Quantitative and Qualitative Confirmation of Pesticides in Beet Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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Application Note 617

Key Words
Q Exactive Focus, Orbitrap, pesticides, high resolution, accurate mass, quantitation, target screening, unknown screening, retrospective data analysis

Goal
To describe a method for the analysis of pesticides, showing the utility of a full-scan data-dependent MS/MS workflow to achieve regulatory levels while providing a complete targeted and screening analysis using a high-resolution, accurate mass (HRAM) spectral library for identification and confirmation.

Introduction
As world agricultural trade has expanded and concerns over food safety have grown, the enforcement of stricter pesticide regulations has become of utmost importance. In 2006, Japan introduced the Positive List System that established maximum residue levels (MRLs) for hundreds of agricultural chemicals in food, including approximately 400 pesticides, and set a uniform limit of 10 µg/kg (ppb) for chemicals for which MRLs have not been determined.[1] In 2008, the European Parliament implemented Regulation (EC) No. 396/2005, which harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 10 µg/kg for all pesticide/commodity combinations for which no MRLs have been set.[2] A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon its completion in 2009, led to the approval of only about 250 substances and effectively set the permissible levels of over 700 de-listed pesticides to the default limit.[3] The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residues.

Here, a method utilizing the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer is described. It consists of a generic chromatographic method and a full-scan data-dependent MS/MS (FS-ddMS^2) mass spectrometric method with library searching and fragment confirmation. The FS-ddMS^2 approach was used to generate calibration curves and analyze samples for targeted known compounds. In the typical acquisition setup demonstrated here, a simple full-scan data-dependent MS/MS experiment was associated with new preset confirmation settings for easier and faster method development (Figures 1 and 2).

For evaluation of the method, spiked matrix samples were analyzed by high-resolution, accurate-mass LC-MS/MS.

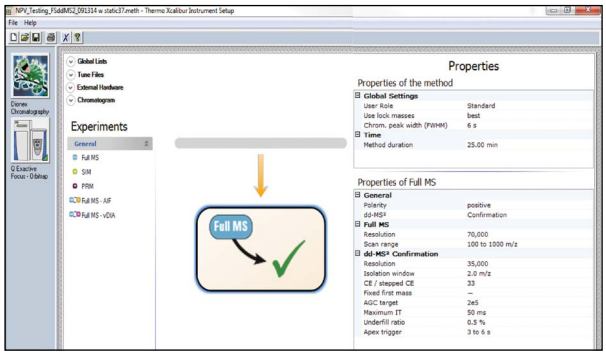

Figure 1. Instrument Setup page, showing full-scan data-dependent MS/MS.

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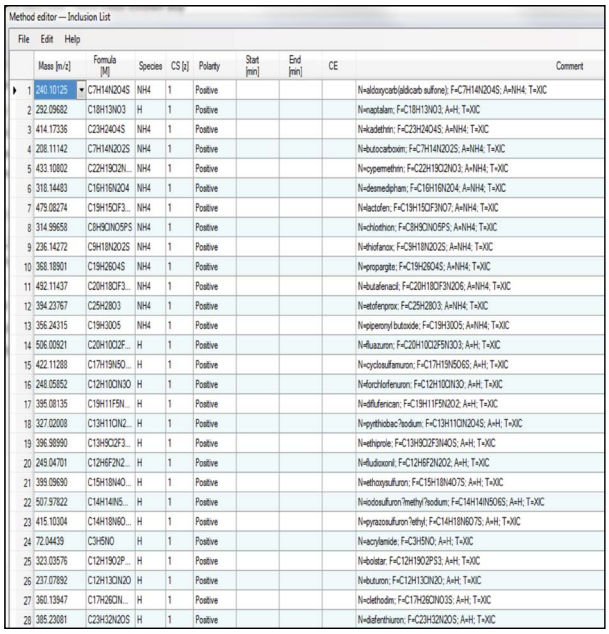


	Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	CE	Comment
1	240.10125	C7H14N2O4S	NH4	1	Positive				N=aldoxycarb/aldicarb sulfone); F=C7H14N2O4S; A=NH4; T=XIC
2	292.09682	C18H13NO3	H	1	Positive				N=naptalam; F=C18H13NO3; A=H; T=XIC
3	414.17336	C23H24O4S	NH4	1	Positive				N=kadethrin; F=C23H24O4S; A=NH4; T=XIC
4	208.11142	C7H14N2O2S	NH4	1	Positive				N=butocarboxim; F=C7H14N2O2S; A=NH4; T=XIC
5	433.10802	C22H19Cl2N...	NH4	1	Positive				N=cypermethrin; F=C22H19Cl2NO3; A=NH4; T=XIC
6	316.14483	C16H16N2O4	NH4	1	Positive				N=desmedipham; F=C16H16N2O4; A=NH4; T=XIC
7	479.08274	C19H15OF3...	NH4	1	Positive				N=lactofen; F=C19H15OF3NO7; A=NH4; T=XIC
8	314.99658	C8H9ClNO5PS	NH4	1	Positive				N=chlorthion; F=C8H9ClNO5PS; A=NH4; T=XIC
9	236.14272	C9H18N2O2S	NH4	1	Positive				N=thiofanox; F=C9H18N2O2S; A=NH4; T=XIC
10	368.18901	C19H28O4S	NH4	1	Positive				N=propargite; F=C19H28O4S; A=NH4; T=XIC
11	492.11437	C20H18OF3...	NH4	1	Positive				N=butafenacil; F=C20H18OF3N2O6; A=NH4; T=XIC
12	394.23767	C25H28O3	NH4	1	Positive				N=etofenprox; F=C25H28O3; A=NH4; T=XIC
13	356.24315	C19H30O5	NH4	1	Positive				N=piperonyl butoxide; F=C19H30O5; A=NH4; T=XIC
14	506.00921	C20H10O2F...	H	1	Positive				N=fluazuron; F=C20H10O2F5N3O3; A=H; T=XIC
15	422.11288	C17H19N5O...	H	1	Positive				N=cyclosulfamuron; F=C17H19N5O6S; A=H; T=XIC
16	248.05852	C12H10ON3O	H	1	Positive				N=forchlorfenuron; F=C12H10ON3O; A=H; T=XIC
17	395.08135	C19H11F5N...	H	1	Positive				N=diflufenican; F=C19H11F5N2O2; A=H; T=XIC
18	327.02008	C13H11ClN2...	H	1	Positive				N=pyrithiobac?sodium; F=C13H11ClN2O4S; A=H; T=XIC
19	396.98990	C13H9Cl2F3...	H	1	Positive				N=ethiprole; F=C13H9Cl2F3N4OS; A=H; T=XIC
20	249.04701	C12H6F2N2...	H	1	Positive				N=fludioxonil; F=C12H6F2N2O2; A=H; T=XIC
21	399.09690	C15H18N4O...	H	1	Positive				N=methoxysulfuron; F=C15H18N4O7S; A=H; T=XIC
22	507.97822	C14H14IN5...	H	1	Positive				N=iodosulfuron?methyl?sodium; F=C14H14IN5O6S; A=H; T=XIC
23	415.10304	C14H18N6O...	H	1	Positive				N=pyrazosulfuron?ethyl; F=C14H18N6O7S; A=H; T=XIC
24	72.04439	C3H5NO	H	1	Positive				N=acrylamide; F=C3H5NO; A=H; T=XIC
25	323.03576	C12H19O2P...	H	1	Positive				N=bolstar; F=C12H19O2PS3; A=H; T=XIC
26	237.07692	C12H13ClN2O	H	1	Positive				N=buturon; F=C12H13ClN2O; A=H; T=XIC
27	360.13947	C17H26ClN...	H	1	Positive				N=clethodim; F=C17H26ClNO3S; A=H; T=XIC
28	385.23081	C23H32N2OS	H	1	Positive				N=diafenthiuron; F=C23H32N2OS; A=H; T=XIC

Figure 2. An example of an inclusion list that was added for targeted confirmation of known pesticides in the sample.

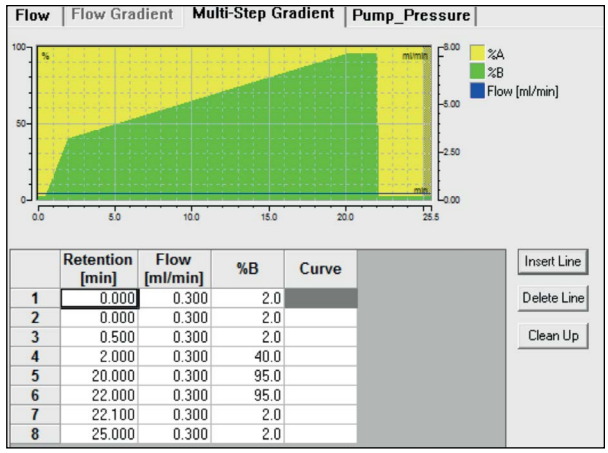
Sample Preparation

Beet samples, provided by the California Department of Food and Agriculture, were extracted using a modified QuEChERS method. Pesticide stock standards (ULTRA Scientific, N. Kingston, RI) were spiked into the QuEChERS extract. Then, the appropriate amount of acetonitrile was added to adjust the organic composition of the final standard solution to 50:25:25 water/matrix/acetonitrile. The concentration of the standards ranged from 0.05 to 200 µg/kg.

Liquid Chromatography Method

A generic LC method was used for all samples:

Instrumentation	Thermo Scientific™ Dionex™ UltiMate™ 3000 LC system, consisting of: · Pump: HPG-3200RS · Autosampler: WPS3000TRS · Column Warmer: TCC3000RS · Degasser: SRD3400
Column	Thermo Scientific™ Accucore™ aQ 100 x 2.1 mm, 2.6 µm particle size (p/n 17326-102130)
Column temperature	30 °C
Mobile phase A	0.1% formic acid, 5 mM ammonium formate in water
Mobile phase B	0.1% formic acid, 5 mM ammonium formate in methanol
Gradient	Refer to Figure 3
Sample injection	10 µL
Instrument run time	25 min



	Retention [min]	Flow [ml/min]	%B	Curve
1	0.000	0.300	2.0	
2	0.000	0.300	2.0	
3	0.500	0.300	2.0	
4	2.000	0.300	40.0	
5	20.000	0.300	95.0	
6	22.000	0.300	95.0	
7	22.100	0.300	2.0	
8	25.000	0.300	2.0	

Figure 3. Flow gradient.

