## APPLICATION NOTE

# 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<sup>™</sup> Orbitrap Exploris<sup>™</sup> 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<sup>™</sup> AcquireX<sup>™</sup> 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  $\mu$ g/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<sup>™</sup> Orbitrap<sup>™</sup> 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<sup>1</sup> 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 <sup>™</sup> HPLC cap/septum	C4010-60A
Thermo Scientific <sup>™</sup> Accucore <sup>™</sup> aQ 100 x 2.1 mm 2.6 µm	17326-102130

## Standards

All pesticide standards were purchased from Agilent Technologies<sup>™</sup>. 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  $\mu$ g/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
- 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
- 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  $\mu m$  filter into a HPLC vial

## Preparation of matrix match samples (MMS)

- 1. Weigh 2 g of each spice into a 50 mL conical tube
- 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  $\mu m$  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<sup>™</sup> Vanquish<sup>™</sup> 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]	<b>A%</b>	В%	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
lon polarity	Pos
Full Scan mass range	<i>m/z</i> 100–1100
Full Scan resolution	60,000
DIA resolution	15,000
Q1 isolation	<i>m/z</i> 200
ddMS <sup>2</sup>	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<sup>™</sup> TraceFinder<sup>™</sup> 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 coextractives prior to acquisition, while using a targeted MS<sup>2</sup> 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<sup>2</sup>), ±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  $\geq$ 60% and a R<sup>2</sup>  $\geq$  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 <sup>2</sup>	LOD (ua/ka)	%RSD	LOQ (ua/ka)	%RSD	<i>m/z</i> (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 <sub>4</sub> )	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	-1.2/34			
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			
Pyriproxyten	8.30	0.9998	0.5	3.5	1.0	3.3	-1.04/3			
Secoumeton	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./	-1.2065			
Spirodicioten	ŏ.//	0.9997	0.5	10.5	1.0	(.)	-0.7003			
	6.30	0.9997	0.5	0.2 0.5	1.0	0.9	-1.1484			
Tebufenozide ( $M-C_4H_7$ )	0.79	0.9996	0.5	9.5	0.1	δ.)	-0.9949			
rebutenpyrad	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 <sup>2</sup>		%RSD		%RSD	m/z	
Tebuthiuron	4 45	0 9997	0.5	47	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	
Tricvclazole	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	
Hudromothylnon	7.94	0.9946	5.0	1.7	5.0	1.7	1.0264	
	0.10	0.9077	5.0	4.3	5.0	4.3	0.0175	
Propiograzole	7.11	0.9907	5.0	5.7	5.0	5.7	-0.2175	
Spiroxamine	6.18	0.0000	5.0	7.0	5.0	7.0	_1.4000	
Thiamethoxam	3 23	0.9958	5.0	8.3	5.0	8.3	-0.6603	
Triadimenol	6.31	0.9985	5.0	5.0	5.0	5.0	-1 0379	
Methoxyfenozide	6 14	0.9947	10	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 <sup>2</sup>	LOD (ua/ka)	%RSD	LOQ (ua/ka)	%RSD	<i>m/z</i> (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 <sub>4</sub> )	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	10.7	5.0	4.9	0.0434	
Fluoiastrobili	6.75	0.9992	1.0	3.4	1.0	3.4	-0.5769	
Fusiazole	5.61	0.9974	0.5	2.9	0.5	2.9	-0.5310	
Furathiocarb	7.04	0.9903	50.0	0.3	50.0	0.3	-0.4160	
Hevaconazole	7.94	0.9990	0.5	3.9	0.5	3.9	-0.4100	
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	74	5.0	74	-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 <sup>2</sup>	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	<i>m/z</i> (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 <sub>4</sub> H <sub>7</sub> )	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<sup>2</sup> experiments are not performed on matrix background signals. As a result, more cycle time is spent on triggering MS<sup>2</sup> 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<sup>™</sup> mzCloud<sup>™</sup> curated spectra libraries to online ChemSpider<sup>™</sup> 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.

