Delivering the Right Results

Analysis of Pesticide Residues in JonaGold Apples Using QuEChERS Approach and Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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INTRODUCTION

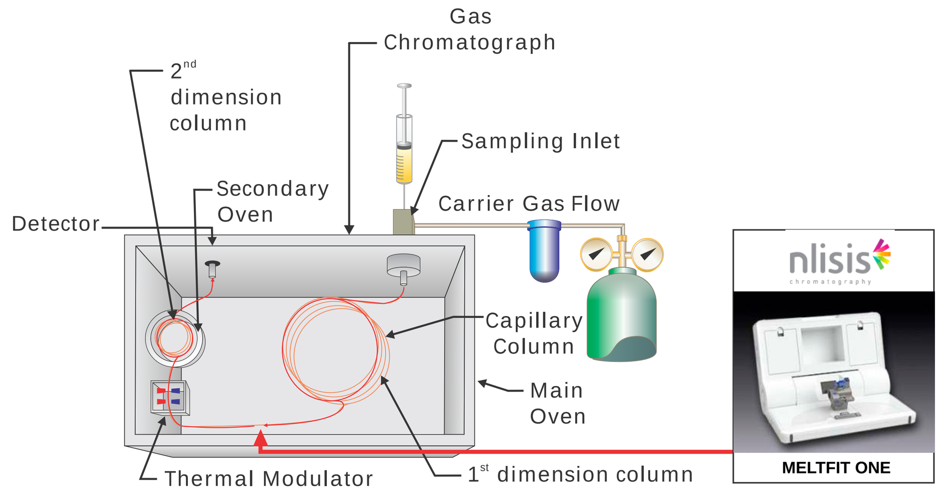
Screening and quantitation of pesticide residues in fruits and vegetables is of utmost importance for public health and safety concerns. These commodities can contain hundreds-to-thousands of analytes, making them extremely difficult for the screening and accurate quantitation of pesticide residues. Many current methodologies incorporate the use of complex, time-consuming sample cleanup techniques to eliminate much of the matrix interference prior to analysis by GC-MS. 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.

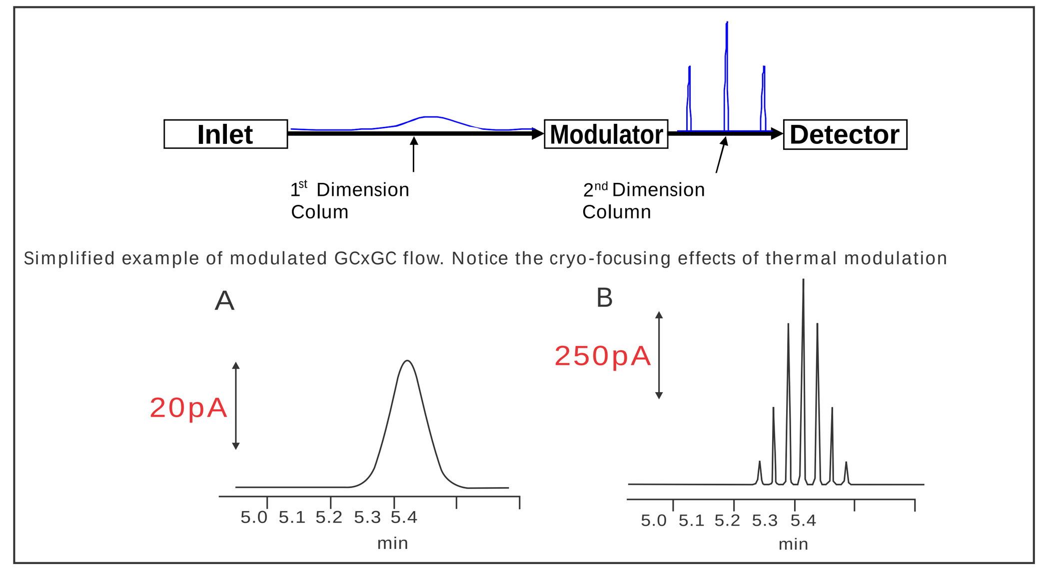
Time-of-Flight Mass Spectrometry (TOFMS) provides a valuable solution through its ability to acquire full range mass spectra without sacrificing speed or sensitivity. There is no need for SIM to enhance detection limits. TOFMS offers fast acquisition speeds (up to 500 full mass range spectra/second) 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.

