APPLICATION NOTE 65983

Quantitation of pesticide residues in garlic and cumin using an Orbitrap Exploris 120 high-resolution mass spectrometer

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Goal

To develop and pre-validate a multi-residue instrumental method that can be applied for high-throughput quantitation of pesticide residues in garlic and cumin at or below the current legislative requirements. The Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer was operated in two different workflows: the first workflow using full-scan Data-Independent Acquisition (FS-DIA) for quantitation and confirmation, and the second using a Thermo Scientific™ AcquireX™ intelligent data acquisition background exclusion workflow for full spectrum filtering, retrospective analysis, and multi-parameter-based compound identification. These methods were tested for a targeted list of pesticides, with an option for future extension to a larger number of analytes.

Introduction

The demand for quick and simple analysis for a multi-class list of pesticides in large numbers of diverse food samples in agricultural applications 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. Government agencies typically set maximum residue levels for pesticides in different products of plant and animal origin at low part per billion (ppb or µg/kg) levels. The regulations present significant analytical challenges with respect to the low limits of quantification and high number of target analytes.

Currently, routine LC-based methods are typically based on triple quadrupole mass spectrometry. In recent years, Thermo Scientific™ Orbitrap™ mass spectrometers have

become available, providing higher confidence in compound identification with quantitative capabilities comparable to triple quadrupole MS/MS. Mass accuracy (typically below 5 ppm) minimizes interferences from co-eluting analytes and matrix co-extractives, and thus reduces the potential for false positive and false negative results. Sample preparation is also a critical part of the workflow. The use of QuEChERS (Quick Easy Cheap, Effective, Rugged, and Safe) methods have been widely adopted for the extraction of pesticide residues from a wide range of food matrices including spices.

This work describes the method performance parameters using the latest benchtop LC - Orbitrap instrument, the Orbitrap Exploris 120 mass spectrometer for the quantitation of a targeted list of pesticides (Table 2) at or below legislative levels (maximum residue levels—MRLs) in both cumin and garlic matrices. The optimized method was verified according to SANTE/12682/2019[1] guidelines and evaluated for compliance with the EU MRL requirements.

Experimental

Consumables

Reagents	Part number
Acetonitrile, UHPLC-MS grade	A9561
Ammonium Formate > 99%	A115-50
Methanol, UHPLC-MS grade	A4581
Formic Acid, extra pure for HPLC	28905
Water, UHPLC-MS grade	W8-1

Consumables	Part number
Thermo Scientific™ HPLC vial	A4954-010
Thermo Scientific™ HPLC cap/septum	C4010-60A
Thermo Scientific™ Accucore™ aQ 100 x 2.1 mm 2.6 µm	17326-102130

Standards

All pesticide standards were purchased from Agilent Technologies™. See results table for the identity of all pesticides investigated for targeted analysis.

Sample preparation

Cumin and garlic were purchased from a local market and analyzed for background levels of pesticides.

Calibration standard preparation

A standard mega mix stock was prepared in 100% Acetonitrile with a final concentration of 1 µg/mL. A 6-level matrix-matched calibration series, over the range 0.5—100 ng/mL, was prepared by post spiking blank extracts. All levels of the extracted matrix match sample (MMS) calibrants were injected eight times/level while the individual matrix extracted sample (MES, n = 5) calibrants were individually tested for extraction efficiency and reproducibility.

Preparation of blank samples

1. Weigh 2 g of each powder spice into a 50 mL conical tube
2. Add 15 mL of water with 1% Acetic acid to step 1 and mix for 5 min and let stand at room temperature for 10 min soaking
3. Add 15 mL of Acetonitrile to the above mixture
4. Mix vigorously for 1 min on a benchtop vortexer
5. Add QuEChERS (6 g Magnesium Sulfate, 1.5 g Sodium Acetate) to the tube and shake by hand vigorously for 1 min
6. Place in benchtop vortexer for 5 min
7. Centrifuge at 4,500 rpm for 5 min at ambient temperature
8. Transfer supernatant layer into 15 mL conical tube
9. Aspirate 2 mL from step 8 and filter through a 0.45 µm filter into a HPLC vial

Preparation of matrix match samples (MMS)

1. Weigh 2 g of each spice into a 50 mL conical tube
2. **For MES samples**—spike samples of pesticide mega mix at 1 µg/mL for the levels required at 0.5, 1, 5, 10, 50 and 100 ng/mL final; let spiked sample sit at room temperature for 30 min
3. For MMS samples go to next step
4. Add 15 mL of water with 1% Acetic acid to step 1 and mix for 5 min and sit at room temperature for 10 min soaking
5. Add 15 mL of Acetonitrile to the above mixture
6. Mix vigorously for 1 min on a benchtop vortexer

7. Add QuEChERS salt to the tube and shake by hand vigorously for 1 min
8. Place in benchtop vortexer for 5 min
9. Centrifuge at 4500 rpm for 5 min at ambient temperature
10. Aspirate or pour top layer into 15 mL vial
11. Aspirate 2 mL from step 8 and filter through a 0.45 µm filter into a 15 mL conical vial
12. Aliquot 1 mL into individual HPLC vials and make calibration levels at 0.5, 1, 5, 10, 50, and 100 ng/mL using mega mix stock

A 1 µL sample was then injected into the LC - Orbitrap Exploris 120 mass spectrometer for analysis.

Instrument analysis

Sample analysis was carried out on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system coupled to an Orbitrap Exploris 120 mass spectrometer.

Separation	
Column	Accucore aQ column, 100 x 2.1 mm, 2.6 µm
Column temperature	25°C
Flow rate	0.300 mL/min
Injection volume	1 µL
Mobile phase	A: Water with 5 mM ammonium formate, 0.1% formic acid B: Methanol with 5 mM ammonium formate, 0.1% formic acid
Gradient	Table 1

Table 1. UHPLC gradient program.

Time [Min]	Flow Rate [mL/min]	A%	B%	Curve
0.0	0.300	98	2	5
1.0	0.300	98	2	5
2.0	0.300	50	50	5
9.0	0.300	2	98	5
12.0	0.300	2	98	5
12.1	0.300	98	2	5
15.0	0.300	98	2	5

Orbitrap Exploris 120 MS Settings	
Spray voltage	3.5 kV
Sheath gas	30 arb
Aux gas	6 arb
Sweep gas	1 arb
Capillary temp.	290°C
Vaporizer temp.	350°C
Ion polarity	Pos
Full Scan mass range	*m/z* 100–1100
Full Scan resolution	60,000
DIA resolution	15,000
Q1 isolation	*m/z* 200
ddMS^2	15,000
HCD collision energy	Stepped nCE 18 ,35, 60
RF Lens	60

Data acquisition and processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software to ensure full automation from instrument setup to raw data collection, processing, and reporting.

Experiment 1: Data acquired from FS-DIA were analyzed with an extraction mass tolerance of ±5 ppm for both precursor and product ions. Analytes were quantified based on full scan precursor accurate mass. In addition, confirmation of target pesticides was performed by DIA fragment matching using a curated high-resolution spectral library.

Experiment 2: The samples were then analyzed for other contaminants, using a new 'data-mining' software function called AcquireX intelligent data acquisition workflow. This functionality has several workflows. One such workflow is called Background Subtraction and uses a blank matrix to automatically generate an exclusion list of matrix co-extractives prior to acquisition, while using a targeted MS^2 inclusion list with retention times for added specificity for the targeted pesticides. Data were extracted with a mass tolerance of 5 ppm for both precursor and product ions of targeted pesticides. Analytes were first quantified using the full scan precursor mass trace and then identified using a targeted list of pesticides from a compound database and matched with a spectral library. All data were evaluated against SANTE Guidelines criteria using EC SANTE/12682/2019.[1]

Results and discussion

Experiment 1: Simplified in-house validation for screening and quantitative methods was carried out for targeted pesticides. The linearity of the calibration curves for MMS was assessed over the range from 0.5 to 100 ng/mL to demonstrate the potential of the method for quantitative analysis. Method selectivity and sensitivity was evaluated by comparing the blanks (garlic and cumin) and MMS (garlic and cumin) (respectively). The evaluation was based on accurate mass of the analyte at the specified retention time window (±0.1 min). Full MS scan acquisition-based quantitation using mono-isotopic match, presence of fragment ions (FI), and a high resolution curated pesticide spectral library match (LS) were additionally applied for identification according to References 1 and 2. Acceptance values were set ≤5 ppm for mass accuracy (FS, DIA and ddMS^2), ±0.1 min for retention time, reproducibility at limit of quantitation (LOQ) RSD ≤15% and limit of detection (LOD) between 15–20% RSD with at least one fragment ion (FI) present and ≥50% for LS matching, however reporting standards were set at ≥60% and a $R^2 \geq 0.9800$. The established values are shown in Table 2 for cumin and Table 3 for garlic. Figure 1 shows some select pesticides across the retention time range of the method (1–10 min); while Figure 2 demonstrates sufficient scans across each peak for accurate quantitation. Recoveries were checked for both cumin (Table 2) and garlic (Table 3) to confirm the extraction protocol was universal for both matrices at 3 different concentration levels (1.3, 6.6, and 13.3 µg/kg) and n = 5 replicates/concentration. The results show excellent recoveries between 70–120%. Some compounds in 60% range showed excellent precision between replicates and thus are allowable under SANTE guidance, Figure 3A and 3B (respectively).

