Utilization of High Resolution LC-MS for Screening and Quantitative Analysis of Pesticides in Food Matrix using a Q Exactive Bench Top Orbitrap Platform

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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 the governmental authorities typically at low part per billion (ppb) or low part per trillion (ppt)levels. Traditionally, triple quadropoles has been used by the food industries for the identification and quantitation of these residues. The introduction of the Q Exactive provides high resolution accurate mass 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 Full scan data dependent ms/ms with a targeted list can deliver the ability to detect as many analytes as necessary in combination with screening for an unlimited number of compounds in a targeted list as well as untargeted approach, using only one chromatographic run. A bench top Q Exactive with the proven power of the Orbitrap mass analyzer and a novel software application for unified quantitative, confirmation and screening data processing fulfills these demands with higher confidence and precision.

Methods

Sample Preparation

Green Bell Peppers were prepared for analysis by using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, which is a sample preparation procedure used to extract pesticides from food. The QuEChERS extracts were obtained from 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 60 pesticides with different starting concentration was also provided by CDFA to make the standard calibration curve in neat matrix plus spiking calibration in bell pepper matrix to determine if there are ion suppression.

Liquid Chromatography

Chromatographic analysis was performed using the Thermo Scientific Accela 1250 U-HPLC system. The autosampler was an Open Accela EQuan Max HTC-Pal Autosampler (CTC Analytics, Zwingen, Switzerland). The chromatographic conditions were as follows:
Column: Thermo Scientific Hypersil GOLD aQ C18 column (100 x 2.1 mm, 1.9 µm particle size)
Mobile Phase A: Water with 0.1% formic acid and 10 mM ammonium formate
Mobile Phase B: Methanol with 0.1% formic acid and 10 mM ammonium formate
Flow Rate: 300 µL/min
Column Temperature: 40 °C
Sample Injection Volume: 5 µL

Gradient:

Time 0.00 min 98%A, 2%B

Time 0.25 min 70%A, 30%B

Time 10.0 min 0%A, 100%B

Time 12.5 min 0%A, 100%B

Time 13.0 min 98%A, 2%B

Time 18.0 min 98%A, 2%B

Mass Spec Conditions

Full MS Scan (Targeted List)
Mass Range -120 to 1000
Positive ion mode
High Resolution: 75,000
Heated ElectroSpray Ion Source
- Spray Voltage 3800v
- Capillary Temp 295ºC
- Sheath Gas: 32
- Aux Gas: 7
- Vap. Temp 295ºC

Full Scan MS Data Dependent MS/MS (Targeted List)
Mass Range – 120 to 1000
Positive ion mode
Resolution: 35,000 (Data Dependent), 75,000 (Full Scan)
Heated ElectroSpray Ion Source

Optimization of mass transitions and collision energies for each compound was not performed as full ms scan was used on a high resolution accurate mass spectrometer. Table 1 shows the list of targeted 60 pesticides analyzed.

Results

Data processing was carried out with Thermo Scientific ExactFinder software for Quantitation, Confirmation and Screening workflows. Specificity of analysis was achieved by applying a mass window of 5ppm to the theoretical mass of the analytes. All analytes gave very good linear response in the calibration range (0.01 to 0.1 ng/mL depending on starting concentration in mixture) and didn't show any interference with other analytes or matrix components (see Fig. 1, 2 and 3), the quantification data showed good reproducibility and good recovery rates.

FIGURE 1. ExactFinder displaying Boscalid Calibration Curve Plot of Matrix vs Neat, R^2, List of Compounds, Chromatogram and Delta ppm

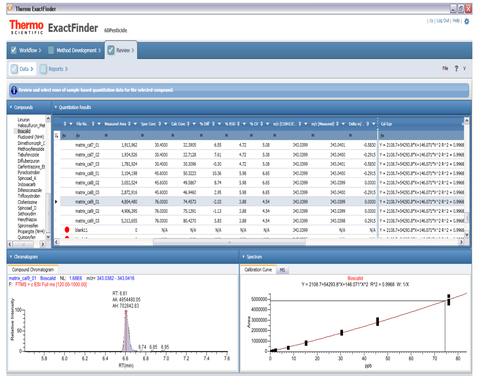


FIGURE 2. ExactFinder displaying Diuron Calibration Curve Plot of Matrix vs Neat, R^2, List of Compounds, Chromatogram and Delta ppm