As sample loads increase, analysts are forced to find ways to increase the speed of sample preparation and analysis while maintaining high quality of the analytical results.

This poster shows the successful use of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) for effective screening and quantitation of pesticides in apples. JonaGold apples from Southwest Michigan were analyzed for potential pesticide residues following preparation by a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction approach. A cryogen-free thermal modulator was utilized for these analyses.

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY OVERVIEW


Gas Chromatograph
2nd dimension column
Sampling Inlet
Secondary Oven
Carrier Gas Flow
Detector
Capillary Column
Main Oven
Thermal Modulator
1st dimension column
nlisis
MELTFIT ONE


Inlet
Modulator
Detector
1st Dimension Colum
2nd Dimension Column
Simplified example of modulated GCxGC flow. Notice the cryo-focusing effects of thermal modulation
A
B
20pA
250pA
5.0 5.1 5.2 5.3 5.4
min

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.

SAMPLES



The QuEChERS extraction approach was used to prepare skins from JonaGold apples for GCxGC-TOFMS analysis. This extraction was performed in the following manner: A 10g sample of homogenized apple skins was placed into a 50 mL centrifuge tube. A 10mL aliquot of acetonitrile was added and the mixture was shaken for 1 minute. Buffer salts (4g magnesium sulfate, 1g sodium chloride, 1g trisodium citrate dihydrate, and 0.5g disodium hydrogen citrate sesquihydrate) were added prior to shaking for an additional minute. The mixture was placed in a centrifuge to separate solids and a portion of the liquid was removed for analysis. The extracts were analyzed by GCxGC-TOFMS and the mass spectra obtained were searched against a pesticide reference library to facilitate rapid screening.

EXPERIMENTAL CONDITIONS

Gas Chromatograph
Agilent 6890 w/LECO thermal Consumable-Free (CF) modulator and secondary oven
Injection: 1 µL, Splitless (60 s Purge Time) @ 250°C
Carrier: Helium at 1.5 ml/min, corrected constant flow
Primary Column: Rxi-5 Sil MS, 15 m x 0.25 mm x 0.25 µm (Restek Corporation, Bellefonte, PA)
Primary Oven: 90°C hold 1 min, 5°C/min to 300°C, hold 10 min
Secondary Column: Rtx-200, 1.25 m x 0.18 mm x 0.18 µm (Restek Corporation, Bellefonte, PA)
Secondary Oven: +25°C offset from primary oven
Modulator Offset: 15ºC (relative to the secondary oven)
Modulation Period: 5 s
Transfer Line Temp: 300°C
Note: The nlisis Meltfit One was used to connect the first and second dimension columns.

MS: LECO Pegasus® 4D
Saved Mass Range: 45-550 m/z
Acquisition Rate: 100 spectra/s
Source Temperature: 225ºC



