Rapid Pesticide Screening and Identification Using the High Resolution All Ions MS/MS Technique

Application Note

Food Safety

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Abstract

The All Ions MS/MS technique was used to rapidly screen, quantify, and identify pesticides in food matrices. This analytical method uses a high resolution Time-of-Flight (TOF) or Quadrupole-TOF mass spectrometer to rapidly analyze samples and generate quantitative information for target compounds.

To validate the effectiveness of this new methodology in complex matrices and at low concentrations, three different food matrices were spiked with a comprehensive pesticide standard and were analyzed using the All Ions MS/MS technique. This technique helps eliminate false positives, and has the speed and accuracy to significantly improve the productivity of pesticide screening and quantitation.

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Introduction

Consumers continue to be concerned about the public health impacts of pesticide residues in food. Especially with the expanding global trade in food products, detection of pesticides has pushed forward regulations such as European Commission (EC) Regulation 396/2005[1] and 40 CFR Part 180 in the United States[2]. However, pesticide residues continue to be a quality issue in food. For example, in 2009, there were 173 alert notifications in the European Union[3] related to pesticide residues entered in the Rapid Alert System for Food and Feed (RASFF)[4].

State-of-the-art pesticide screening requires the consideration of more than 1,000 pesticides and their metabolites. Of these, approximately 600 to 800 compounds are included in routine monitoring programs and approximately 150 are typically detected in food commodities. The analytical method used for pesticide screening needs to be validated and must comply with quality standards laid down in the SANCO 12495/2011[5] guidelines.

The most widely-used sample preparation method for pesticide screening is the Quick Easy Cheap Effective Rugged Safe (QuEChERS)[6] for sample preparation. This is widely accepted as a universal extraction procedure. The analytical method of choice is liquid chromatographic separation followed by detection using a triple quadrupole mass spectrometer. While the use of Multiple Reaction Monitoring (MRM) is the most sensitive technique for the analysis of pesticides in complex matrices, this technique has drawbacks. The MRM technique requires thorough method development, constant maintenance, need for expert knowledge, and days of tedious work to enter MRM transitions for all compounds and estimate their retention times. In addition, identification of a compound requires the acquisition of two or more product ions with a constant ratio, hence limiting the number of compounds that can be acquired in one single analytical method. Most importantly, the ability to re-interrogate data for new and unexpected residues without reacquisition is not possible.

Agilent Technologies has developed the All Ions MS/MS technique for the screening and identification of pesticides in a single analytical run. The technique uses an accurate mass LC/TOF or Q-TOF and features easy setup of the acquisition method, verification of the pesticide compounds using MS/MS spectral libraries, and chromatographic coelution of the precursor and product ions, and rapid development of a quantitative method including product ions as qualifiers. The user can quickly verify the identities of compounds with high resolution accurate mass data, and then create a quantitative method for the compounds of interest in minutes. Potential false positives can be eliminated by assessing the quality of the product ion chromatograms. With this technique, hundreds of pesticides can be quantified in a single analysis.

Experimental

Sample preparation

Tomato, avocado, and lemon samples obtained from a local grocery store were prepared according to the citrate buffered QuEChERS method, using Agilent BondElut QuEChERS kits (p/n 5982-5650). Sample extracts were cleaned up using Agilent BondElut QuEChERS EN dispersive SPE tubes (p/n 5982-5256). Before the cleanup, only the lemon extracts were neutralized by adding sodium hydroxide solution. After the cleanup, all samples were acidified using 5% formic acid in acetonitrile to improve the stability of the target pesticides. Samples were spiked at three relevant concentrations with a comprehensive pesticide standard containing more than 190 pesticides.

System configuration

Separation was carried out using an Agilent 1290 Infinity UHPLC System consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C).

An Agilent 6540 UHD Q-TOF was operated with MassHunter Acquisition Software rev. B.05.01 using 2 GHz extended dynamic range mode with an acquisition rate of three scans/s in MS and two discrete collision energies for the All Ions MS/MS method. The use of precursor scan with any collision energies and MS/MS scans with two collision energies resulted in alternating spectra with a low energy channel containing the precursor ion and two high energy channels containing the precursor and product ions.

