Quantitative and Qualitative Confirmation of Pesticides in Beet Extract Using High Resolution Accurate Mass (HRAM) Mass Spectrometry

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Introduction

The demand for quick and simple analysis of large numbers of samples in agriculture analysis is growing year by year. Throughout the world, pesticides are used to control pests that are harmful to crops, humans, and animals. These substances can pose a significant health threat and therefore, need to be accurately detected at the lowest levels requested by governmental authorities, typically at low part per billion (ppb) or low part per trillion (ppt) levels. Traditionally, triple stage quadrupole mass spectrometers (MS-MS) have been used by the food industries for the identification and quantitation of these residues. The introduction of the Thermo Scientific™ Q Exactive™ Focus benchtop mass spectrometer provides high-resolution, accurate mass (HRAM) to unequivocally identify compounds without time-consuming MS-MS optimization. The results of this unique solution are improved sensitivity and precision, as well as unmatched throughput. Mass spectrometric detection with HRAM technology using full scan experiments, or variable data independent acquisition (vDIA), can detect as many analytes as necessary in combination with screening for an untargeted approach, using only one chromatographic run and no targeted lists. The Q Exactive Focus benchtop mass spectrometer with the proven power of the Thermo Scientific™ Orbitrap™ mass analyzer and a novel software application for unified quantitative, confirmation, and screening data processing, fulfills these demands with higher confidence and precision than a triple stage quadrupole.

Methods

Sample Preparation: Beet extracts were prepared for analysis by using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, which is a sample preparation procedure used to extract pesticides from food. The QuEChERS extracts were obtained from the California Department of Food and Agriculture (CDFA). For the QuEChERS extraction, 15 g of homogenized sample and 15 mL of acetonitrile were used. Then, 200 µL of final QuEChERS extract, 300 µL of acetonitrile, and 500 µL of water were transferred into an autosampler vial, spiked with 20 µL of the pesticides standard, and mixed well. A mixture of 400+ pesticides with similar starting concentrations was used to make the standard calibration curve in neat matrix plus spiking calibration in beets matrix to determine if there is ion suppression.

Liquid Chromatography: Chromatographic analysis was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system, which consists of a column warmer, an auto sampler, and a high-pressure pump. The chromatographic conditions were as follows:

Column:	Thermo Scientific™ Accucore™ aQ column (100 × 2.1 mm, 2.6 µm particle size)
Mobile Phase A:	Water with 0.1% formic acid and 5 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 5 mM ammonium formate
Flow Rate:	300 µL/min
Column Temp.:	40° C
Sample Inj. Vol.:	5 µL

Gradient:

Time (min)	%A	%B
0.00	98%	2%
0.5	98%	2%
2.00	60%	40%
20.00	0%	95%
22.00	0%	95%
22.10	98%	2%
25.00	98%	2%

Mass Spec Conditions:	Full MS Scan	vDIA
Mass Range:	100 to 1000	100 – 205 195 – 305 295 – 405 395 – 505 495 – 1000
AGC:	1e6	1e6
Ion Mode:	mix mode	mix mode
Resolution:	70,000	17,500
Ion Source:	Heated Electrospray	Heated Electrospray
Spray Voltage:	3500v	2500v
Capillary Temp:	325° C	325° C
Sheath Gas:	35	35
Aux Gas:	10	10
Vaporizer Temp:	350° C	350° C
HCD:		33ev

No Optimization of mass transitions and collision energies for each compound was needed to performed this analysis like that of traditional triple quadrupoles.

Results

Data processing was carried out with Thermo Scientific™ TraceFinder™ software for quantitation, confirmation, and screening workflows. A new HRAM MS/MS Spectra Library which contains over 1,700 compounds and over 7,000 MS/MS spectra all collected at 140,000 resolution (FWHM at *m/z* 200) and at 5 different energies. Specificity of analysis was achieved by applying a mass window of 5 ppm to the theoretical mass of the analytes. All analytes gave very good linear response in the calibration range from 0.01 to 0.1 ng/mL depending on starting concentration in mixture (Figures 1, 2, 3), and the quantification data showed good reproducibility and good recovery rates while the usage of fragment ions was used to confirm the compound as well as the spectra library as a third confirmation to support the confirmation of the compound.