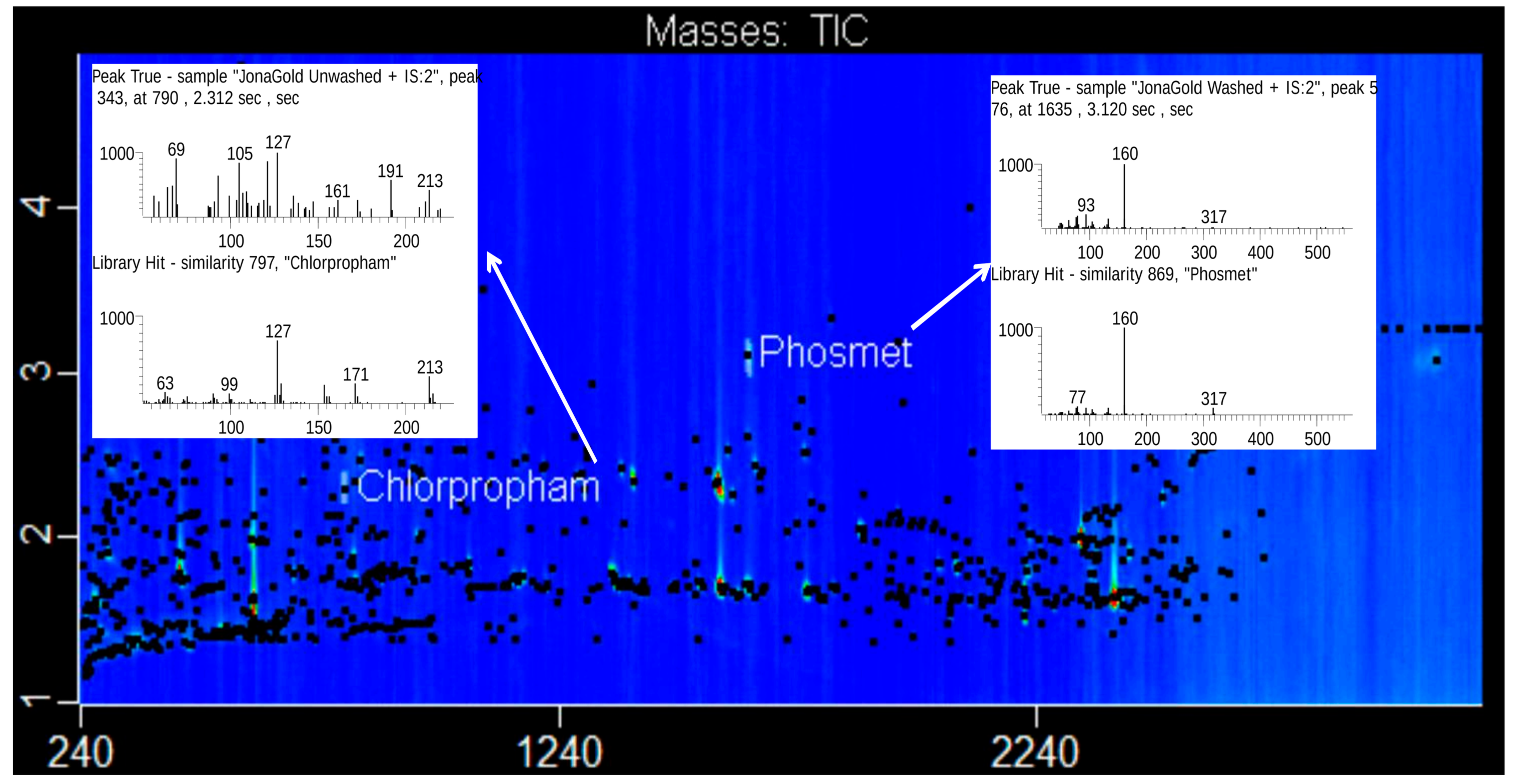

Masses: TIC
Peak True - sample "JonaGold Unwashed + IS:2", peak
343, at 790 , 2.312 sec , sec
Library Hit - similarity 797, "Chlorpropham"
Peak True - sample "JonaGold Washed + IS:2", peak 5
76, at 1635 , 3.120 sec , sec
Library Hit - similarity 869, "Phosmet"
Phosmet
Chlorpropham
240
1240
2240

Figure 1. GCxGC total ion contour plot for the JonaGold apple skin extract. The Phosmet and Chlorpropham peaks are labeled and their resulting mass spectra and library matches are shown.