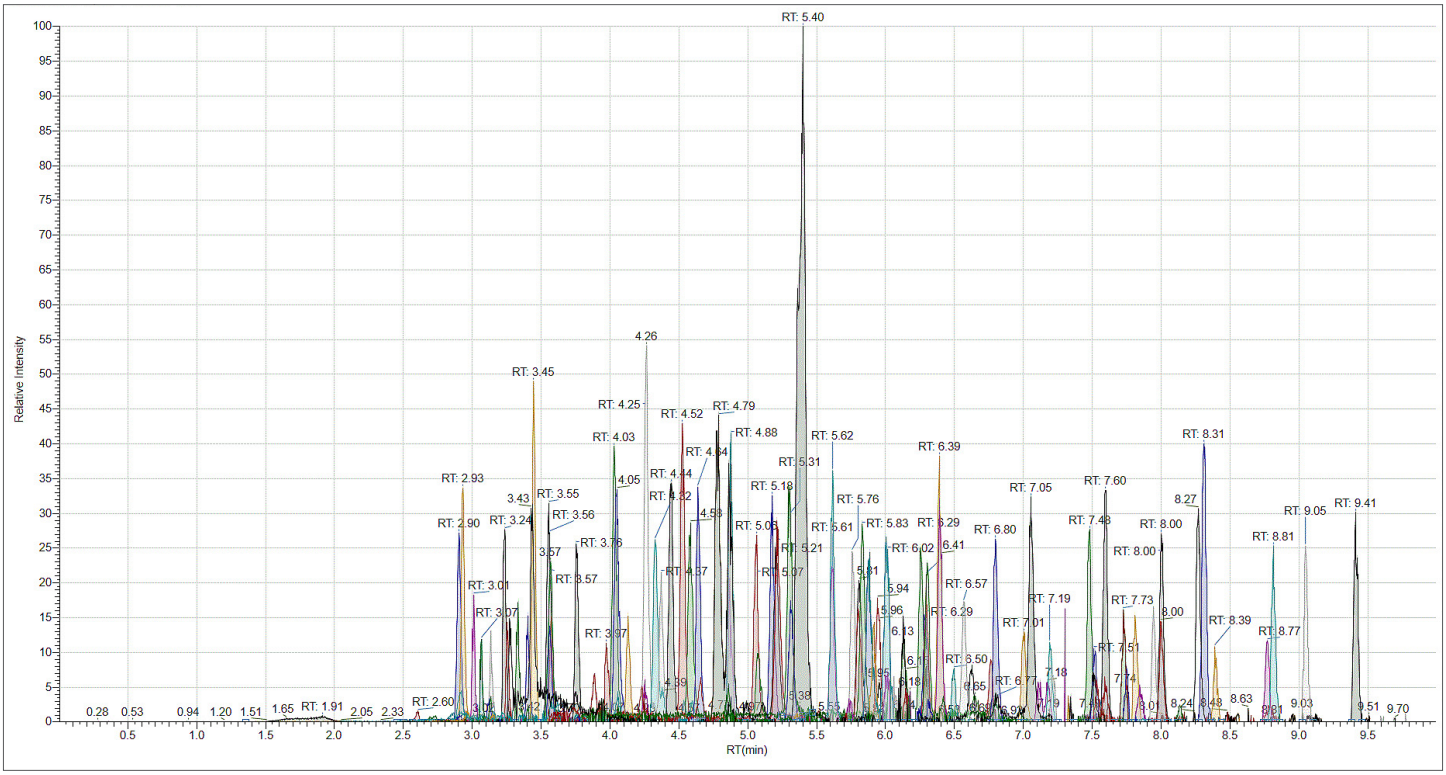


Figure 1. Robust LC-MS shows a 10 ppb spiked pesticides in garlic (MMS) across the retention time range of the method (1–10 min) with extracted mass tolerance of 5 ppm.

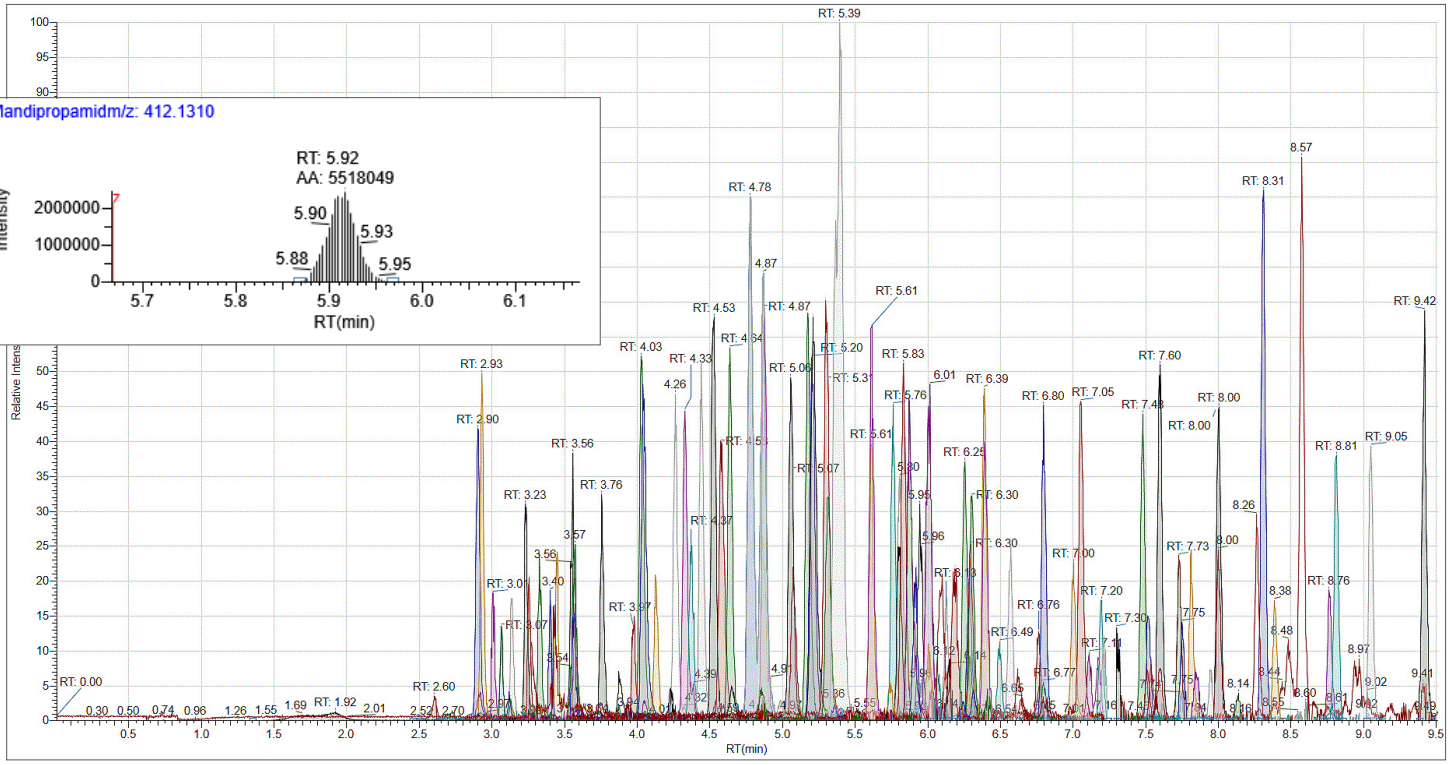


Figure 2. Chromatogram of all pesticides in 15 min in cumin MMS spiked at 10 ppb. The peak highlighted at 5.92 min is mandipropamid, showing over 11 scans across the full scan quantitation ion used for the analysis.