Mass Spectrometry Method

A generic FS-ddMS^2 method on a Q Exactive Focus MS system was used for all samples as described below:

Full Scan	
Resolution setting	70,000 (FWHM) at *m/z* 200
Mass range	100–1000 *m/z*
ddMS^2	
Resolution setting	35,000 (FWHM) at *m/z* 200
Isolation windows	2.0 *m/z*
Spray voltage	3500 V
Sheath gas	35 arb
Aux gas	10 arb
Sweep gas	1 arb
Capillary temperature	325 °C
Heater temperature	350 °C
RF-lens level	50
HCD collision energy	33 eV

Data Processing

Data processing was performed using Thermo Scientific™ TraceFinder™ software version 3.2. For generation of extracted ion chromatograms, an extraction window of 5 ppm was used. For targeted screening, a built-in compound database (>1500 compounds), consisting of compound name, precursor and fragment *m/z* values, and retention time, was used together with a spectral library (>7500 spectra) for confirmation of targeted residues.

Results and Discussion

Data analysis was performed within TraceFinder software with the help of green, yellow, and red flags that can quickly be sorted for review. Figure 4 demonstrates the capability of the flagging feature within TraceFinder software, which can identify issues with compounds and help the analyst make quick decisions if the sample contains that compound.

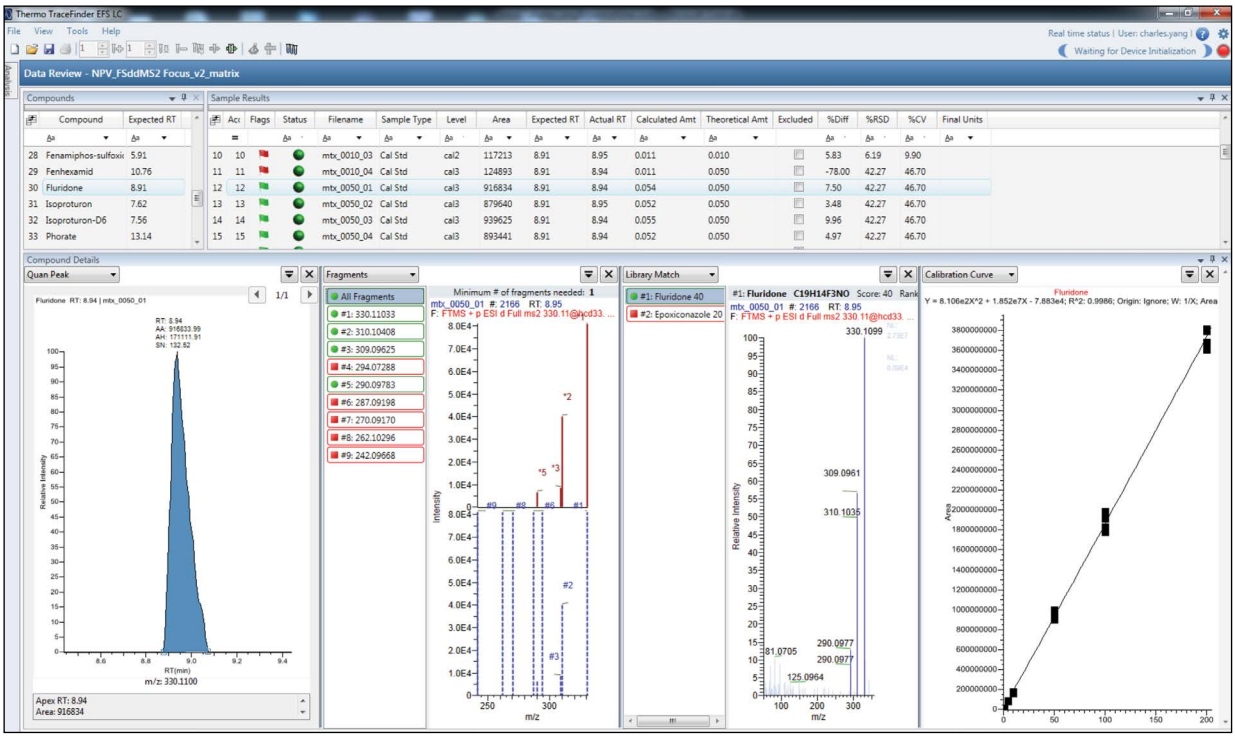


Figure 4. Flagging feature, showing that one fluridone sample has a green flag while the other sample has a red flag. A green flag indicates that all of the parameters were met and there was no issue with the calibration curve. A red flag indicates that there was a problem with the sample from the library search, fragment ion confirmation, or the calculated amount was out of range. The flagging details describe the issues with the sample.

Figure 5 demonstrates the compound details in the Quan Peak, Fragment Matching, Spectra Matching, and Calibration Curve views of TraceFinder software, which can assist the analyst in quickly looking through the data set for confirmation.