RESULTS

After GCxGC-TOFMS screening determined the presence of Phosmet and Chlorpropham, quantitative determinations were carried out by preparing calibration curves with pure standards of the two pesticides. Triphenyl phosphate was used as an internal standard. Standards were analyzed at concentrations ranging from 60 to 600 ng/mL (ppb). The calibration curves for Phosmet and Chlorpropham exhibited r^2 values of 0.997 and 0.999, respectively. The calibration curves and 3D surface plots for Phosmet and Chlorpropham are shown below in Figures 2 through 5.

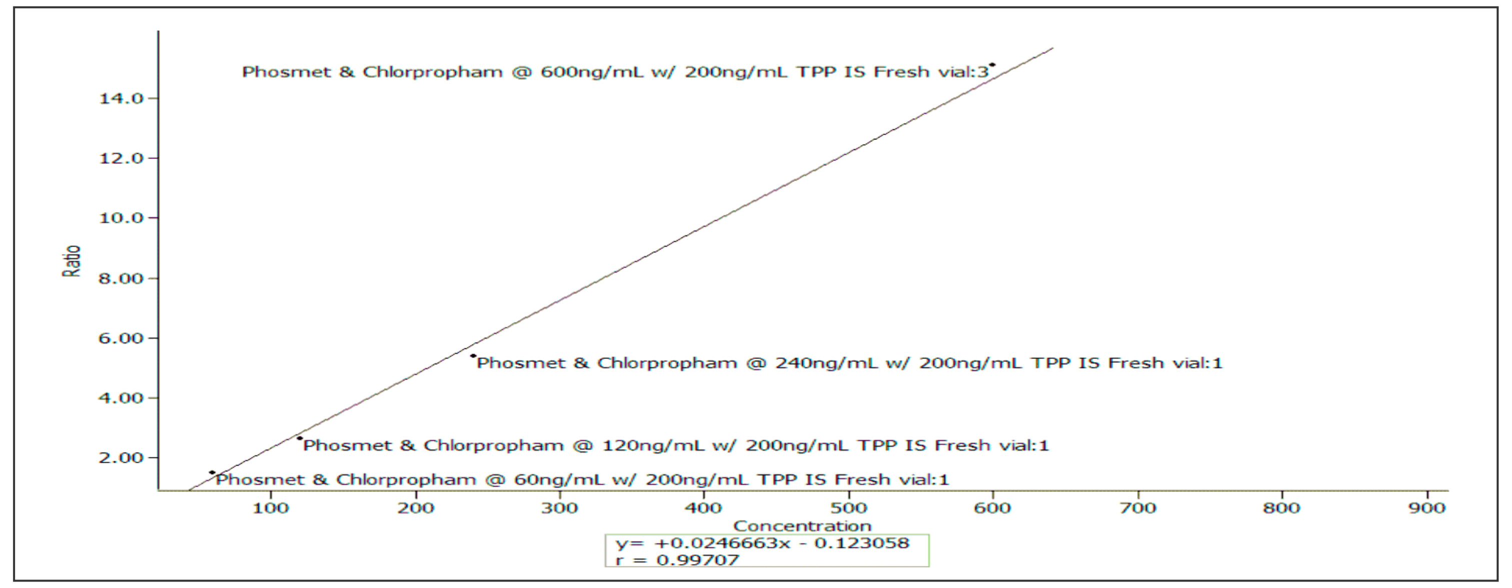

Phosmet & Chlorpropham @ 600ng/mL w/ 200ng/mL TPP IS Fresh vial:3
Phosmet & Chlorpropham @ 240ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Phosmet & Chlorpropham @ 120ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Phosmet & Chlorpropham @ 60ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Ratio
Concentration
y= +0.0246663x - 0.123058
r = 0.99707

Figure 2. Calibration curve for Phosmet ranging from 60 to 600 ng/mL .