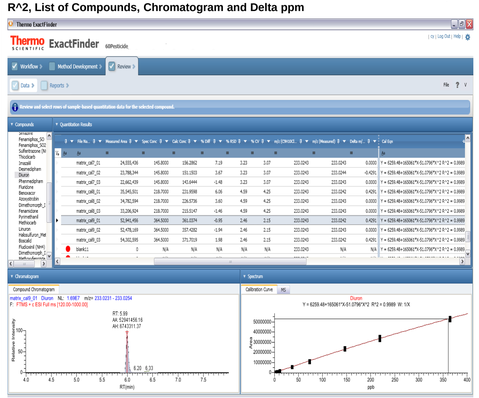
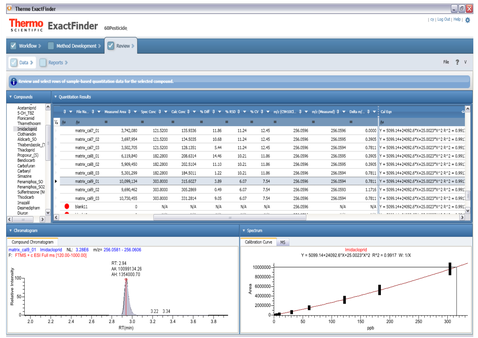


FIGURE 3. ExactFinder displaying Imidacloprid Calibration Curve Plot of Matrix vs Neat, R^2, List of Compounds, Chromatogram, Delta ppm



In addition to the quantitation a targeted and unknown screening was applied, using exact mass and retention time as identification criteria in the targeted screen. Confirmation of identity was achieved by automated matching of the given elemental composition with the isotopic pattern of the determined signal. Additional criteria, unused here, are occurrence of up to 5 fragment ions, library spectra match and internet database search via Chemspider. (Figure 4 and 5)
The remaining signals automatically occurred as unknowns, which again were screened against a larger compound list as unknown screen. For all signals elemental compositions were calculated on base of the isotopic distribution after defining a list of elements to be used for this calculation..

FIGURE 4. Targeted Screening hit of Difenoconzole with MS/MS Spectra Library Matching (see lower right, top spectra theoretical, lower ms/ms actual)

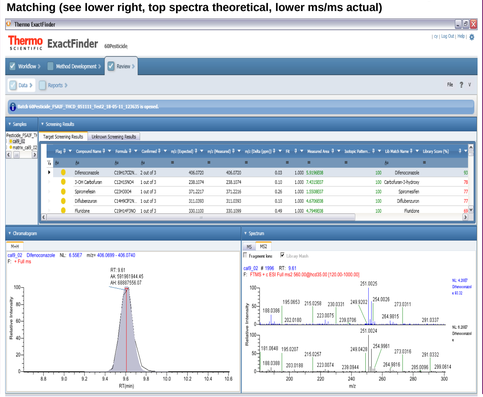
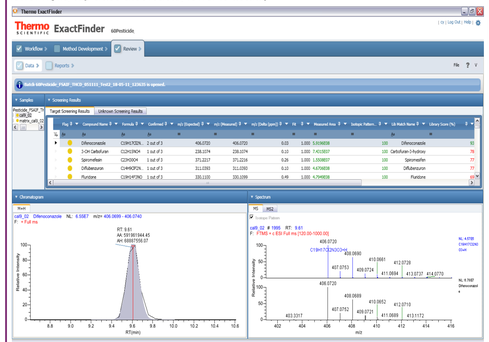


FIGURE 4. Targeted Screening hit of Difenoconzole Isotopic Pattern Matching (shown lower right, top trace theoretical, lower is actual)



The untargeted screen yielded additional compounds present in the samples. For most of the signals elemental compositions could be determined (see Fig.4). All 60 analytes of interest could be easily quantified and were assigned as knowns in the automated screen. The unknown screen yielded additional identifications of analytes without additional analytical effort. Elemental compositions were assigned to most of the so far unknown signals, leading the path into a versatile and easy to do general unknown screening (Fig. 5).

FIGURE 5. Unknown Screening hit of Chlorpyrifos oxon determined by Isotopic Pattern match using a larger pesticide database.

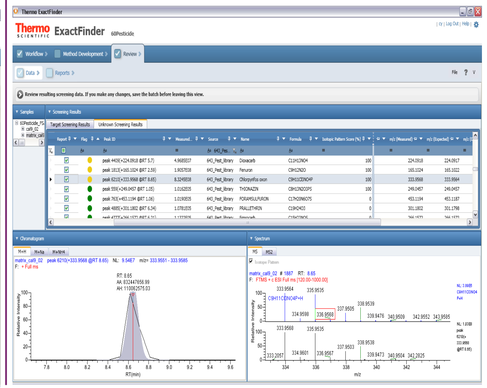


Table 1. List of 60 pesticides and it associated retention times.