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lethod Development 🚽 🖣	Method View - CuminGarlic_PE_Ax_FSddMS2_V1*
Method View	Calibration file last used: [Local] - PesticideSpices\Pesticide Cumin Ax MMS_MES_012721
Acquisition	Mass Tolerance: 5.00 💌 🔿 MMU 💿 PPM
Quantitation	Threshold Override 0
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Thermo TraceFinder EFS LC     File Local Method Tools Help				– 🗖 Real time status į User: charles.yang į 🍘	×
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Analysis 👻 🖗	Local Method View - CuminGarlic_PE_Ax_FSddMS2_ScreenerV1				
<ul> <li>Batch View</li> </ul>	Master method: <u>CuminGarlic PE Ax FSddMS2 ScreenerV1</u> Peak Settings	Library Settinger Database Settinger Element Settinger ChemSnider Settings			
Samples	Peak Detection Settings	ChemSpider Settings			, ą
<ul> <li>Data Review</li> </ul>	Autocalc defaults from rawfiles C:\Users\charles.yang\Desktop\AcquireXGarlicV2\AxGarlicV2\ME5	Internet Search Options			
Sample View Compound View	256,000 Minimum MS Signal Threshold 1,000,000,000 Maximum MS Signal Threshold	Available Databases	Refresh	Cached Entries Expire After v Active Databases	
Unknown Screening View Report View	Looucould Maximum MS Signal Threehold     O00 Min Peak Width     O38 Max Peak Width     O48 RT Shift (minutes)     3000 RT Window (seconds)     Vise RT Limits     Search from 1.00 minutes     to 11.00 minutes     Mass tolerance 5.00 Ppm ●     Alignment and Gap Filling     Ali Peaks     Top Peaks     Top Peaks     Top Peaks     So ●     Search Coptions     Search Coptions     Search     Ubtray Search     Ubtray Search     Ditabase Search     Uithery Search     Window (seconds)     Highest Point Analysis     Bethaustive Search     Simple Search	Available Vatibases ber Acros Organics Acros Acros Scientific Action Aggregated Computational Toxicology Resource Advanced ChemBlocks AK Scientific AK Scientific Affa Aeaar Affa Aeaaar Affa Aeaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa		Active variabases DDMAD, Epapartment of Agri-Food Molecular Sciences, University of Milano, Italy EPA Torcsat Food and Agriculture Organization of the United Nations FoodB Particide Common Names Sigma-Aldrich Toxin, Toxin-Target Database FDA	
Acquisition	Number of top matches 3 🖨				
Analysis					
Method Development					_

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.