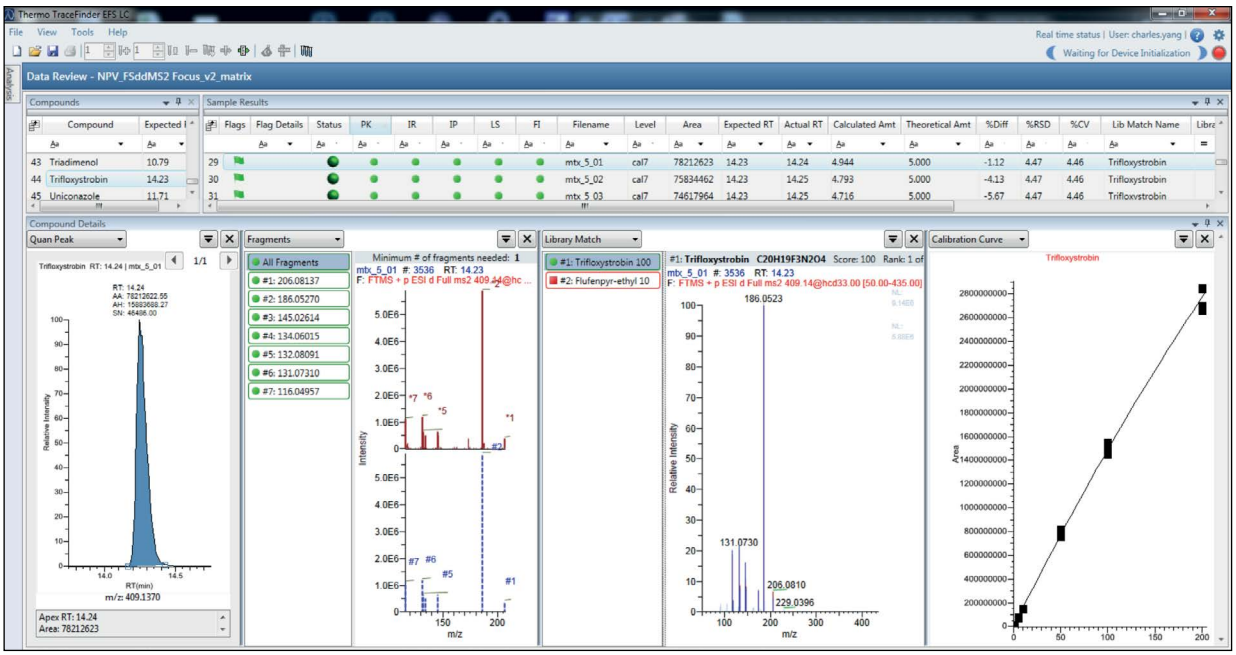


Figure 5. Tryfloxystrobin at 5 ppb, showing compound details in lower portion to highlight quick data review.

The detection results of the pesticides analyzed in the beet matrix are shown in Table 1. Detection limits varied depending on the compound. The determination of the limit of quantitation (LOQ) was based on the presence of a minimum of one fragment ion as well as reproducibility at each level as stated by the EU SANCO regulations.[4] Table 1 also shows the %RSD of n=4 at each level and available EU regulation limits for listed pesticides.[4] All RSDs were found to be well below the guidelines which require RSDs of less than 15% in order to be accepted as the LOQ.

All compounds showed good calibration curves with R^2 better than 0.99 as shown in Table 2.

Table 1. LOD/LOQ based on fragment confirmation and %RSD values compared to available EU regulation limits for pesticides.

Compound	LOD (µg/kg)	%RSD	LOQ (µg/kg)	%RSD	EU Regulation Limits (µg/kg)
Allethrin	0.91	6.25	5.13	3.27	
Atrazine	0.10	6.09	0.58	10.34	50
Azoxystrobin	0.10	3.99	0.55	11.77	15,000
Bendiocarb	0.73	6.88	0.73	6.88	
Benoxacor	0.09	6.99	0.53	8.99	
Bioresmethrin	0.77	4.44	5.24	4.98	
Boscalid	0.10	4.37	0.55	12.19	30,000
Bupirimate	0.10	3.83	0.10	3.83	50
Cadusafos	0.44	11.63	0.88	6.11	10
Carbendazim	0.53	12.39	1.04	5.58	100
Chloropyrifos	0.44	11.79	0.91	5.41	50
Coumaphos	0.10	6.00	0.60	13.19	
Cyazofamid	0.44	11.26	0.86	4.66	10
Cyproconazole	0.44	11.98	0.90	5.65	50
DEF	0.09	5.67	0.59	8.80	
Dimethenamid	0.10	2.23	0.57	11.82	10
DMST	0.09	3.46	0.58	10.71	
Fenamiphos-sulfone	0.09	5.97	0.57	12.16	20
Fluoridone	0.09	5.00	0.53	12.34	
Isoproturon	0.10	7.37	0.63	12.76	10
Phorate	0.91	5.23	4.92	2.46	10
Propetamphos	0.73	7.66	4.92	3.19	
Rotenone	0.05	42.34	0.10	4.72	10
Sulprofos	0.45	9.10	0.86	2.88	
Spirodiclofen	0.44	9.98	0.86	5.59	20
Thiobencarb	0.47	8.55	0.91	3.61	10
Triadimenol	5.12	2.93	5.12	2.93	100
Trifloxystrobin	0.10	2.91	0.10	2.91	20
Uniconcazole	0.45	13.48	0.87	4.54	

Table 2. R^2 results of 29 pesticides in beet matrix.