Chromatographic conditions		
UHPLC column	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 150 mm, 1.8 µm	
Column temperature	30 °C	
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol	
Gradient program	Min	% B
	0	5
	0.2	5
	2.2	30
	10.5	100
	13.0	100
	13.5	5
Stop time	15 minutes	
Flow rate	0.50 mL/min	

Results and Discussion

Data analysis was performed using the MassHunter Qualitative Analysis Software (B.06.00) using the Find by Formula (FbF) algorithm. FbF uses a formula and determines if a compound with that formula is present in the high resolution mass spec data. The FbF algorithm has been updated to support the All Ions MS/MS technique. The mass peaks in the low energy channel were first searched against the Pesticide Personal Compound Database and Library (PCDL) (B.04.01) for compounds that had the same *m/z* values. A set of putative identifications was then compiled. Figure 1 shows that carbendazim was confidently identified from the samples by using the FbF algorithm.

For the identified compounds, the fragment ions in the MS/MS spectra from the PCDL were compared to the ions in the high energy channel to confirm the presence of the correct fragments (Figure 2).

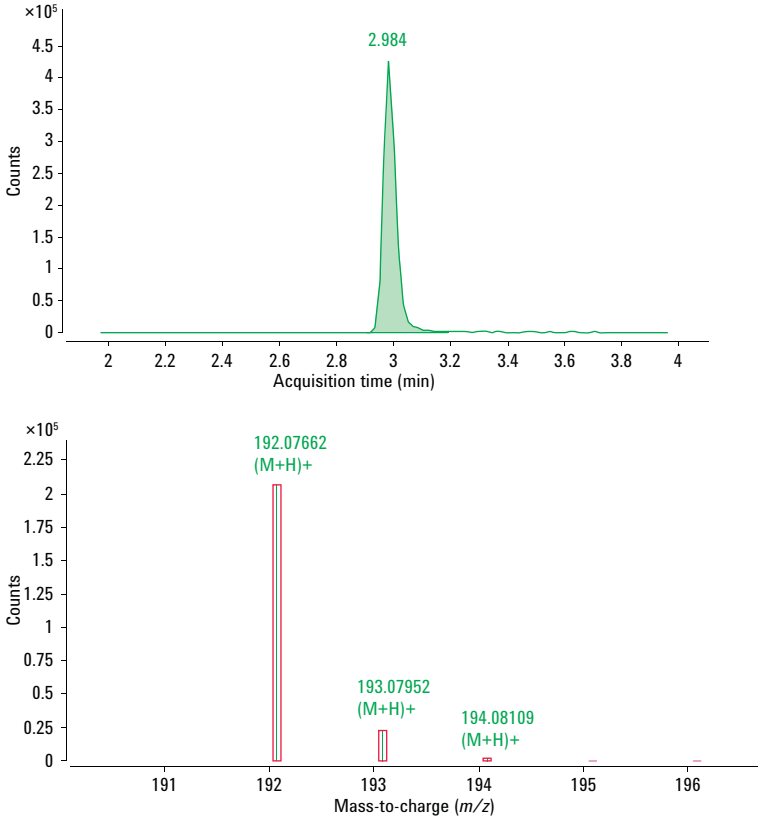


Figure 1. Identification of carbendazim using the Find by Formula (FbF) algorithm.

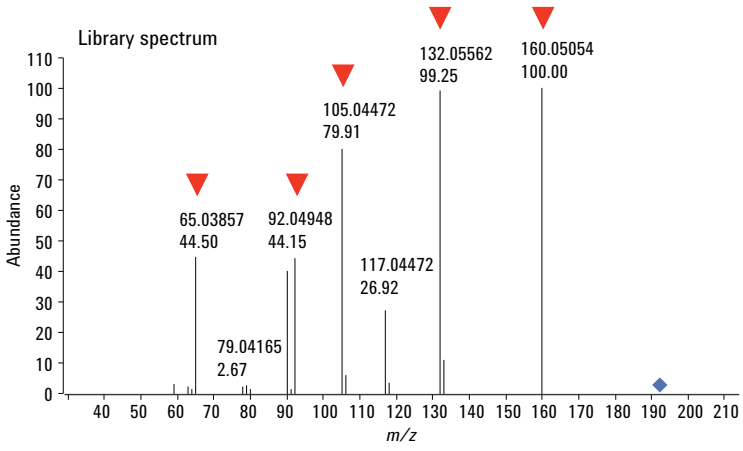


Figure 2. High energy (40 eV) spectrum of carbendazim.