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FIGURE 1. Showcasing Boscalid at 0.5 ppb calibration curve plot in beets with R^2, quantitation peak, fragment ion matching, and spectra library scoring.

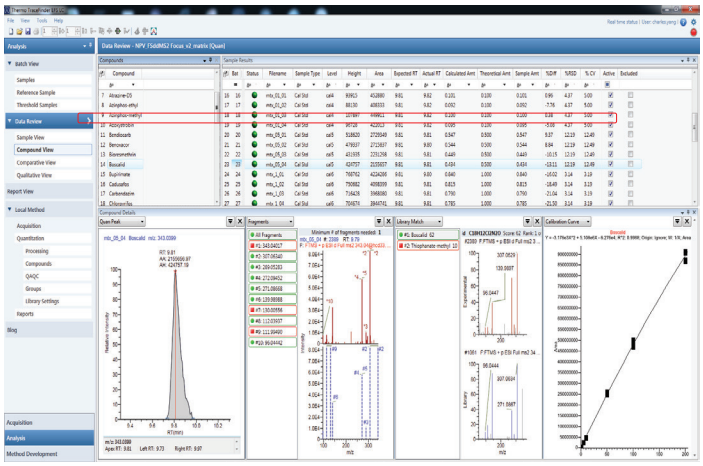


FIGURE 2. Showcasing Cyazofamid at 5 ppb with calibration curve, R^2, fragment matching, quantitation peak, and spectra library matching.

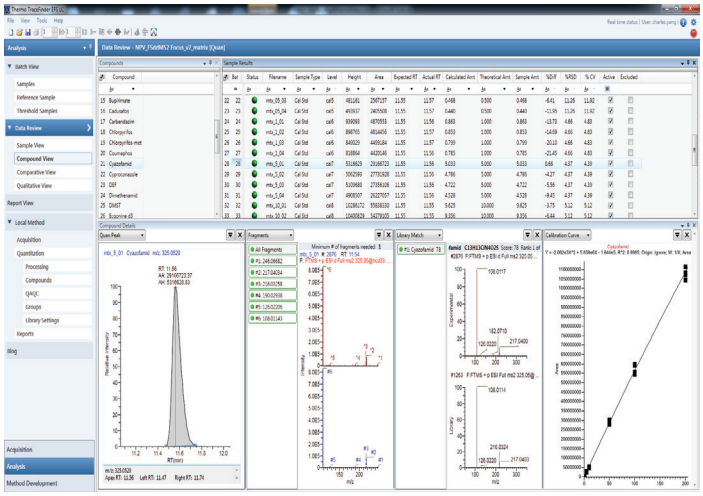
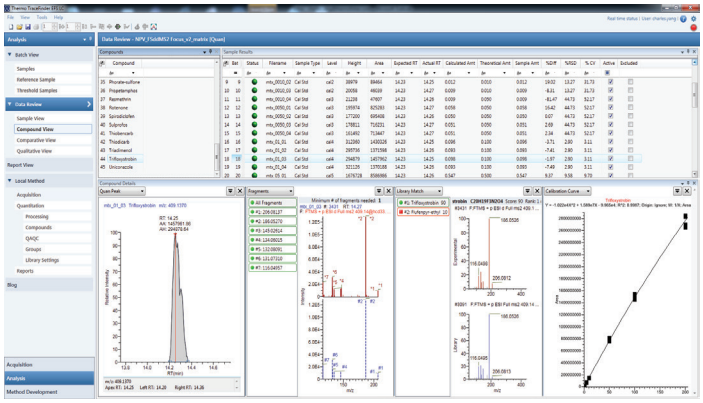


FIGURE 3. Showcasing Trifloxystrobin at 0.1ppb calibration curve R^2, quantitation peak, fragment ion matching, and spectra library scoring.