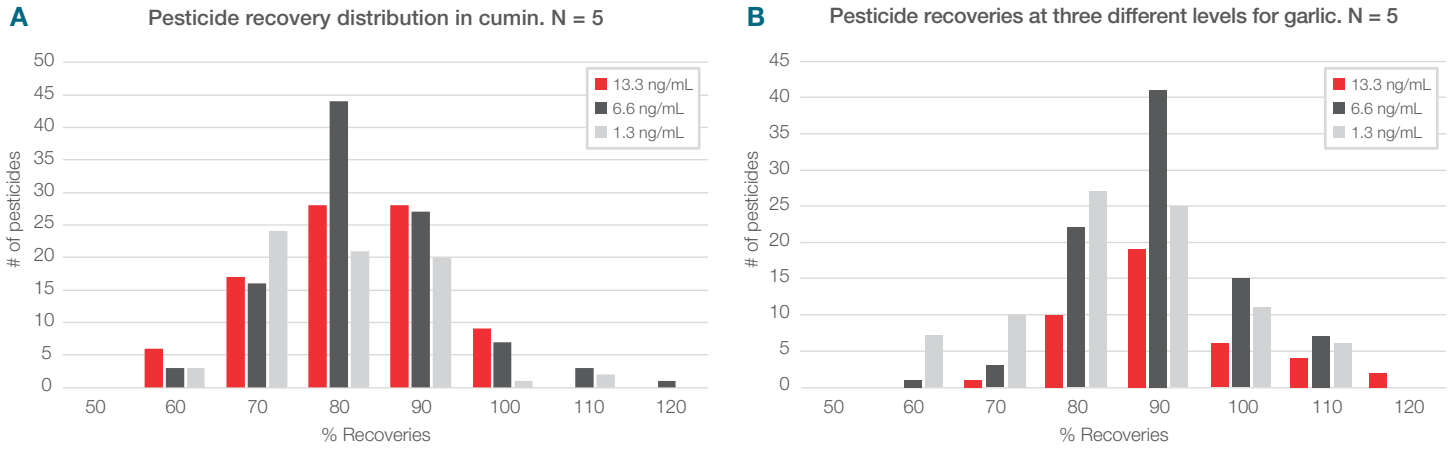


Figure 3. Pesticide recoveries in cumin and garlic at 1.3, 6.6 and 13.3 ng/mL for n = 5 replicates.

Table 2. Table 2: Results for cumin in matrix match samples.

Compound	RT	R^2	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	*m/z* (Delta)
Acetamiprid	3.57	0.9958	0.5	5.6	1.0	7.9	–0.5084
Ametryn	5.21	0.9998	0.5	4.0	1.0	3.4	–1.0413
Aminocarb	2.92	0.9912	0.5	4.5	1.0	2.1	–0.7369
Azoxystrobin	5.61	0.9988	0.5	10.9	1.0	6.8	–1.2467
Bupirimate	6.38	0.9995	0.5	9.4	1.0	10.2	–0.7869
Buprofezin	8.00	0.9997	0.5	10.9	1.0	6.3	–0.9227
Butafenacil ($M+NH_4$)	6.28	0.9995	0.5	12.1	1.0	7.1	–1.2822
Carboxin	4.63	0.9999	0.5	4.0	1.0	3.7	–0.6692
Chloroxuron	6.39	0.9997	0.5	7.1	1.0	5.5	–0.9520
Difenoconazole	7.63	0.9995	0.5	19.5	1.0	12.1	–0.3241
Dimethoate	3.56	0.9940	0.5	8.7	1.0	6.3	–0.9419
Diniconazole	7.51	0.9995	0.5	8.7	1.0	5.9	–0.0527
Epoxiconazole	6.57	0.9998	0.5	4.3	1.0	4.4	–0.7599
Fenamidone	5.76	0.9989	0.5	11.4	1.0	4.6	–0.9582
Fenpyroximate	8.81	0.9997	0.5	3.6	1.0	5.2	0.2121
Fluometuron	4.87	0.9997	0.5	7.4	1.0	4.9	–1.5277
Fluoxastrobin	6.29	0.9997	0.5	4.2	1.0	5.9	–1.1107
Furalaxyl	5.61	0.9989	0.5	7.6	1.0	5.3	–1.4410
Hexythiazox	8.39	0.9997	0.5	8.6	1.0	7.4	–0.2012
Isoproturon	5.17	0.9995	0.5	4.9	1.0	3.3	–1.2755
Mandipropamid	5.90	0.9991	0.5	7.5	1.0	6.3	–0.7357
Mefenacet	6.25	0.9997	0.5	3.4	1.0	3.3	–1.0224
Methabenzthiazuron	5.31	0.9994	0.5	5.7	1.0	4.5	–1.6961
Methamidophos	1.90	0.9998	0.5	4.0	1.0	4.6	–1.4253
Methoprotryne	5.20	0.9998	0.5	6.2	1.0	2.8	–0.5563
Metribuzin	4.37	0.9997	0.5	7.4	1.0	5.1	–1.5369
Monocrotophos	3.25	0.9995	0.5	11.3	1.0	9.0	–1.1697
Monolinuron	4.86	0.9995	0.5	11.7	1.0	9.1	–1.5527
Nitenpyram	3.14	0.9992	0.5	5.9	1.0	6.0	–0.7434
Omethoate	2.89	0.9992	0.5	3.5	1.0	2.6	0.4249
Penconazole	7.00	0.9998	0.5	6.4	1.0	6.2	–1.0242
Pencycuron	7.48	0.9996	0.5	7.2	1.0	5.1	–0.9575
Picoxystrobin	6.76	0.9996	0.5	11.7	1.0	3.2	–0.9320
Pirimicarb	4.04	0.9996	0.5	7.0	1.0	3.4	–0.6881
Prometon	4.76	0.9998	0.5	1.4	1.0	2.7	–0.5873
Prometryn	6.00	0.9989	0.5	20.0	1.0	4.6	–1.2734
Pyracarbolid	4.52	0.9998	0.5	3.2	1.0	4.2	–0.6584
Pyridaben	9.05	0.9997	0.5	9.5	1.0	5.0	–1.0661
Pyriproxyfen	8.30	0.9998	0.5	3.5	1.0	3.3	–1.0473
Secbumeton	4.93	0.9998	0.5	4.4	1.0	3.1	–0.9921
Siduron	5.80	0.9983	0.5	11.9	1.0	6.7	–0.7823
Simetryn	4.57	0.9998	0.5	2.5	1.0	2.7	–1.2065
Spirodiclofen	8.77	0.9997	0.5	10.5	1.0	7.1	–0.7003
Spirotetramat	6.30	0.9997	0.5	6.2	1.0	6.9	–1.1484
Tebufenozide ($M-C_4H_7$)	6.79	0.9996	0.5	9.5	1.0	7.8	–0.9949
Tebufenpyrad	8.00	0.9995	0.5	20.0	1.0	12.6	–0.3030

Table 2. Results for cumin in matrix match samples. (continued)