Both the precursors and product ions were extracted as chromatograms (Figure 3A) and evaluated using a coelution score. The coelution score was derived from a technique similar to UV chromatography's Peak Purity[7], where the software calculates a number that takes into account multiple factors, such as abundance, peak shape (symmetry), peak width, and retention time. The scores were plotted and were easily viewable as a coelution plot (Figure 3B).

The software analysis reported that carbendazim was found with five valid qualifier fragments from the PCDL MS/MS spectrum (Figure 4).

A parallel analysis was performed for all other putative precursor ions found in the low energy channel and were searched against the 741 compounds with MS/MS spectra in the Pesticides PCDL.

As a validation study, 190 pesticides were spiked into three different matrices (tomato, lemon, and avocado) at increasing concentration levels. Table 1 shows the results for 50 important and frequently found pesticides. Most of the compounds were found in the lowest levels of all matrices and their presence was verified by at least one additional fragment ion (as indicated by green cells). In some cases, the compounds were found by the FbF algorithm, but the fragment ions were not qualified (yellow cells).

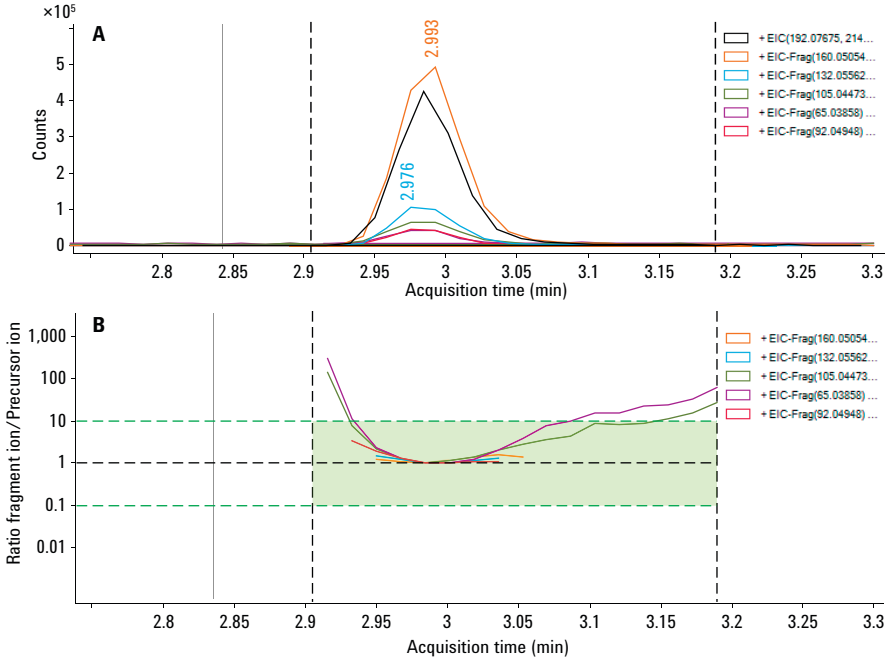


Figure 3. Overlaid ion chromatograms (A) and calculated coelution plot (B).