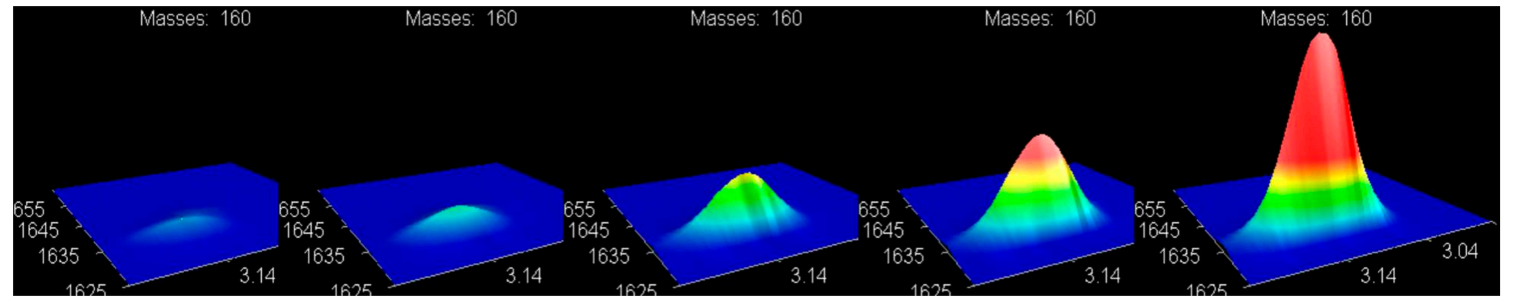

Masses: 160

Figure 3. 3D extracted ion surface plots for Phosmet standards ranging from 60 to 600 ng/mL.