Compound	R^2
Allethrin	0.9989
Atrazine	0.9988
Azoxystrobin	0.9983
Bendiocarb	0.992
Benoxacor	0.9987
Bioresmethrin	0.9989
Boscalid	0.9988
Bupirimate	0.9984
Carbendazim	0.9982
Chlorpyrifos	0.9987
Coumaphos	0.9989
Cyazofamid	0.9985
Cyproconazole	0.9987
DEF	0.9988
Dimethenamid	0.9987
DMST	0.9989
Fenamiphos-sulfone	0.9988
Fluridone	0.9986
Isoproturon	0.9985
Phorate	0.9984
Propetamphos	0.9985
Rotenone	0.9986
Spirodiclofen	0.9983
Sulprofos	0.9989
Thiobencarb	0.9989
Thiodicarb	0.9986
Triadimenol	0.9977
Trifloxystrobin	0.9987
Uniconazole	0.9988

Figure 6 shows the capability of the Q Exactive Focus MS to scan quickly with polarity switching at 10 ppb. Due to the many pesticides that were spiked into the matrix, it was necessary to include internal standards to check and correct for shifts in retention times, as shown in Figures 7, 8, and 9.

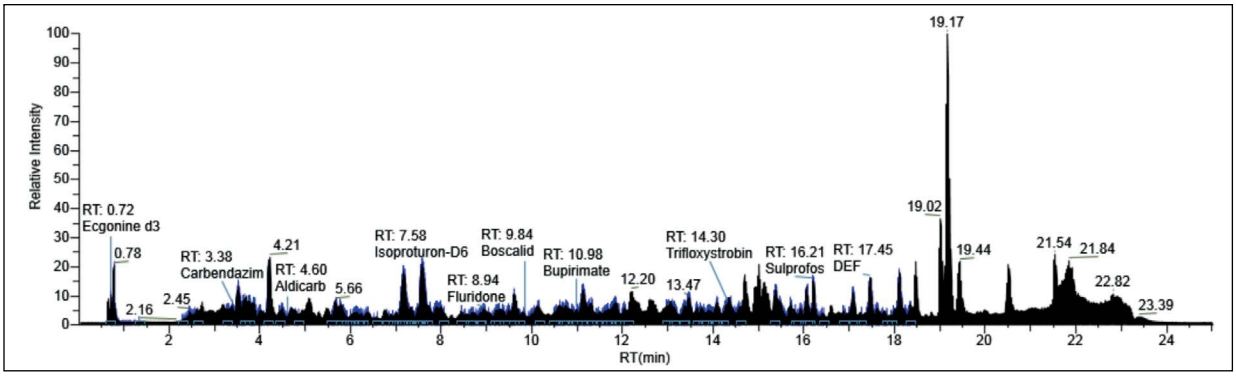


Figure 6. Total ion current (TIC) chromatogram of spiked pesticides in beets at 10 ppb.

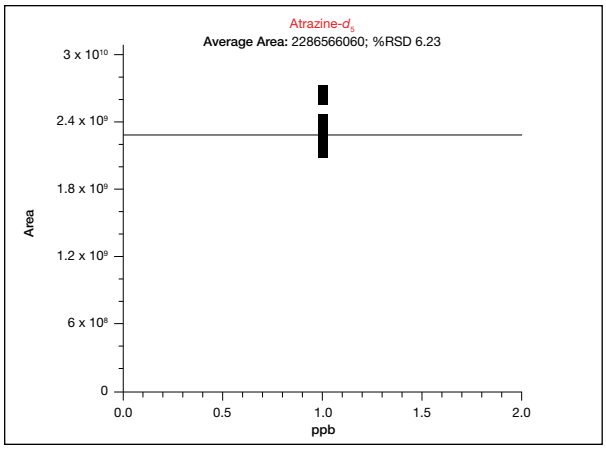


Figure 7. Atrazine-d_5, %RSD = 6.23 (beet matrix).

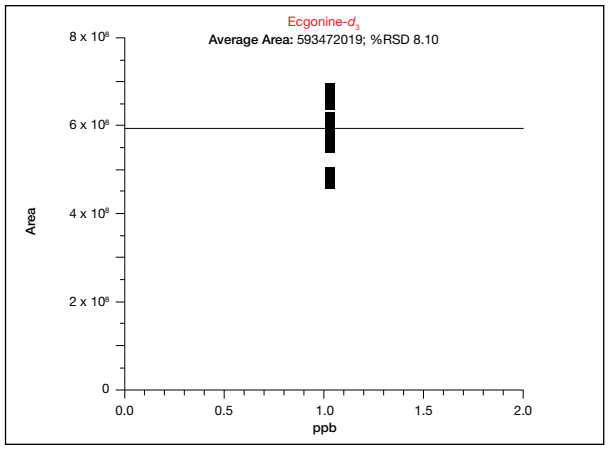


Figure 8. Ecgonine-d_3, – early eluter, %RSD = 8.10 (beet matrix).