ID Techniques Applied

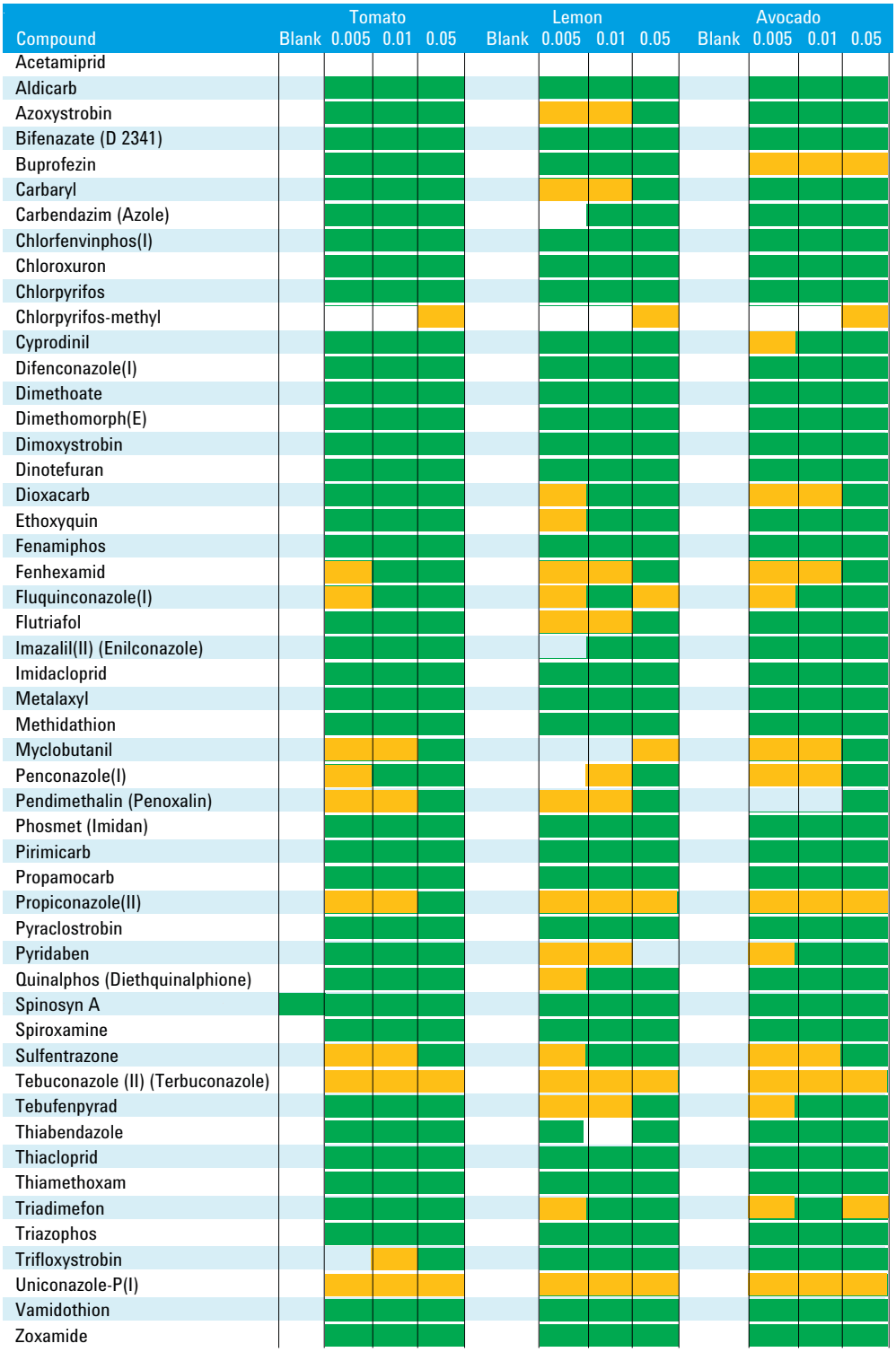
FBF

Name	Species	Formula	m/z	Score	Diff (ppm)	CAS ID	Best	Mass	RT	Hits (DB)
Carbendazim	(M+H)+ (M+Na)+	C9 H9 N3 O2	192.07662 214.05833	99.37	0.67	10605-21-7		191.06935	2.984	

Coelution Score	m/z	SNR	CE	Flags(Fls)	Height	Compound Name
99	132.05562	273.6	40	Qualified	104535	Carbendazim
98.8	160.05054	2286.2	20	Qualified	493827.6	Carbendazim
97.7	105.04473	7.9	40	Qualified	62636.4	Carbendazim
97.3	92.04948	45.7	40	Qualified	44105.7	Carbendazim
97.2	65.03858	11.8	40	Qualified	36319.8	Carbendazim

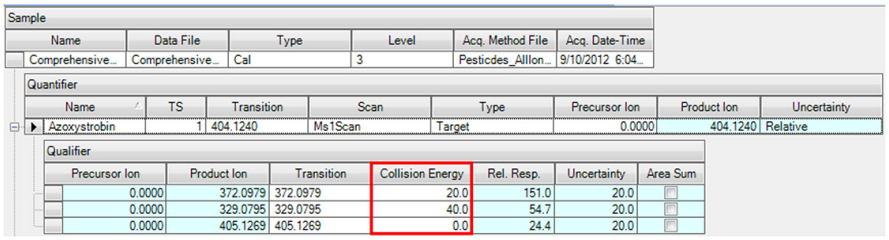
Figure 4. Compound identification results.

