Application Note 579

Use of UHPLC and High-Resolution MS for Quantitative Analysis of Pesticides in Onion Matrix

Charles Yang, Leo Wang, Dipankar Ghosh, Thermo Fisher Scientific, San Jose, CA

Key Words

Exactive Plus, TraceFinder, high resolution, accurate mass, food safety, UltiMate UHPLC

Goal

To demonstrate the ability of a high-resolution, accurate-mass UHPLC-MS system, combined with appropriate application-specific workflow software, to provide fast, confident, and precise screening and quantitative analysis of pesticides in onion matrix.

Introduction

Monitoring for pesticide and other chemical residues in produce is essential to maintaining a safe food supply. Monitoring data can also be used to better understand the relationship of pesticide residues to agriculture practices, enhance integrated pest management, and support the export of U.S. commodities. Monitoring is typically done by public agencies, but budget restrictions have increased pressure on these agencies to improve productivity while lowering costs.

Traditionally, triple quadrupole mass spectrometers have been used for the identification and quantitation of pesticide and chemical residues. However, MS/MS analysis with triple quadrupole mass spectrometers requires time-consuming selection of mass transitions and optimization of collision energies. The introduction of affordable benchtop, Orbitrap™-based, high-resolution, accurate-mass (HR/AM) mass spectrometers has provided an alternative method for unequivocal identification of trace contaminants without time-consuming MS/MS optimization.

A liquid chromatography/mass spectrometry methodology employing ultrahigh performance liquid chromatography (UHPLC) and HR/AM mass spectrometry makes it possible to identify, quantify, and confirm more trace-level contaminants in complex mixtures in a single analytical run. The results of this unique solution are improved sensitivity and precision, as well as unmatched throughput.

Experimental

Sample Preparation

Onion was 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. 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 thoroughly. A mixture of 120 pesticides with different starting concentrations was prepared in neat matrix (70:30 methanol/water) to make the standard calibration curve and spiked into onion matrix to determine if there was any ion suppression.

Liquid Chromatography

Chromatographic analysis was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC UHPLC system with high-pressure mixing binary pump and 35 µL gradient mixing kit. High-purity Fisher Chemical LC/MS solvents were used.

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The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD aQ™ column (50 x 2.1 mm, 1.9 µm)
Oven:	TCC-3300RS
Autosampler:	WPS-3000RS thermostated autosampler
Pump:	HPG3200RS binary with 35 µL gradient mixing kit, SRD-3400 solvent rack, and degasser
Mobile Phase A:	Water with 0.1% formic acid and 4 mM ammonium formate
Mobile Phase B:	Methanol with 0.1% formic acid and 4 mM ammonium formate
Flow Rate:	300 µL/min
Column Temperature:	40 °C
Sample Injection Volume:	5 µL

Gradient:	Gradient Time (min)	%A	%B
	-2.50	98	2
	0.00	98	2
	0.25	70	30
	10.00	0	100
	12.49	0	100
	12.50	98	2

Mass Spectrometry

All samples were analyzed on a Thermo Scientific Exactive™ Plus benchtop Orbitrap mass spectrometer.

The MS conditions were as follows:

Ion Source:	Heated electrospray (HESI-II)
Ion Mode:	Positive/Negative
Capillary Temperature:	280 °C
Vaporizer Temperature:	295 °C
Spray Voltage:	2200 V
Sheath Gas:	32 arbitrary units
Aux Gas:	7 arbitrary units
Scan Type:	Full MS scan
Mass Range:	*m/z* 120–1000
Mass Resolution:	70,000

Unlike triple quadrupole mass spectrometers, the high-resolution, accurate-mass Exactive Plus instrument required no optimization of mass transitions or collision energies for each analyte. Therefore, the effort for method development was significantly reduced. Table 1 lists the pesticides targeted in this analysis.

Data Analysis

Data processing was carried out with Thermo Scientific TraceFinder™ software for quantitation and targeted-screening workflows. Specificity of analysis was achieved by applying a mass extraction window of 5 ppm to the theoretical mass of the analytes.