Compound	RT	R^2	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	*m/z* (Delta)
Tebuthiuron	4.45	0.9997	0.5	4.7	1.0	4.0	–0.6862
Terbumeton	4.95	0.9997	0.5	3.3	1.0	3.2	–0.3174
Terbutryn	5.86	0.9987	0.5	7.8	1.0	3.7	–0.9583
Thiabendazole	3.55	0.9950	0.5	4.0	1.0	7.0	–1.3843
Thiacloprid	3.74	0.9995	0.5	4.0	1.0	3.6	–0.9874
Triadimefon	6.12	0.9992	0.5	12.2	1.0	11.8	–0.0261
Tricyclazole	4.04	0.9994	0.5	4.3	1.0	3.4	–0.5886
Trifloxystrobin	7.59	0.9998	0.5	6.1	1.0	6.0	–0.8379
Triflumizole	7.81	0.9995	0.5	13.1	1.0	7.8	–1.0148
Zoxamide	7.19	0.9996	0.5	7.7	1.0	8.3	–0.6911
Bifenazate	6.29	0.9995	1.0	13.9	1.0	13.9	–2.4550
Carbofuran	4.32	0.9988	1.0	2.3	1.0	2.3	–1.2252
Cycluron	5.29	0.9988	1.0	14.8	1.0	14.8	–0.4284
Hexaconazole	7.21	0.9994	1.0	14.6	1.0	14.6	0.1396
Metalaxyl	5.05	0.9986	1.0	6.2	1.0	6.2	–0.5540
Spinetoram 1	7.84	0.9989	1.0	11.8	1.0	11.8	–2.9761
Tetraconazole	6.49	0.9995	1.0	9.5	1.0	9.5	–0.9881
Imidacloprid	3.40	0.9898	0.5	18.6	5.0	12.5	–1.5872
Acephate	2.73	0.9995	1.0	15.6	5.0	3.0	–0.3301
Benalaxyl	7.05	0.9991	1.0	13.0	5.0	3.7	–0.5327
Carbendazim	3.33	0.9944	1.0	19.7	5.0	7.1	–0.7061
Carbetamide	4.15	0.9993	1.0	20.2	5.0	3.2	–1.8131
Clethodim	7.74	0.9991	1.0	15.3	5.0	5.3	0.4107
Dinotefuran	3.00	0.9829	1.0	15.8	5.0	13.4	–1.0205
Fenazaquin	9.42	0.9993	1.0	15.9	5.0	1.9	–1.4203
Fenuron	3.57	0.9893	1.0	17.8	5.0	6.5	–0.9612
Imazalil	5.07	0.9991	1.0	7.3	5.0	1.5	–0.5035
Ipconazole	7.73	0.9996	1.0	9.3	5.0	2.1	–0.3943
Oxadixyl	3.98	0.9981	1.0	12.1	5.0	6.6	–2.0355
Benzoximate	7.33	0.9941	5.0	12.9	5.0	12.9	–0.9921
Cyromazine	2.60	0.9957	5.0	2.4	5.0	2.4	–0.7102
Dimethomorph	6.00	0.9983	5.0	12.4	5.0	12.4	–1.4102
Fenpropimorph	5.82	0.9961	5.0	3.4	5.0	3.4	–0.6058
Flusilazole	6.75	0.9994	5.0	5.8	5.0	5.8	–1.2709
Furathiocarb	7.94	0.9946	5.0	1.7	5.0	1.7	1.5751
Hydramethylnon	7.86	0.9877	5.0	4.5	5.0	4.5	1.0364
Piperonyl-butoxide	8.13	0.9987	5.0	7.7	5.0	7.7	–0.2175
Propiconazole	7.11	0.9993	5.0	5.7	5.0	5.7	–1.4365
Spiroxamine	6.18	0.9987	5.0	7.2	5.0	7.2	–1.7801
Thiamethoxam	3.23	0.9958	5.0	8.3	5.0	8.3	–0.6603
Triadimenol	6.31	0.9985	5.0	5.1	5.0	5.1	–1.0379
Methoxyfenozide	6.14	0.9947	1.0	17.0	10.0	11.5	–2.1080
Chlorantraniliprole	5.36	0.9987	5.0	11.8	10.0	5.2	–0.4684
Cyproconazole	6.07	0.9968	5.0	15.8	10.0	8.7	–0.4033
Tebufenozide	6.79	0.9570	10.0	8.8	10.0	8.8	0.7806

Table 3. Results for garlic in matrix match samples.

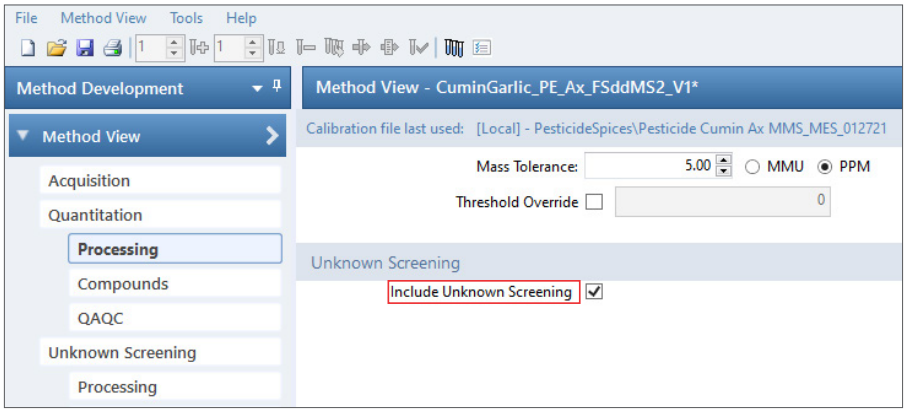
Compound	RT	R^2	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	*m/z* (Delta)
Acephate	2.73	0.9988	5.0	10.8	5.0	10.8	–0.8276
Acetamiprid	3.57	0.9765	5.0	3.0	5.0	3.0	–0.6452
Ametryn	5.21	0.9934	0.5	2.0	1.0	1.8	–0.2387
Aminocarb	2.92	0.9951	5.0	16.3	10.0	14.4	–1.4665
Azoxystrobin	5.61	0.9962	0.5	3.2	1.0	1.4	–0.1139
Benalaxyl	7.05	0.9995	0.5	2.7	1.0	1.6	0.1222
Benzoximate	7.33	0.9986	1.0	6.7	1.0	6.7	–0.1539
Bifenazate	6.29	0.9988	0.5	3.5	1.0	1.7	–1.2390
Bitertanol	7.35	0.9994	0.5	6.4	1.0	3.5	2.3752
Bupirimate	6.38	0.9995	0.5	2.8	0.5	2.8	–0.0171
Buprofezin	8.00	0.9990	0.5	2.6	0.5	2.6	–0.2250
Butafenacil ($M+NH_4$)	6.28	0.9996	0.5	3.3	0.5	3.3	1.5084
Carbetamide	4.15	0.9890	5.0	1.3	5.0	1.3	–0.9122
Carbofuran	4.32	0.9880	5.0	2.6	5.0	2.6	–1.1565
Carboxin	4.63	0.9930	0.5	15.5	5.0	3.4	–0.6046
Chlorantraniliprole	5.36	0.9892	5.0	3.2	5.0	3.2	0.3547
Chloroxuron	6.39	0.9997	0.5	3.8	0.5	3.8	–0.6375
Clethodim	7.74	0.9992	0.5	4.6	0.5	4.6	–1.3688
Clothianidin	3.44	0.9592	5.0	6.8	5.0	6.8	–1.3778
Cycluron	5.29	0.9941	5.0	8.8	5.0	8.8	0.0312
Cyproconazole	6.07	0.9976	0.5	4.2	1.0	2.4	0.4325
Cyromazine	2.60	0.9969	0.5	14.8	1.0	7.6	–3.2670
Difenoconazole	7.63	0.9989	0.5	4.6	1.0	3.3	0.1268
Dimethoate	3.56	0.9844	5.0	3.3	5.0	3.3	–0.9419
Dimethomorph	6.00	0.9976	0.5	3.0	1.0	2.4	–0.4667
Diniconazole	7.51	0.9981	0.5	7.6	1.0	3.6	–0.0527
Dinotefuran	3.00	0.9928	1.0	6.7	1.0	6.7	0.1815
Epoxiconazole	6.57	0.9997	0.5	6.5	1.0	6.9	–0.4826
Fenamidone	5.76	0.9966	0.5	2.5	1.0	1.7	0.3129
Fenazaquin	9.42	0.9995	0.5	6.3	1.0	6.0	0.1693
Fenbuconazole	6.66	0.9976	50.0	4.1	50.0	4.1	0.3916
Fenpropimorph	5.82	0.9978	0.5	3.6	1.0	1.7	–0.1043
Fenpyroximate	8.81	0.9992	0.5	4.7	0.5	4.7	–0.2939
Fenuron	3.57	0.9799	5.0	2.9	5.0	2.9	–0.9612
Fluometuron	4.87	0.9959	1.0	16.7	5.0	4.9	0.0434
Fluoxastrobin	6.29	0.9992	0.5	3.4	0.5	3.4	–0.5789
Flusilazole	6.75	0.9974	1.0	2.9	1.0	2.9	0.9495
Furalaxyl	5.61	0.9963	0.5	2.6	0.5	2.6	–0.5319
Furathiocarb	7.94	0.9998	50.0	0.3	50.0	0.3	–0.4160
Hexaconazole	7.21	0.9998	0.5	3.9	0.5	3.9	–1.2207
Hexythiazox	8.39	0.9997	0.5	1.4	0.5	1.4	–0.1148
Hydramethylnon	7.86	0.9960	1.0	18.0	5.0	6.9	0.8515
Imazalil	5.07	0.9916	5.0	10.3	10.0	8.8	–1.6335
Imidacloprid	3.40	0.9512	5.0	7.4	5.0	7.4	–0.7529
Ipconazole	7.73	0.9993	0.5	3.9	0.5	3.9	–1.1249
Isoproturon	5.17	0.9938	0.5	12.6	1.0	3.1	–1.1282
Mandipropamid	5.90	0.9987	0.5	3.2	0.5	3.2	–0.3655