Table 1. Compound confirmation results.



The data were then exported to MassHunter Quantitative Analysis rev. B.05.02 using a Compound Exchange Format (CEF) file. The CEF file contained information necessary to rapidly set up a quantitative processing method including compound name, retention time, precursor ion, fragment ions (to create qualifiers required by regulation), collision energy, and relative abundances. The Quantitative Analysis software automatically selected the major precursor and fragment ions with a relative abundance above 10% for each compound, saving tedious manual labor and time. Fragment ions with different collision energies were selected and used by the software (Figure 5).

The Quantitative Analysis software extracted chromatograms for the quantifier (target), qualifier ions, and isotopic cluster of the pseudo molecular ion. The isotope pattern can be confirmed by viewing an overlay with the theoretical pattern (Figure 6).



Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Comprehensive...	Comprehensive...	Cal	3	Pesticdes_Alllon...	9/10/2012 6:04...

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	Uncertainty
Azoxystrobin	1	404.1240	Ms1Scan	Target	0.0000	404.1240	Relative

Qualifier

Precursor Ion	Product Ion	Transition	Collision Energy	Rel. Resp.	Uncertainty	Area Sum
0.0000	372.0979	372.0979	20.0	151.0	20.0	
0.0000	329.0795	329.0795	40.0	54.7	20.0	
0.0000	405.1269	405.1269	0.0	24.4	20.0	

Figure 5. Quantitative method setup with multiple collision energies.

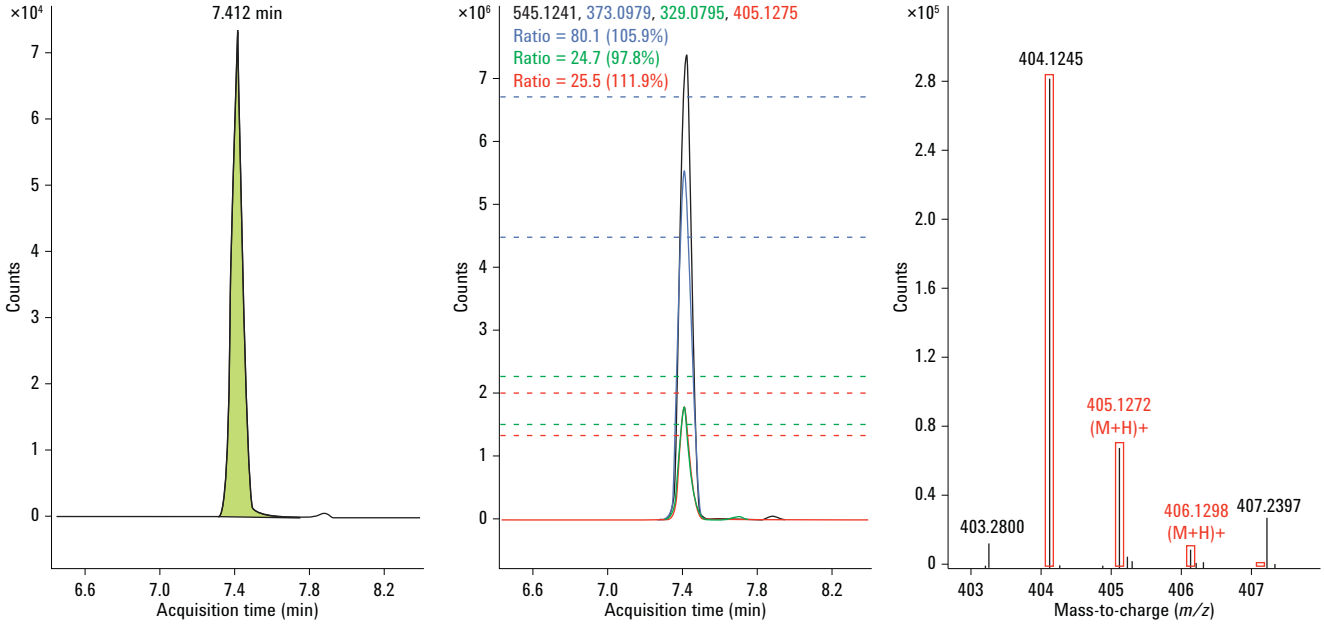


Figure 6. Compound information with quantifier, qualifiers, and isotopic cluster.