Mass [m/z]	Polarity	Start [min]	End [min]	nCE	CS [z]	Comment
255.13390	Positive	4.85	6.85		1	3-OH Carbofuran
218.03830	Positive	2.41	4.41		1	5-OH TBZ
223.07452	Positive	3.32	5.32		1	Acetamiprid
208.11140	Positive	2.23	4.23		1	Aldicarb
223.07473	Positive	3.32	5.32		1	Aldicarb SO
207.07981	Positive	1.78	3.78		1	Aldicarb SO2
404.12412	Positive	6.72	8.72		1	Azoxystrobin
224.09176	Positive	4.85	6.85		1	Bendiocarb
260.02398	Positive	6.48	8.48		1	Benoxacor
343.03997	Positive	7.03	9.03		1	Boscalid
202.08628	Positive	4.93	6.93		1	Carbaryl
222.11249	Positive	4.85	6.85		1	Carbofuran
429.07023	Positive	7.96	9.96		1	Carfentrazone Et
303.01990	Positive	8.87	10.87		1	Clofentezine
250.01602	Positive	2.93	4.93		1	Clothinidin
301.11776	Positive	6.30	8.30		1	Desmedipham
406.07200	Positive	8.83	10.83		1	Difenoconazole
311.03936	Positive	7.98	9.98		1	Diflubenzuron
388.13104	Positive	6.90	8.90		1	Dimethomorph I
388.13104	Positive	7.22	9.22		1	Dimethomorph II
203.11389	Positive	1.81	3.81		1	Dinotefuran
233.02432	Positive	6.35	8.35		1	Diuron
392.16046	Positive	8.33	10.33		1	Famoxadone
312.11653	Positive	6.81	8.81		1	Fenamidone
320.10802	Positive	5.00	7.00		1	Fenamiphos SO
336.10293	Positive	5.18	7.18		1	Fenamiphos SO2
230.05360	Positive	2.25	4.25		1	Flonicamid
266.07353	Positive	7.15	9.15		1	Fludioxonil
330.11005	Positive	6.60	8.60		1	Fluridone
222.12373	Positive	1.65	3.65		1	Formetanate
435.04845	Positive	7.07	9.07		1	Halosulfuron Methyl
353.10798	Positive	9.59	11.59		1	Hexythiazox
297.05562	Positive	6.13	8.13		1	Imazalil
256.05960	Positive	2.86	4.86		1	Imidacloprid
528.07801	Positive	8.74	10.74		1	Indoxacarb
249.01923	Positive	6.97	8.97		1	Linuron
226.08910	Positive	6.90	8.90		1	Methiocarb
163.05360	Positive	1.68	3.68		1	Methomyl
369.21729	Positive	7.21	9.21		1	Methoxyfenozide
237.10156	Positive	1.97	3.97		1	Oxamyl
163.05360	Positive	1.68	3.68		1	Oxamyl Oxime
301.11776	Positive	6.47	8.47		1	Phenmedipham
368.18898	Positive	9.71	11.71		1	Propargite
210.11249	Positive	4.76	6.76		1	Propoxur [S]
200.11825	Positive	6.92	8.92		1	Pyrimethanil
308.00400	Positive	9.87	11.87		1	Quinoxyfen
328.19357	Positive	9.12	11.12		1	Sethoxydim
202.08542	Positive	4.93	6.93		1	Simazine
732.46815	Positive	8.67	10.67		1	Spinosad A
746.48380	Positive	9.06	11.06		1	Spinosad D
371.22116	Positive	9.67	11.67		1	Spiromefesin
404.01567	Positive	5.00	7.00		1	Sulfentrazone
202.04337	Positive	3.50	5.50		1	TBZ
353.22238	Positive	7.89	9.89		1	Tebufenozide
253.03094	Positive	3.81	5.81		1	Thiacloprid
292.02659	Positive	2.34	4.34		1	Thiamethoxam
409.13699	Positive	8.75	10.75		1	Trifloxystrobin

Conclusion

ExactFinder software coupled with the bench top Q Exactive provided easy access to full quantitative, confirmation and screening data in one package. The unknown search provided by the software led to the identification of a number of untargeted compounds to which in most cases elemental compositions could be assigned or by searching a larger compound database. In this case the finding of Chlorpyrifos Oxon in the unknown matrix samples shows the need to quickly move to newer technologies to help determine what we are not seeing by triple quadropole systems.

Acknowledgements

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