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▼ Data Review >	37 Fenpropimorph	5.82	⊕ 64	✓	MES_1ppb_03	Chk Std	N/F	N/F		0.133	N/F	NaN	0.00	0.00	N/F	N/F		N/A	64 N/F	N/A	
	38 Fenpyroximate	8.81	⊕ 65	~	MES_1ppb_04	Chk Std	N/F	N/F		0.133	N/F	NaN	0.00	0.00	N/F	N/F		N/A	65 N/F	N/A	
Sample View	39 Fenuron	3.57		✓	MES_1ppb_05	Chk Std	N/F	N/F		0.133	N/F	NaN	0.00	0.00	N/F	N/F		N/A	66 N/F	N/A	
Compound View	40 Fluazinam	6.72	⊕ 67	✓	MES_5ppb_01	Chk Std	303929	493714		0.660	0.592	-10.26	4.20	5.14	.5735 (ppm)	0.00	•	91	67 422.2077	Fenpyroximate	
	41 Flubenzimine	7.74	± 68		MES_5ppb_02	Chk Std	272420	527461		0.660	0.625	-5.27	4.20	5.14	.7903 (ppm)	0.00	•	91	68 422.2078	Fenpyroximate	
Report View	43 Eluometuron	4.87	± 69		MES_5ppb_03	Chk Std	352162	518690		0.660	0.617	-6.57	4.20	5.14	-1.4504 (ppm	) 0.00	•	97	69 422.2068	Fenpyroximate	
T Lord Mathed	44 Fluoxastrobin	6.29	. 70	✓	MES_Sppb_04	Chk Std	276989	515928		0.660	0.614	-6.97	4.20	5.14	4385 (ppm)	0.00		91	70 422.2072	Fenpyroximate	
+ Local Metriou	45 Flusilazole	6.75			MES_SPPD_US	Chk Std	2/1884	403104		0.000	0.502	-14.78	4.20	5.14	.0457 (ppm)	0.00		97	71 422.2077	Fenpyroximate	
Acquisition	46 Furalaxyl	5.61	. 72	<b>V</b>	MES_TOPPD_01	Chk Std	480408	900780		1.330	1.048	-21.23	5.90	0.03	.5012 (ppm)	0.01		95	72 422-2070	Fenpyroximate	
Quantitation	47 Furathiocarb	7.94	0 74	▼	MES_10ppb_02	Chk Std	598248	1156622		1.330	1.100	-13.10	5.90	6.52	1209 (npm)	0.00		01	73 422-2079	Fenpyroximate	
	48 Hexaconazole	7.21	0 74	<u>v</u>	MES_10ppb_03	Chik Std	529240	1060511		1,330	1.145	12.02	5.00	6.52	.1350 (ppm)	0.00		100	74 422.2013	Fenpyroximate	
Processing	49 Hexythiazox	8.39	@ 76	· ·	MES_10ppb_04	Chik Std	569272	1067406		1 220	1.140	12.41	5.00	6.52	0771 (ppm)	0.00		07	75 422.2014	Fennyroximate	
Compounds	50 Hydramethylnon	7.86	a 77	•	MES_TOPpb_03	Chik Std	2016650	5652227		6.660	5.622	-15.50	2.07	2.11	- 2020 (ppm)	0.00	- 1	100	70 422.2013	Fennyroximate	
QAQC	51 Imazalil	5.07	0 70		MES_SOppb_07	Chik Std	2725515	5624691		6 660	5 504	-16.01	2.07	2.11	- 0049 (ppm)	0.00		100	78 422.2073	Fennyroximate	
Reports	52 Imidacloprid	3.40	9 70		MES_S0ppb_02	Chk Std	2044184	5712949		6 660	5.690	-14 72	2.07	2.11	1209 (nnm)	0.00		100	79 422.2074	Fennyroximate	
	53 Ipconazole	7.73	E 80		MES_50ppb_03	Chk Std	2942604	5932363		6.660	5 894	-11.51	2.07	2.11	6457 (ppm)	0.00		100	80 422 2077	Fennyroximate	
	54 Isoproturon	5.17	· · · · ·	2	MES 50ppb_01	Chk Std	2926482	5748267		6.660	5.714	-14.20	2.07	2.11	.4289 (nnm)	0.00		100	81 422,2076	Fennyroximate	
	55 Manaipropamia	5.90	E 82		MES 100mpb 01	Chk Std	5727551	11499522		13,300	11.320	-14.89	1.31	1.32	1.0071 (nnm)	0.00		100	82 422,2079	Fennyroximate	
	57 Metalavyl	5.05	+ 83		MES 100ppb 02	Chk Std	5713260	11301064		13,300	11,127	-16.34	1.31	1.32	.4289 (ppm)	0.00		100	83 422.2076	Fenpyroximate	
	58 Methabenzthiazuron	5.31	⊕ 84	~	MES 100ppb 03	Chk Std	5539564	11624657		13.300	11,442	-13.97	1.31	1.32	.0675 (ppm)	0.00		100	84 422.2075	Fenpyroximate	
	59 Methamidophos	1.90	. 85	1	MES 100ppb 04	Chk Std	5950707	11700400		13.300	11.516	-13.41	1.31	1.32	.2121 (ppm)	0.00		100	85 422.2075	Fenpyroximate	~
	Compound Details		11																		<b>-</b> ↓×
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	Quarreak							iginenti			Mi	nimum # of	fragments p	adad: 1	• ~	Contrary more		#1.5		Convertion Reals 1 of	2 14 00102
	Fenpyroximatem/z: 422	2074						All Fragments	MMS	S_10ppb_0	07 #: 51	84 RT: 8.	80	ceded. I	0100000	#1: Fenp	pyroximate	100 #5184	4 F'ETMS + c ESI Full ms2	400 0000@hcd37 67 [50	0000-53
				T 8 81				#1: 366.1451	F: F	TMS + c E	SI Full n	ns2 400.000	00@hcd37.6	7 [50.0000-53	35.1000]	🔵 #2: Fenp	oyroximate	89 100	0-		
				AA: 10141	727		10	#2: 231.1004		6.0E6								Tage 100	E I		
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	1000000-								5	1.560								#5015		152 422.00@01040.00[10	0.00-45
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Mathed Davalanment	Apex RT: 8.81 Left R	T: 8.77 R	ight RT: 8.8	5						1	50	200	250	300	350				100 200	300 400 50	10
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Figure 6. A quick overview of Fenpyroximate quantitation with library scoring of 100% and  $\Delta$ ppm of  $\leq$  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.