After analyzing a large batch of samples, it is possible to easily review the results by sample and compound in the Compounds-at-a-glance module. Figure 7 shows chromatograms with compounds, annotated in red, that were outside of user defined outlier limits.

Conclusions

Samples containing pesticides spiked into food matrices were used to rapidly generate a quantitative data processing method for a Q-TOF mass spectrometer. The All Ions MS/MS technique was used to screen for the presence of compounds prior to the creation of the quantitative method. With the All Ion MS/MS technique, analysts benefit by gaining increased productivity as they do not have to enter hundreds of compound names or select specific product ions. In addition, large batches of sample results can easily be reviewed at a time. The data can also be re-interrogated at a later time by adding more compounds to the screen using an expanded PCDL. The Quantitative Analysis software provides an added level of confidence in the results by providing the interface to view quantifier and qualifier ions, including scoring the quality of identifications with accurate mass metrics.

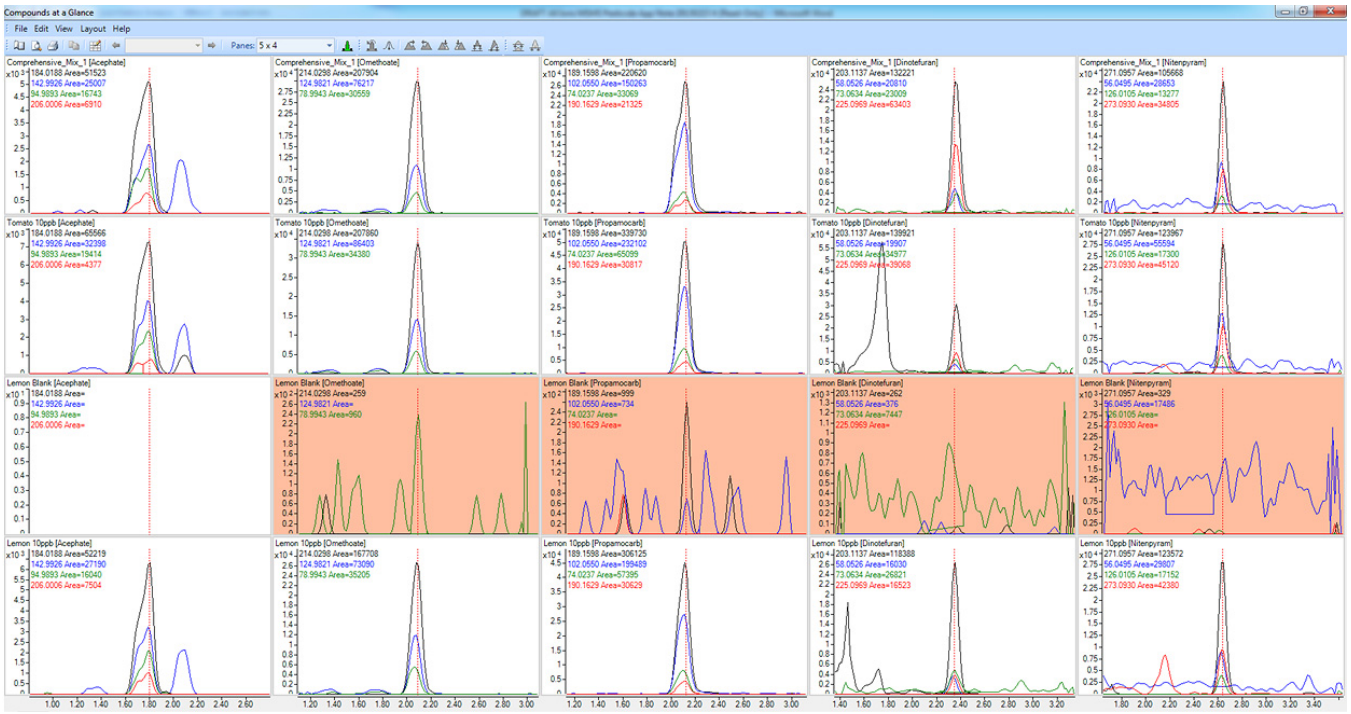


Figure 7. Compounds-at-a-glance view.

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Published in the USA, May 6, 2013
5991-2295EN

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