Table 3. Results for garlic in matrix match samples. (continued)

Compound	RT	R^2	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	*m/z* (Delta)
Mefenacet	6.25	0.9987	0.5	3.3	0.5	3.3	–0.0021
Metalaxyl	5.05	0.9919	5.0	16.0	10.0	7.1	–1.2076
Methabenzthiazuron	5.31	0.9938	0.5	2.5	1.0	2.0	–0.5967
Methamidophos	1.90	0.9990	5.0	0.9	10.0	1.4	–0.9955
Methoprotryne	5.20	0.9938	0.5	3.3	5.0	3.7	–0.2199
Methoxyfenozide	6.14	0.9983	0.5	5.6	1.0	8.1	0.1237
Metribuzin	4.37	0.9890	1.0	10.5	5.0	2.5	–0.4019
Mexacarbate	3.54	0.9746	5.0	9.6	5.0	9.6	–0.6659
Monolinuron	4.86	0.9947	5.0	17.2	10.0	8.8	–1.0561
Nitenpyram	3.14	0.9977	0.5	18.2	5.0	11.9	–0.9685
Omethoate	2.89	0.9994	0.5	7.3	1.0	2.2	–0.2881
Oxadixyl	3.98	0.9870	5.0	1.7	5.0	1.7	–0.1769
Penconazole	7.00	0.9998	0.5	2.5	0.5	2.5	1.0170
Pencycuron	7.48	0.9992	0.5	2.1	0.5	2.1	–0.7721
Picoxystrobin	6.76	0.9993	1.0	5.1	1.0	5.1	2.1354
Piperonyl-butoxide	8.13	0.9988	5.0	2.8	5.0	2.8	–0.8172
Pirimicarb	4.04	0.9895	0.5	15.9	5.0	1.1	–1.3899
Prochloraz	7.26	0.9993	1.0	4.1	1.0	4.1	–0.6600
Prometon	4.76	0.9947	0.5	7.2	0.5	7.2	–0.3174
Prometryn	6.00	0.9982	0.5	2.8	1.0	1.3	0.2390
Propiconazole	7.11	0.9996	0.5	6.2	1.0	4.8	–0.0983
Pyracarbolid	4.52	0.9927	0.5	11.1	1.0	4.9	–0.3087
Pyraclostrobin	7.31	0.9992	0.5	2.9	0.5	2.9	–0.7722
Pyridaben	9.05	0.9996	0.5	4.3	1.0	4.5	0.6890
Pyrimethanil	5.80	0.9967	0.5	2.0	1.0	1.4	–0.0508
Pyriproxyfen	8.30	0.9994	0.5	1.7	0.5	1.7	–0.3842
Secbumeton	4.93	0.9945	5.0	16.3	10.0	7.9	–0.7222
Siduron	5.80	0.9974	0.5	2.9	1.0	2.0	0.1993
Simetryn	4.57	0.9933	1.0	19.8	5.0	3.1	1.0027
Spinetoram 1	7.84	0.9986	0.5	4.9	1.0	3.2	–1.0191
Spirodiclofen	8.77	0.9968	0.5	2.2	1.0	2.3	–0.1807
Spirotetramat	6.30	0.9995	0.5	2.9	0.5	2.9	–0.1698
Spiroxamine	6.18	0.9991	0.5	12.2	1.0	3.4	–0.0407
Tebufenozide	6.79	0.9995	1.0	3.5	5.0	4.0	–0.0834
Tebufenozide (M-C_4H_7)	6.79	0.9997	0.5	2.2	1.0	1.0	0.2374
Tebufenpyrad	8.00	0.9997	0.5	2.7	1.0	1.3	0.4276
Tebuthiuron	4.45	0.9915	0.5	6.8	1.0	4.5	0.3128
Terbumeton	4.95	0.9944	5.0	16.4	10.0	7.7	–1.5993
Terbutryn	5.86	0.9978	1.0	1.5	5.0	1.3	–0.5802
Tetraconazole	6.49	0.9994	0.5	8.6	1.0	2.8	–0.9881
Thiabendazole	3.55	0.9793	1.0	18.4	5.0	3.1	–0.0249
Thiacloprid	3.74	0.9817	5.0	2.2	5.0	2.2	0.0981
Triadimefon	6.12	0.9984	0.5	3.4	1.0	2.9	–0.2337
Triadimenol	6.31	0.9985	1.0	7.1	1.0	7.1	–1.7593
Tricyclazole	4.04	0.9880	0.5	18.7	5.0	1.6	–0.8294
Trifloxystrobin	7.59	0.9990	0.5	4.4	0.5	4.4	–0.1666
Triflumizole	7.81	0.9987	0.5	2.7	0.5	2.7	0.2197
Zoxamide	7.19	0.9993	0.5	3.9	0.5	3.9	0.7620

Experiment 2: The implementation of the AcquireX Background Exclusion workflow also helps in identification of targeted and unknown contaminates using a unique routine to automatically create an exclusion list based on LC-MS analysis of the matrix blank. The instrument method is automatically updated with the exclusion list, so when subsequent samples are analyzed, MS^2 experiments are not performed on matrix background signals. As a result, more cycle time is spent on triggering MS^2 on the relevant ions of interest. This is groundbreaking for data processing because it minimizes false-positives and -negatives. TraceFinder software can efficiently process these new complex data files and extract results for both targeted quantitation and unknown screening workflows. TraceFinder software can easily go from a targeted quantitation workflow to unknown screening workflow by simply checking a box (Figure 4). The software can quickly utilize multi-search options, from custom spectral libraries to the multiple Thermo Scientific™ mzCloud™ curated spectra libraries to online ChemSpider™ database searching, utilizing the Exhaustive Search feature, which can move from one search option to the next to make sure the best results are displayed from each to search criteria (Figure 5).

Figure 4. TraceFinder software is easily configurable to perform either a targeted quantitative or unknown screening workflows.