Data	Data Review - Pesticide Cumin Screener [Quan with Unknown] *																			
Heat Ma	р																			
(P)	Retention T	lime	M/Z		Mass	MES_blnk_03 MS Area	MES_100ppb MS Area	.00												^
	-				61 .	= ·	-	•												
13		4.28		250.1799	249.3115	1,353,335,727	1,207	,233,613												_
14		6.16		231.1380	230.2688	1,263,136,908	1,261	,133,688												
15		6.40		233.1537	232.2866	1,262,546,596	1,125	,307,895												
16		6.80		424.1968	423.3794	1,224,185,988	1,091	,537,105												
17		8.19		279.2320	278.3892	1,189,383,617	977	,251,174												
18		5.45		271.0599	270.1930	1, 149, 728, 185	1,069	,735,027												
19		5.87		322.2011	321.3589	1,132,042,781	1,033	676,122												
20		10.39		423.3260	422.5613	1,081,397,582	866	,235,631												
21		4.72		264.1957	263.3354	1,038,278,438	934	,640,507												
22		9.37		372.3112	371,4937	1,010,604,586	789	418,967												
23		5.19		298.1799	297.3442	1,007,824,335	843	,882,524												
24		3.67		350.2171	349.3627	976,124,226	841	,086,289												
Heat Ma	Cross Sam	10 20	ist	A 75. A 760	6.7A 0000	060 000 000	14.1	1000												
Sample	int	-	_	_			P × Peak I	int.	_	_			V X Reak	denti	fe stirm.	_	_			- 0 ×
(E Bat	Status	Filen	ame	Sample	ID Sample N	lame Sample Type	80	Selecto	d a M/Z -	Mass 👳	Mono Isotopic Mass 👍	Retention Time	Pc · at r		Selected	ID Source	ID Source	e Detail	Match Repult Name	hormul
-	<u>6</u>	<u>é</u> a	- 6	ря	- <u>6</u> +	· 64 ·		6× •					6		Au +	Aa •	Aa	-	Aa -	Aa
1 1	•	MES_bi	nk_03 1		MES_blnk_03	Matrix Blank	26		233.1537	232.2066	232.1464	6.40	3 1		- 7	ChemSpider	4445949		Elaidolinolenic acid	C18H300;
2 2	•	MES_10	0ppb_03 1		MES_100ppb	_03 Chk Std	27		251.1643	250.2979	250.1570	6.41	3 2			ChemSpider	4444437		Alpha-Linolenic acid	C18H300;
							20		424.1968	423.3794	423.1895	6.80	6 3			ChemSpider	4444436		Gamma-Linolenic acid	C18H300.
							29		235.1694	234.3036	234.1621	7.10	3							
							30		252.1960	251.3242	251.1887	7.25	3							
							31		199.1482	198.2731	198.1410	7.26	3							
							32		119.0856	118.0782	118.0783	7.26	3							
							33		189.1639	188.1564	188.1566	7.27	3							
							34		207.1744	206.1670	205.1672	7.27	3							
							35		408.2020	407.3850	407.1948	7.76	6							
							36		291.1230	290.2649	290.1158	7.95	5							
							37		279.2320	278.3892	278.2248	8.19	3 🗸							
													> <							>
Group A	verages									- 4	× Cross Sample Peak C	lverlay								<b>→</b> 9 ×
12060	37930										≥ 100 3					4				4_60
11060	37930															$\Lambda$				ME5_100 ppb_03
10560	37930										1 1					/ \			()	
10060	37930														_		~			
9660	1950			1				2			7.7	7.8 7.9	8.0		8.1	8.2	83	8.4	8.5 8.6	