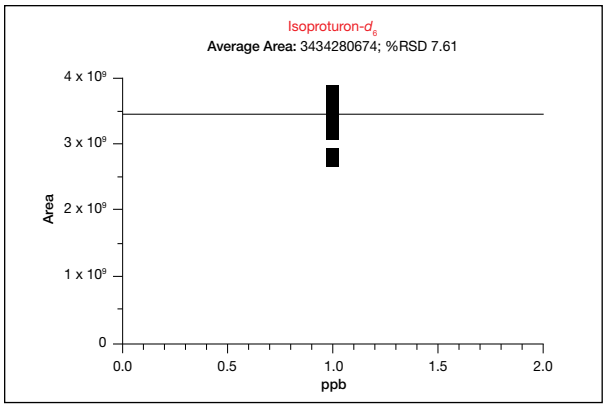


Figure 9. Isoproturon-d_6, %RSD = 7.61 (beet matrix).

Increasingly, more and more compounds are being analyzed in a single run, which can cause issues with co-elutors. A new HRAM MS/MS spectral library and compound database has been generated that is fully integrated and searchable using TraceFinder software to identify compounds with high levels of confidence. The spectral library includes more than five individual, high-resolution spectra for every compound it contains. Each compound was analyzed at multiple collision energies. Figures 10–13 showcase the matching significance of having an extensive spectral library with more than five individual spectra per compound.

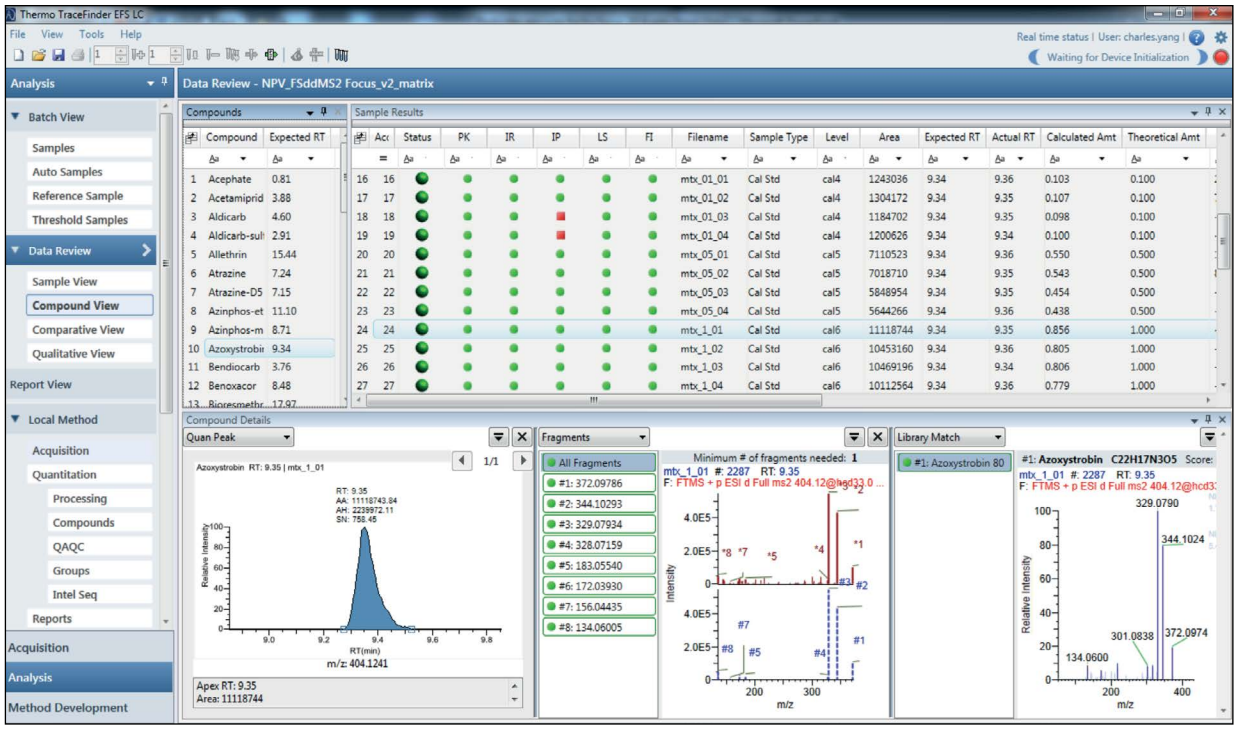


Figure 10. Azoxystrobin library match confirmation with fragmentation confirmation at 1 ppb, showing a library match score of 80% confidence in the lower right pane.