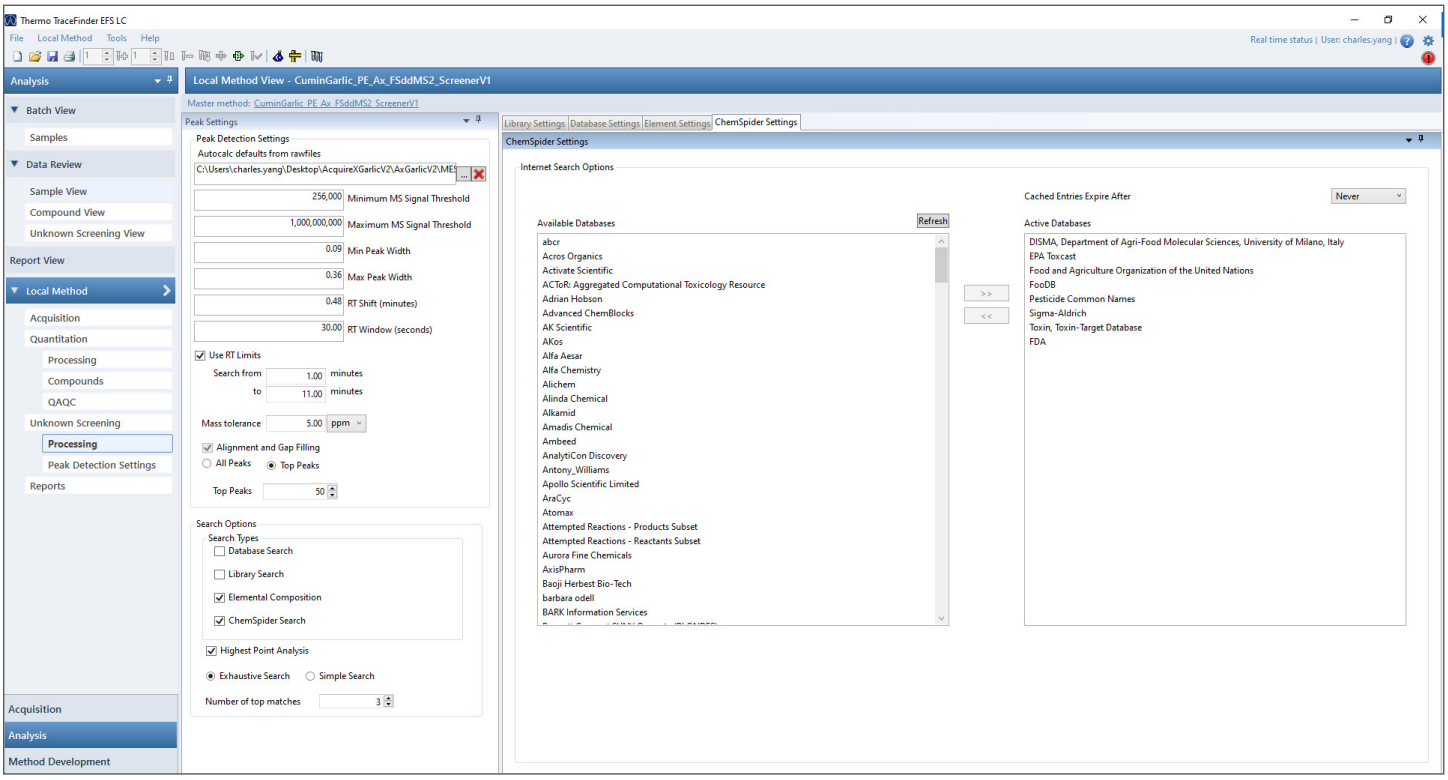


Figure 5. Unknown search parameters are easily activated by checking the box. The capabilities to search multiple mzCloud curated spectral libraries gives you the confidence of the exact match or online targeted database in ChemSpider.

Figure 6 depicts the quick and easy data review section of the quantitation workflow with sortable grids and informative information at the bottom. The highlighted compound Fenpyroximate is shown as an example of consistent mass accuracy of the Orbitrap Exploris 120 mass spectrometer. Only those “not identified” targets from the previous section will be moved down into the unknown section to be identified further using the different online database or local databases which were not used prior.

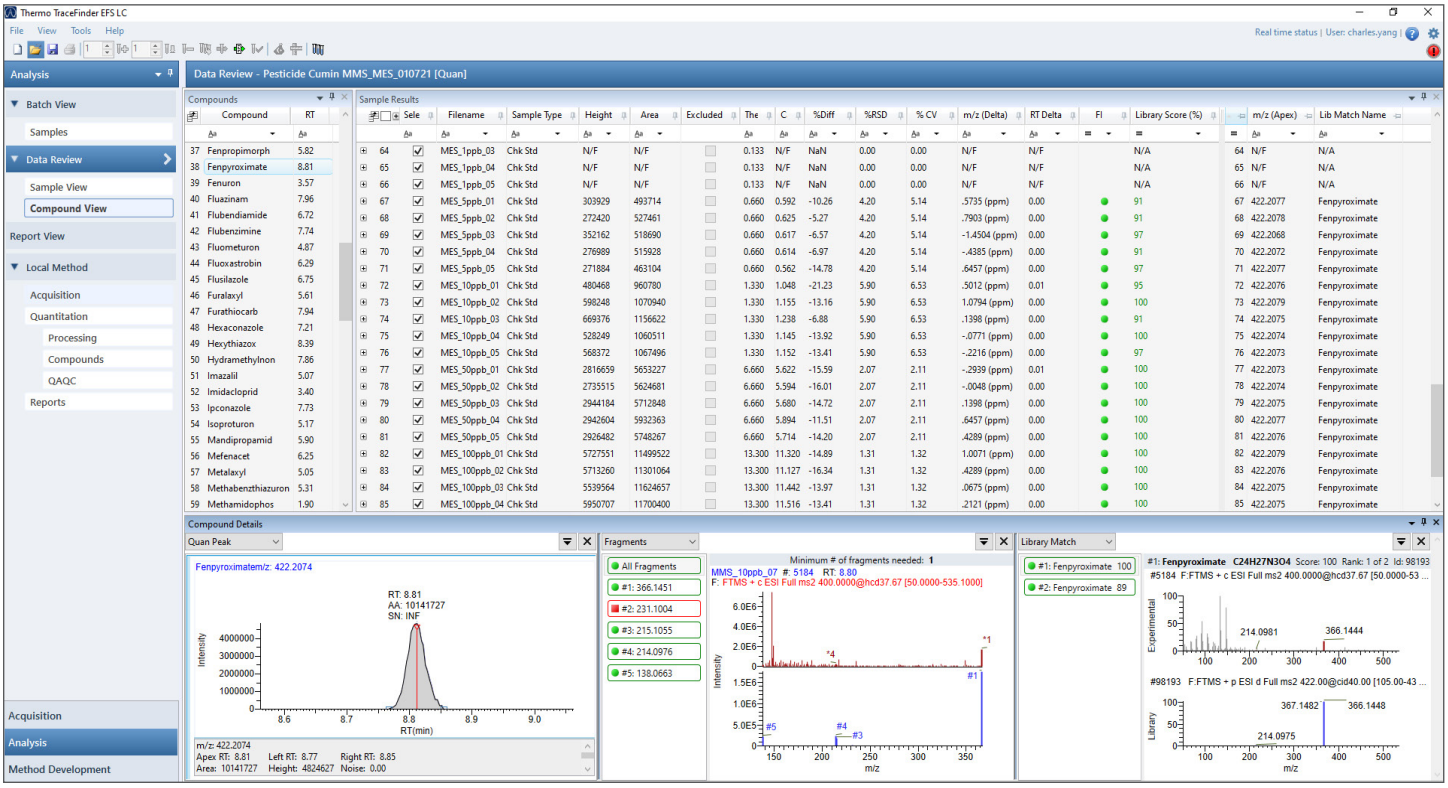


Figure 6. A quick overview of Fenpyroximate quantitation with library scoring of 100% and Δppm of ≤ 1 ppm at 10 ppb concentration level shown above.

In Figure 7, elemental composition and the ChemSpider database were used to identify and label with the probable compound(s) in question. For cumin, an unknown was identified as elaidolinolenic acid (Figure 8).

A unique compound called 1-Dodecyl-2-pyrrolidinone was tentatively identified and more research will need to be done on the compound to confirm its identity (Figure 9). For garlic samples, several compounds were identified; (e.g., octhilinone which is a fungicide and antibacterial agent used for treatment of canker and other fungal and bacterial diseases in fruit trees). Proxan is a non-steroidal anti-inflammatory drug used to treat pain or inflammation in humans which could have found its way through the water supply to the farm. Both compounds were identified through ChemSpider but further research will need to be done to confirm them (Figure 10 and 11 respectively).

Figure 7. Quick overview of unknown screening where identification is quickly listed. Here is a highlighted example of benzylpiperazine found in garlic sample which was not targeted.