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

Data	Review - P	esticide	Cumin Screener	Qu	an with Unknown															
Heat Ma	Hest Map																			
æ	Retention T	lime	M/Z		Mass -	MES_blnk_03 MS Area	MES_100ppl MS Area	b_03												~
	-				6a •		-													
1		9.72	282.2	792	N/A	(	2.000.	512.904												
2		7.26	119.0	856	118.0782	702,395,574	75	3,898,032												
3		3.30	149.0	1960	148.0886	670,714,865	61	1,504,056												
4		5.99	149.0	961	148.0887	695,490,015	72	2,987,076												
5		5.98	163.1	117	162.1043	2,181,872,701	2,22	5,601,869												
6		7.26	189.1	639	188.1564	3,502,934,653	3,50	6,406,835												
7		5.39	199.1	151	198.1076	835,533,318	76	6,713,014												
8		7.26	199.1	482	198.2731	684,576,985	70	1,955,978												
9		7.26	207.1	744	206.1670	2,996,944,212	3,09	3,396,451												
10		6.11	218.1	540	217.1466	1,916,128,125	1,79	2,226,663												
-11		6.16	231.1	380	230.2688	1,263,136,908	1,26	1,133,688												
12		6.40	233.1	537	232.2866	1,262,546,596	1,12	9,307,895												
Heat Ma	P Cross Sam	7 10 pple Peak		KOA.	3000 800	060 101 067	00	1 107 002												
-				-																- 1 - 2
Sample	list	El.		a a la l	D Complet	-	Peak	lict Colorite	4 a 14/7	Maria	Mana Instania Man	Between Trees	Per a	ik Iden	trications			_		
Ra out	Status	riter	ame san	npre	Sample N	sample typ	89	Selecte	a a NVZ -	Midss -	mono isocopic mass	Netenbon time = -	e a		Selected	ID Source	ID Source D	letail 👳 Mate	h Result Name	Formul
		Ba	• 54		• 51	* 64 *		~ ~	-				50 L		<u>A</u> a •	<u>6</u> • •	60	- <u>A</u> a	•	60
		MES_B	ink_us I		MES_DIRK_US	Chi Chi Chi	56		291.1228	290,2030	290.1155	7.96		1		ChemSpider	4446508	(9Z)-9	-Octadecenamide	C18H35N
2 ·	2 🛡	MES_1	Nppb_US 1		MES_TOUPPD	US Chik Sta	3/		279,2317	278.3903	276,2244	0.19		2	~	ChemSpider	39176	5422		C18H35N
							20		207 2422	206 4027	206 2251	9.61		3		ChemSpider	4510066	Elaida	mide	C18H35N
							39		457 2674	456.6100	456 2001	8.92								
							40		619 2020	618 8926	618 2916	9.24								
							47	H	255,2317	254.3742	254,2244	9.27								
							43	H	281,2473	280.4051	280,2400	9.27								
							44		372,3107	371,4950	371,3035	9.37	2							
							45	Ä	496,3399	495,5465	495.3326	9.42								
							46		522.3555	521.5767	521.3483	9.45	3							
							47		282.2792	281,4352	281.2719	9.72	3							
							<						> <							>
Group A	werages		_						_	- 0	× Cross Sample Peak O	verlay								• * ×
2000											100-3									MES. No.
1500											100					N				MED_100
1000											1 50-					I				MM6 <sup>-03</sup>
5000	000000										1910					/ \				
	0			-								94	95	3.0		97 98	99	10.0	10.1	10.2
				1					2					3.0		RT(min)				