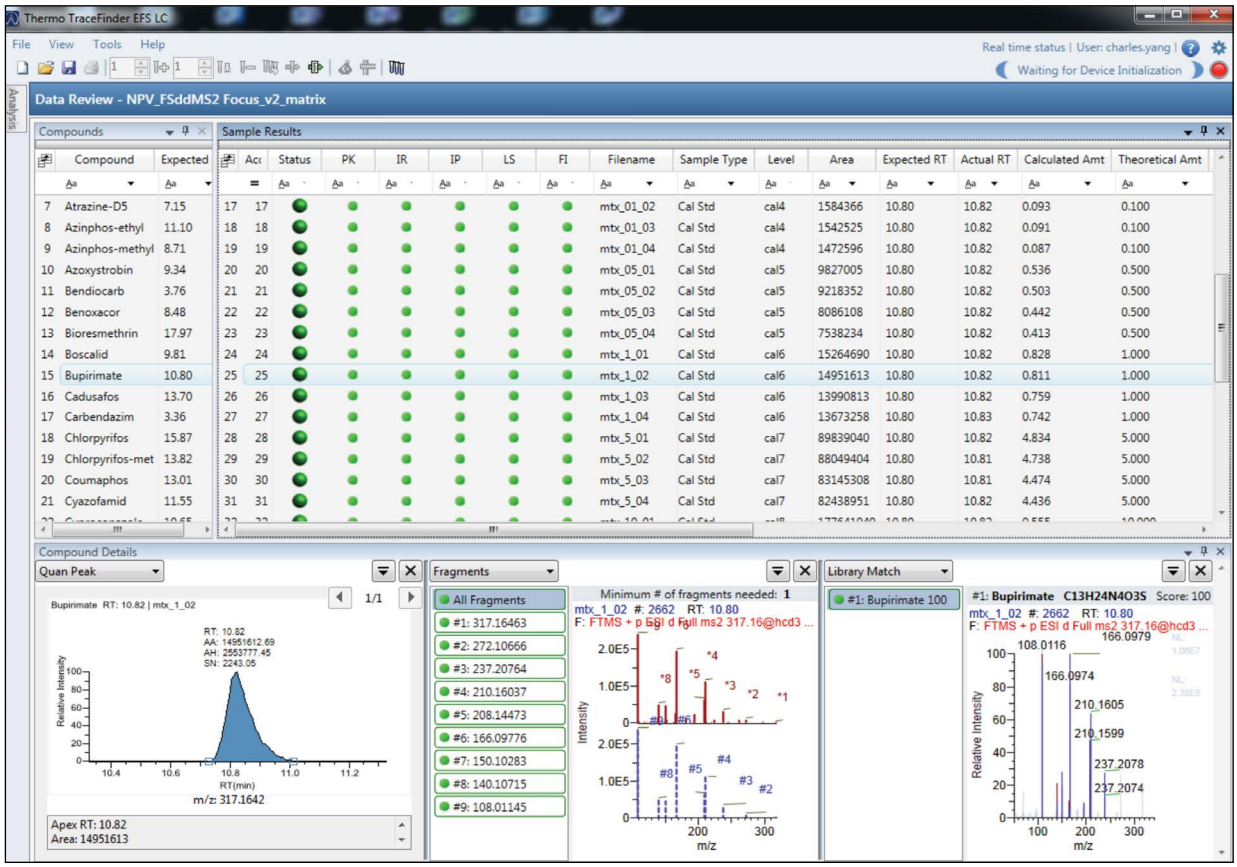


Figure 11. Bupirimate library match confirmation with fragmentation confirmation at 5 ppb, showing a library match score of 100% confidence in the lower right pane.