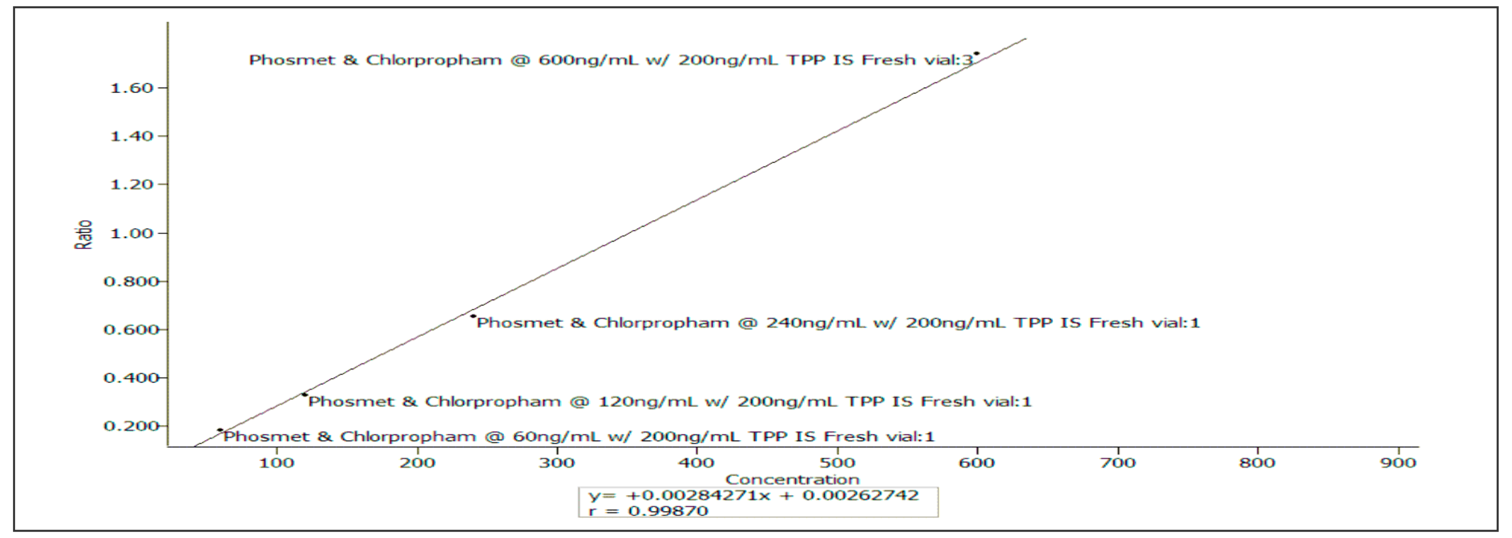

Phosmet & Chlorpropham @ 600ng/mL w/ 200ng/mL TPP IS Fresh vial:3
Phosmet & Chlorpropham @ 240ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Phosmet & Chlorpropham @ 120ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Phosmet & Chlorpropham @ 60ng/mL w/ 200ng/mL TPP IS Fresh vial:1
Ratio
Concentration
y= +0.00284271x + 0.00262742
r = 0.99870

Figure 4. Calibration curve for Chlorpropham ranging from 60 to 600 ng/mL.

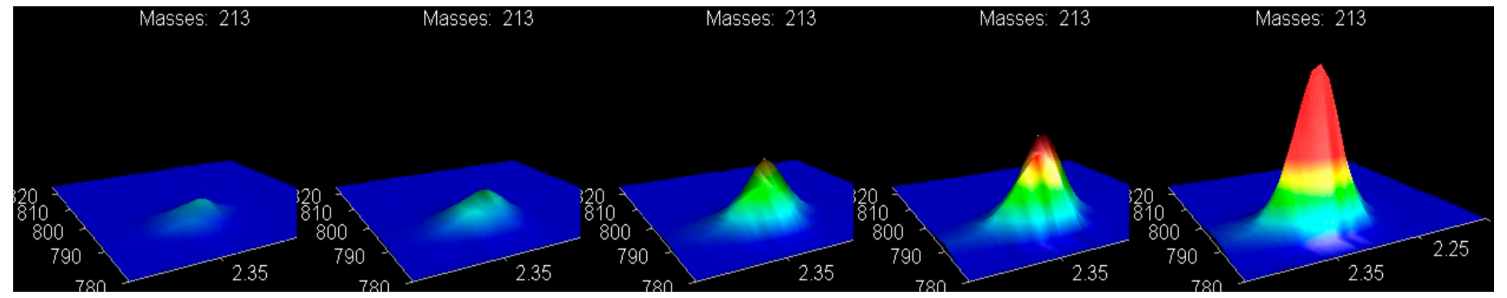

Masses: 213

Figure 5. 3D extracted ion surface plots for Chlorpropham standards ranging from 60 to 600 ng/mL.

The plot shown below in Figure 6 highlights the chromatographic separation on the “Y” axis of the GCxGC contour plot. If this were a one-dimensional separation, Phosmet would have coeluted with the other analytes which are aligned vertically on the contour plot. The interfering compounds are sugars and fatty acids from the apple matrix.