creener rea		oniation																		
	Mass		MES_ MS A	blnk_03 rea	MES_100ppb_04 MS Area															^
	A																			
194,1177		193.11	03	27,354,465	19,384,298															
214,1227		N	(A	0	314.605.234															
251.0230		N	(A	0	683,643,015															
177.1307		176.13	13	22,226,929	23,337,903															
131.0526		N	/A	0	312,472,918															
238.0898		N	A	0	387,011,192															
242.1176		N	/Δ	0	329,594,603															
159.0654		158.05	79	57,513,644	3,186,449															
159.0652		158.05	78	57,513,644	33,957,407															
236.1130		235.10	56	58,840,889	34,452,632															
198.1279		197.25	23	29,239,472	25,397,186															
207.1592		206.15	18	29,909,710	27,721,632															v.
Peak List		_		_			_		_	_	<b>▼</b> 0 ×	Peakide	ntifications							• • ×
E Select	ted 0	M/7 👳	Mass 👝	Mono Isotopic	Mass 😄 Retention T	ime 🚽 💿 Potential ID 🚽	Area 😄	Height a	Database o	mzVault	Elemental C ^	ALC: N	Selected	ID Source	ID Source	Detail	Match Result Name	Formula	Instanic Patter	n Score (%)
Aa						• As •			Aa 🕶	Aa 🕶	As	8-11	buccies -	to bource in	in source	-		to remote to	-	rotore (ny 5
1	1	94 1176	193 1102		193.1104	2.68 3	19 384 298	6 290 976 25	N/A	N/A	N/A	1.1	· ·	C* • •	200222	•			-	
2	1	14 1227	213.1153		213.1155	2.94 3	14 605 234	54 138 464 00	N/A	N/A	N/A			Chemopider	20226		octhilinone [ANSI]	CHIMISNOS		NVA NVA
3		51.0230	250.0156		250.0157	3.05 2	83 643 015	52 026 943.92	N/A	N/A	C3H8O8N4P	6		Chemopider	500000		1212020	CIAITISNO		NVA NVA
4	1	77.1387	176.1313		176.1314	3.15 4	23,337,903	9,193,801.97	N/A	N/A	C11H17NZ			chemaphoer	STREET,		2000210	CHAILING		10011
5		31.0526	130.0452		130.0453	3.17 3	12,472,918	71,218,128.05	N/A	N/A	N/A									
6		38.0898	237.0824		237.0825	3.21 3	87,011,192	71,598,808.01	N/A	N/A	N/A									
7 0		42.1176	241.2477		241.1104	3.26 3	29,594,603	34,046,417.62	N/A	N/A	N/A									
8 [		159.0653	158.0579		158.0580	3.43 3	3,186,449	2,133,602.54	N/A	N/A	N/A									
9 [		59.0653	158.0579		158.0581	3.50 3	33,957,407	18,713,841.10	N/A	N/A	N/A									
10 0		36.1131	235.1056		235.1058	3.50 3	34,452,632	18,749,936.16	N/A	N/A	N/A									
11 D		198.1280	197.1205		197.1207	3.59 0	25,397,186	11,967,173.56	N/A	N/A	N/A									
12 F	7 3	07.1593	206.1519		206.1520	3.72 0	27 721 632	6 869 346.62	N/A	N/A	N/A ~						_			
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	1					2				25	2.6	27	2	8 2	9 RT(min)	3.0	3.1	3.2	3.3	3.4
									-											





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).<sup>2</sup>

#### 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<sup>2</sup> and MS<sup>2</sup> 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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