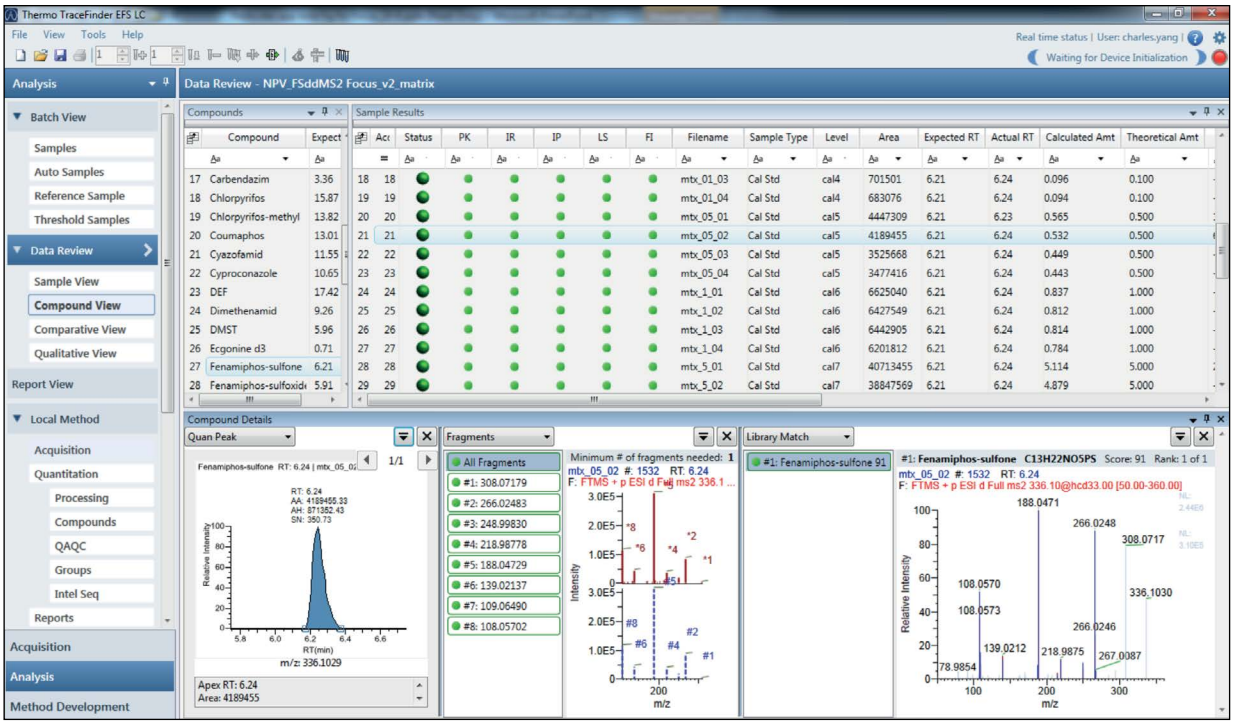


Figure 12. Fenamiphos-sulfone library match confirmation with fragmentation confirmation at 5 ppb, showing a library match score of 91% confidence in the lower right pane.

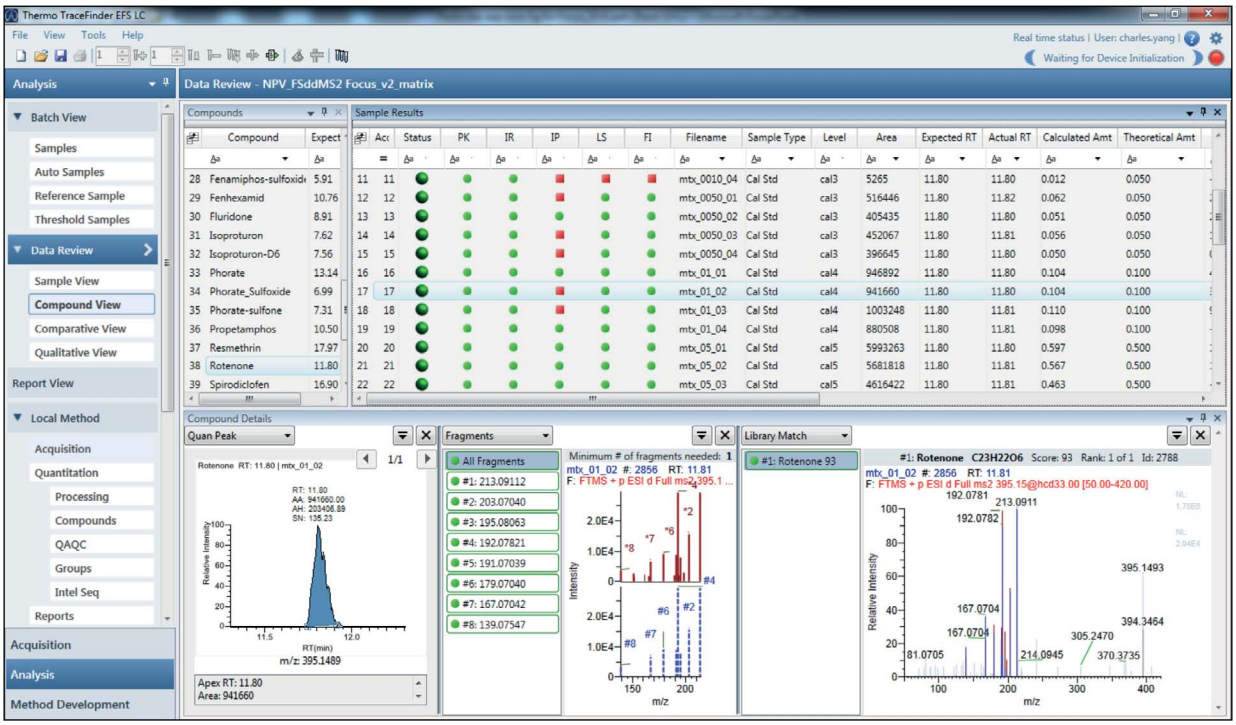


Figure 13. Rotenone library match confirmation with fragmentation confirmation at 1 ppb, showing a library match score of 93% confidence in the lower right pane.

Conclusion

The benchtop Q Exactive Focus MS provided easy access to full quantitative, confirmation, and screening data in a single injection. The high resolution and mass accuracy enabled quantification of the compounds over a wide dynamic range (0.05–200 ng/mL) with linear fit, correlation better than 0.99, and %RSD below 15%. Confirmation by the precursor-selected MS/MS gave an option to use spectral and library matching and pattern recognition within TraceFinder software. The new environmental and food safety HRAM spectral library provided more confidence in the data with its multiple, high-resolution spectra at numerous collision energies for use in any experiment.

References

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