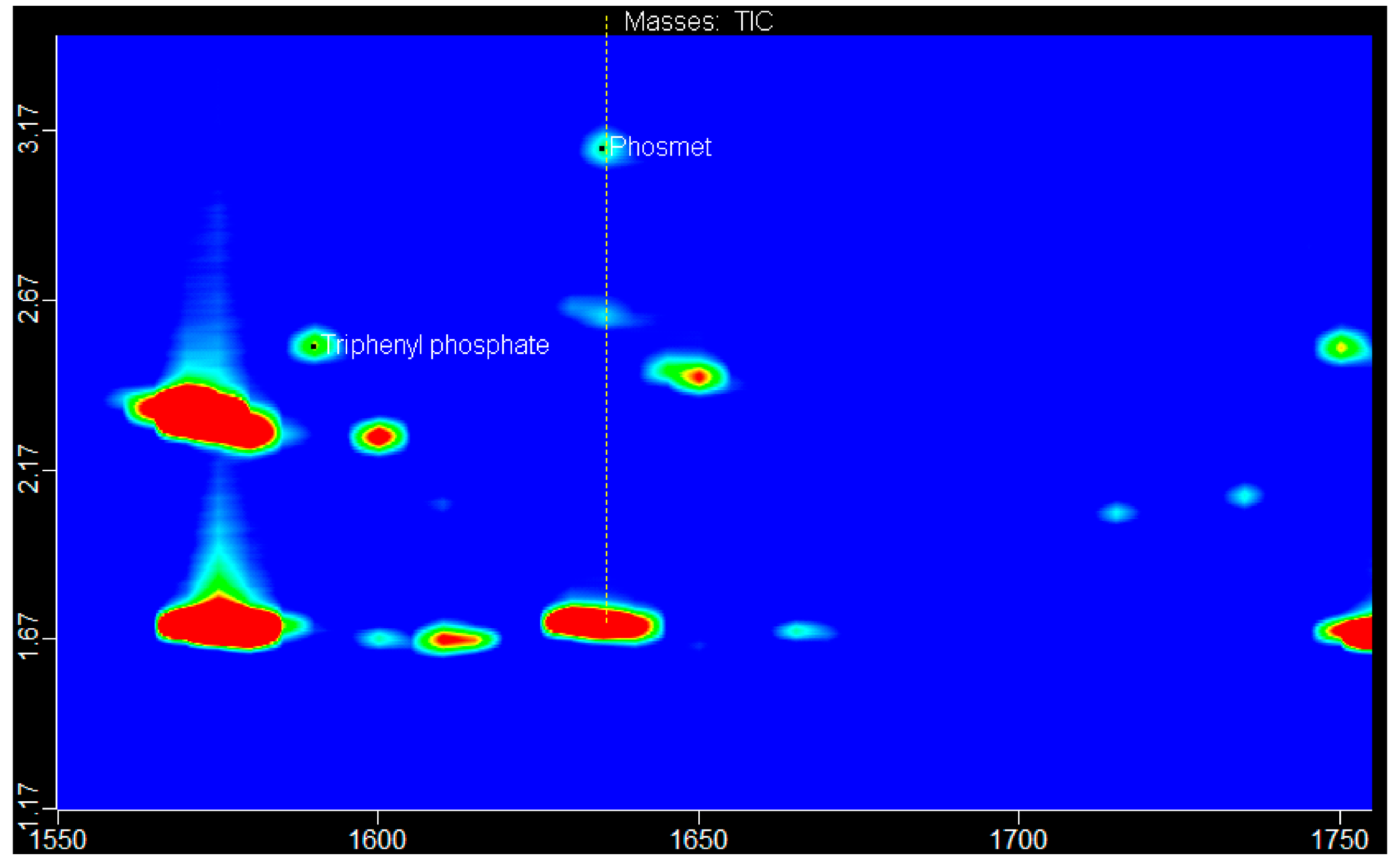

Masses: TIC
Phosmet
Triphenyl phosphate
1550
1600
1650
1700
1750

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

Analyte	Unwashed	Washed
Chlorpropham	0.1	n.d.
Phosmet	18	13

Results reported in ng/gram

CONCLUSIONS

This application shows the utility of GCxGC-TOFMS when combined with a rapid QuEChERS extraction approach. Compounds such as sugars and fatty acids, which can be left behind even after sample cleanup, often lead to chromatographic interferences in a one-dimensional gas chromatography separation. In this example, the enhanced peak capacity of the GCxGC separation is critical to removing the interferences which can ultimately lead to quantitation bias.

In addition to the increase in chromatographic resolution, the use of a Time-of-Flight Mass Spectrometer provides the ability to acquire full mass range spectra without sacrificing speed or sensitivity. This is beneficial for detecting not only target pesticides, but also new and emerging contaminants.