In addition to the targeted quantitation, we also did targeted screened and unknown screening within the same software using exact mass as identification criteria in both cases. Confirmation of identity was achieved by automated matching of the given elemental composition with the isotopic pattern of the determined signal. Additional criteria was to use the HRAM MS/MS Spectra Library which contains of up to five fragment ions, spectra library match, and internet database search via ChemSpider (for unknown screening) (Figures 4, 5, 6). The remaining signals not assigned are automatically occurred as unknowns, which were screened against ChemSpider (of selected databases) to generate a list of unknown screen possibilities. After identification of unknown compound within the TraceFinder software, we can quickly look for the occurrences of the analyte in other matrices through the new heat map visualization which can quickly be sorted for quick data review (Figure 7) where possible contamination or illegal spraying of chemicals where used. The unknown screen yielded additional identifications of analytes without additional analytical effort. Elemental compositions or ChemSpider searching were able to assign most of the unknown signals, leading the path into a versatile and easy-to-do general unknown screening (Figures 8 and 9).

FIGURE 4. Targeted and unknown screening approach in TraceFinder SW, simplified.

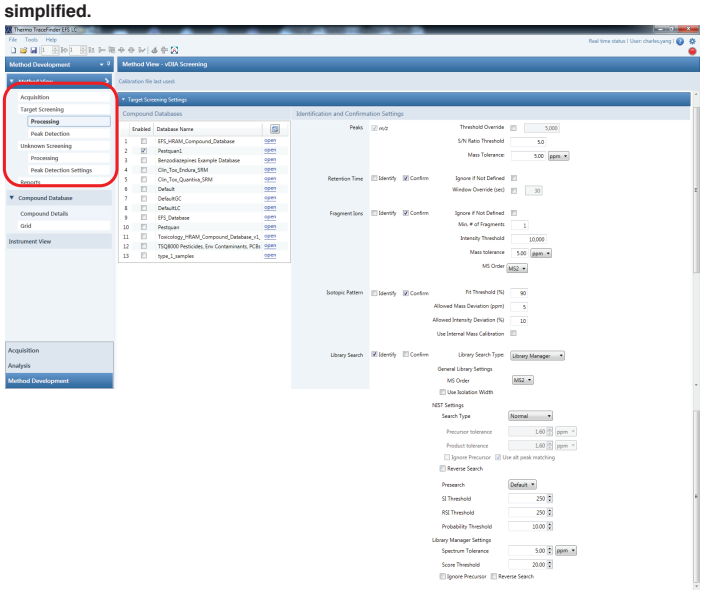


FIGURE 5. Selection of Unknown workflow is activated by checking a box in Targeted Workflow.

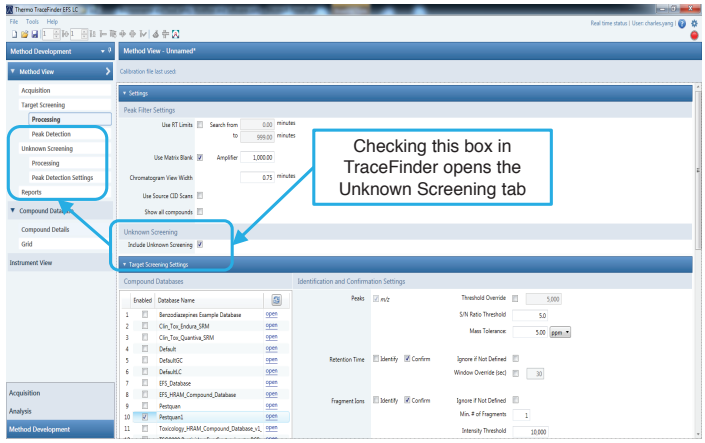


FIGURE 6. Unknown Screening workflow to set up ChemSpider searching.