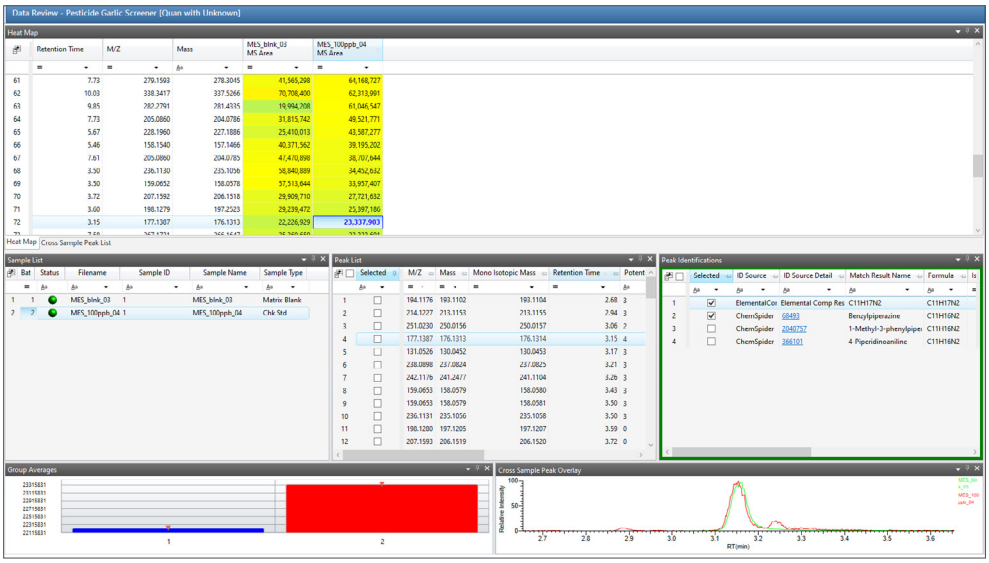


Figure 8. Quick overview of unknown screening which found an unknown and identified as Elaidolinolenic acid.

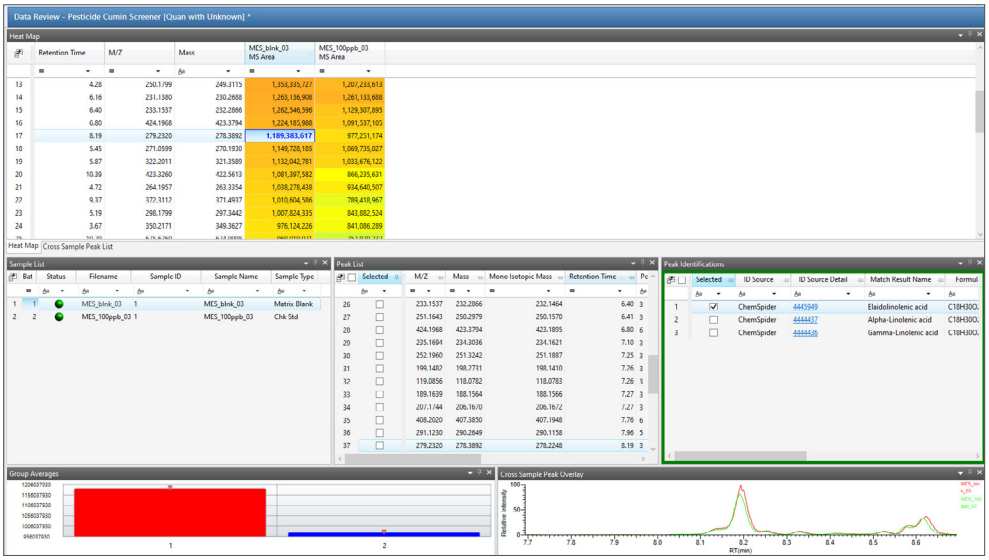


Figure 9. A unique compound called 1-Dodecyl-2-pyrrolidinone was also identified.

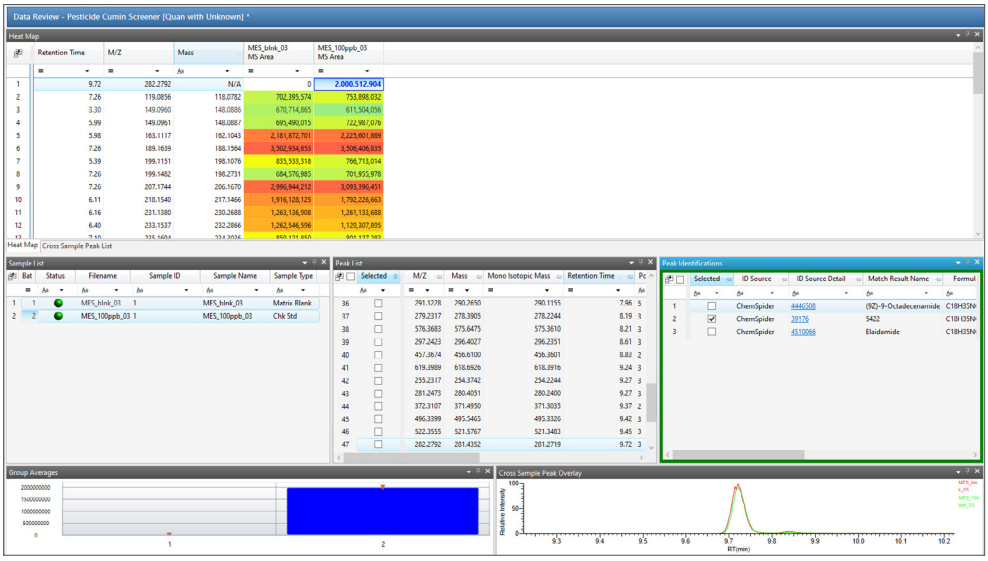


Figure 10. Octhilinone–a fungicide was identified in garlic.

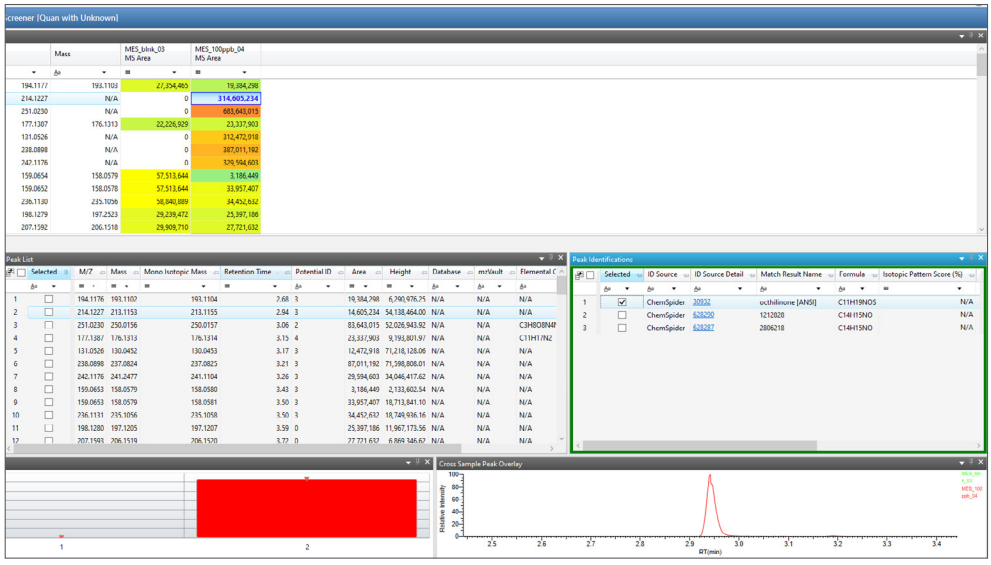


Figure 11. Proxan–anti-inflammatory drug was also identified in garlic.

