOFMS provides the ability to acquire full range mass spectra without sacrificing speed or sensitivity. An added benefit of acquiring full mass range data is that since archived data will contain full mass range spectral information, it is possible to re-evaluate past data for other pesticides in samples that may have degraded or been discarded.