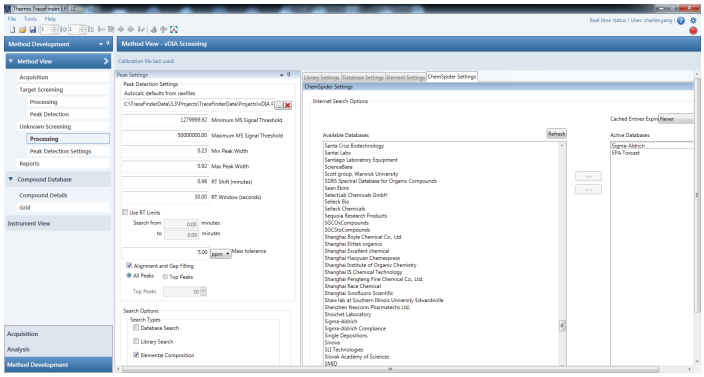


FIGURE 7. Unknown Screening results quickly reviewed through heat map, showing incurrences of the compounds within the different samples.

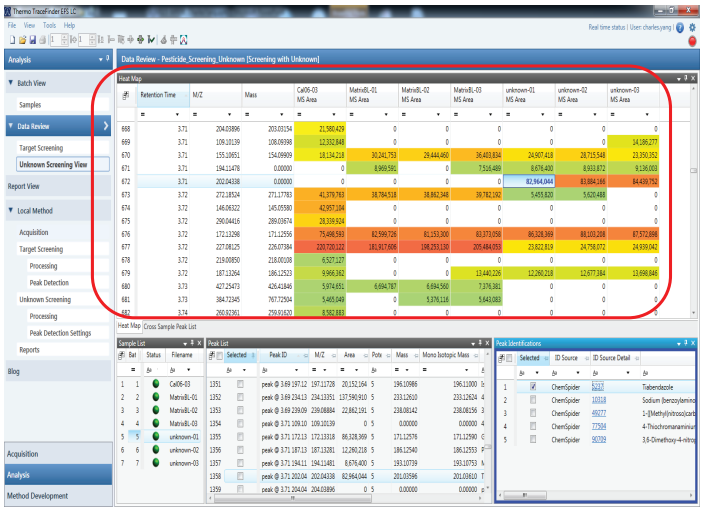
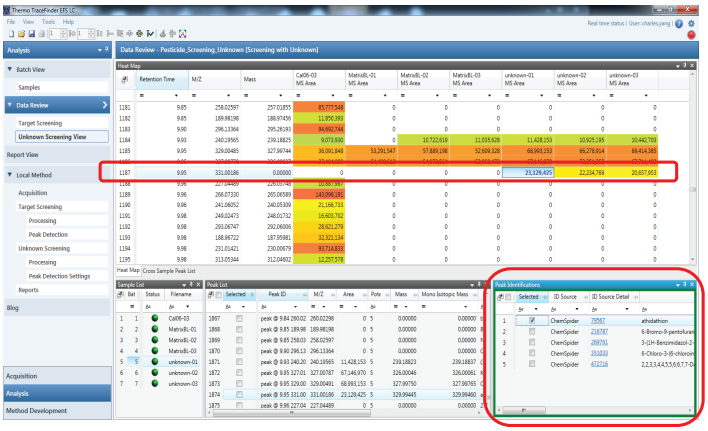


FIGURE 8. Unknown Screening view with ChemSpider search result with a possible hit of anthidiathion (insecticide) which was not present in other samples except for the unknown samples.



Conclusion

TraceFinder software coupled with the Q Exactive Focus benchtop Orbitrap mass spectrometer provided easy access to full quantitative, confirmation, and screening data in one package. Introduction to TraceFinder SW show cases the need to have an all-in-one software to complete the full workflow from quantitation to non-target and unknown screening. The ability to do unknown searching led to the identification of a number of untargeted compounds, and through the ability of online searching through ChemSpider, we were able to identify unknown compounds. In this case, the finding of drepamon, anthidiathion, and thiabendazole in the unknown matrix samples shows the need to quickly move to newer HRAM technologies to help determine what we are not seeing with a triple stage quadrupole system.

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