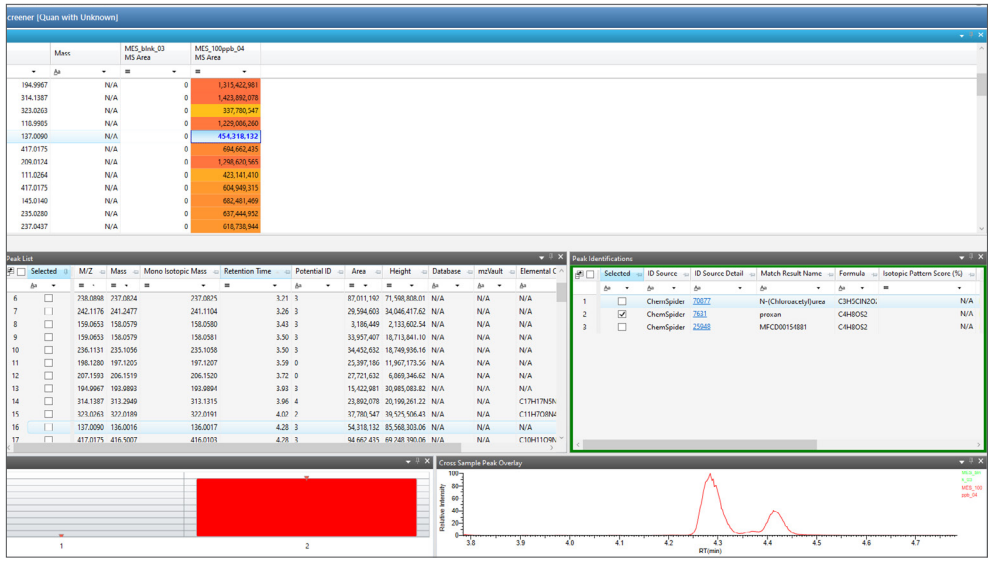
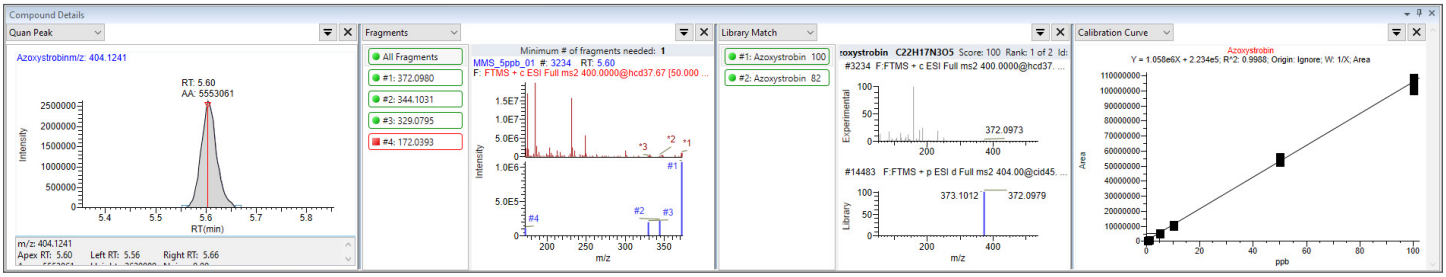


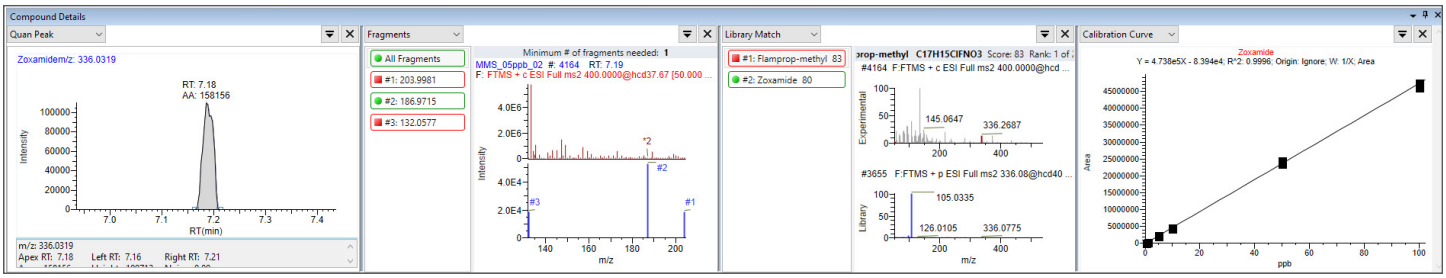
Figure 12A–12D shows typical calibration curves (0.5–100 ng/mL) for Azoxystrobin, Zoxamide, Triflumizolem and Mefenacet in cumin and garlic (respectively). Over 95% of the pesticides studied had calibration curves with $r^2 > 0.990$ (Tables 2 and 3). Confirmation fragment ions are displayed in the middle of each panel at 0.5, 1 and 5 ng/mL for each pesticide, with indicator color (green) highlights that are easily visible to show passing fragment ions and curated mzCloud local spectra library criteria.

A method of 100 pesticides was developed and optimized to ensure that at least one fragment ion was detected per compound while the LODs and LOQs were determined as outlined by the SANTE Guidance (SANTE/12682/2019).[2]

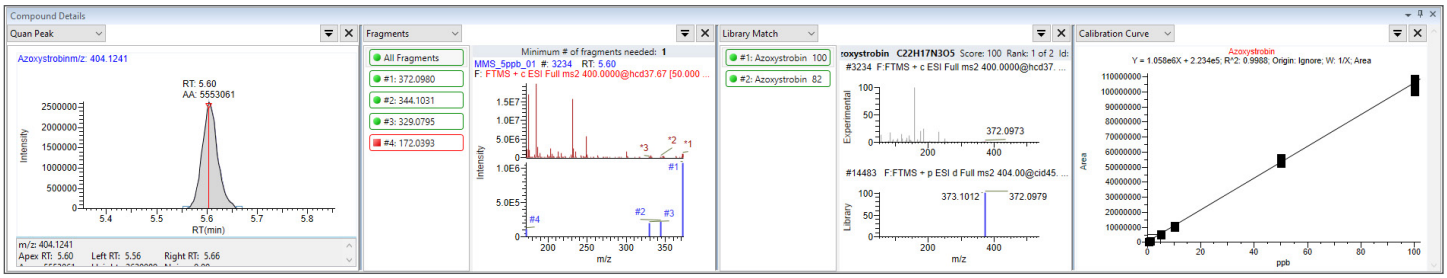
A) Azoxystrobin



B) Zoxamide



C) Triflumizolem



D) Mefenacet

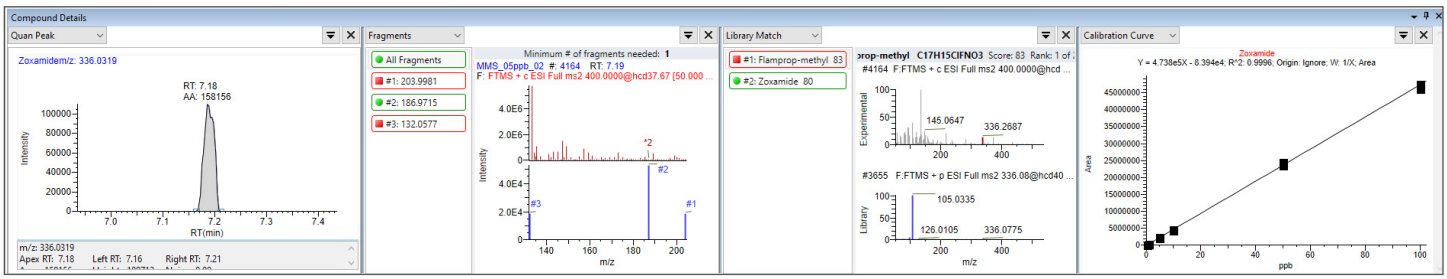


Figure 12. The quantitation and confirmation ions along with calibration range from 0.5 to 100 ppb for (A and B in Cumin) Azoxystrobin at 5 ppb and Zoxamide at 0.5 ppb and (C and D in Garlic) Triflumizolem at 0.5 ppb and Mefenacet at 1 ppb shown in TraceFinder software. All results have excellent R^2 and MS^2 fragment ion matching. The technique allows for confident quantitation and screening with confirmation well below or at the MRL concentration.

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Conclusion

A select targeted panel of pesticides for quantitative analysis at levels below or at EU MRLs have been shown to provide excellent sensitivity and robustness in cumin and garlic. TraceFinder software provides the flexibility to quickly identify unknown contamination within samples using the unknown feature of the software. The capability to search online databases helps to identify unknowns given the excellent mass accuracy and high-resolving power of the Orbitrap Exploris 120 mass spectrometer. These features significantly lower the number of IDs possible and the new AcquireX workflow, utilizing automatic background subtraction, makes identification easier. Ongoing work is required to determine the true unknown chemicals by either chemical synthesis, NMR or other techniques to prove the authenticity of the unknown identifications.

References

1. SANTE Guidelines https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf (accessed Mar. 2021).
2. SANTE Guidelines https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf (accessed Mar. 2021).

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