Table 1. Targeted pesticides and their associated retention times (RT), actual and theoretical *m/z*, and calculated mass errors

Compound	RT	Formula	Theoretical *m/z*	Detected *m/z*	Delta (ppm)
Acetamiprid	2.27	$C_{10}H_{11}ClN_4$	223.0745	223.0746	0.51
Aldicarb	2.80	$C_7H_{14}N_2O_2S$	208.1114	208.1117	1.23
Aldicarb sulfone	1.49	$C_7H_{14}N_2O_4S$	240.1013	240.1013	0.14
Aldicarb sulfoxide	1.55	$C_7H_{14}N_2O_3S$	224.1063	224.1065	0.48
Atrazine	4.38	$C_8H_{14}ClN_5$	216.1010	216.1013	1.03
Azinphos methyl	5.01	$C_{10}H_{12}N_3O_3PS_2$	318.0130	318.0137	1.97
Azinphos methyl OA	2.90	$C_{10}H_{12}N_3O_4PS$	302.0359	302.0359	-0.10
Azoxystrobin	5.40	$C_{22}H_{17}N_3O_5$	404.1241	404.1245	1.03
Bendiocarb	3.52	$C_{11}H_{13}NO_4$	224.0917	224.0918	0.28
Benoxacor	4.97	$C_{11}H_{11}Cl_2NO_2$	260.0240	260.0241	0.57
Bifenazate	6.04	$C_{17}H_{20}N_2O_3$	301.1547	301.1550	0.99
Boscalid	5.61	$C_{18}H_{12}Cl_2N_2O$	343.0399	343.0403	1.07
Buprofezin	7.70	$C_{16}H_{23}N_3OS$	306.1635	306.1637	0.67
Carbaryl	3.88	$C_{12}H_{11}NO_2$	202.0863	202.0864	0.62
Carbofuran	3.52	$C_{12}H_{15}NO_3$	222.1125	222.1126	0.42
Carbofuran, 3-hydroxy	2.19	$C_{12}H_{15}NO_4$	255.1339	255.1339	-0.09
Carboxin	3.77	$C_{12}H_{13}NO_2S$	236.0740	236.0741	0.38
Carfentrazone ethyl	6.62	$C_{15}H_{14}Cl_2F_3N_3O_3$	429.0703	429.0706	0.70
Chlorpyrifos OA	6.37	$C_9H_{11}Cl_3NO_4P$	350.9830	350.9831	0.31
Clofentezine	7.27	$C_{14}H_8Cl_2N_4$	303.0199	303.0200	0.37
Clothianidin	2.03	$C_6H_8ClN_5O_2S$	250.0160	250.0162	0.82
Cymoxanil	2.53	$C_7H_{10}N_4O_3$	199.0826	199.0828	1.07
Difenoconazole	7.40	$C_{19}H_{17}Cl_2N_3O_3$	406.0720	406.0723	0.79
Diflubenzuron	6.50	$C_{14}H_9ClF_2N_2O_2$	311.0393	311.0395	0.39
Dimethomorph	5.80	$C_{21}H_{22}ClNO_4$	388.1310	388.1313	0.63
Dinotefuran	1.49	$C_7H_{14}N_4O_3$	203.1139	203.1140	0.50
Diuron	4.68	$C_9H_{10}Cl_2N_2O$	233.0243	233.0245	0.71
Famoxadone	7.04	$C_{22}H_{18}N_2O_4$	392.1605	392.1608	0.88
Fenamidone	5.46	$C_{17}H_{17}N_3OS$	312.1165	312.1167	0.51
Fenamiphos sulfone	3.77	$C_{13}H_{22}NO_4PS$	320.1080	320.1081	0.22
Fenamiphos sulfoxide	3.93	$C_{13}H_{22}NO_5PS$	336.1029	336.1029	0.09
Fenbuconazole	6.46	$C_{19}H_{17}ClN_4$	337.1215	337.1216	0.57
Fludioxonil	5.71	$C_{12}H_6F_2N_2O_2$	266.0736	266.0737	0.41
Fluridone	5.19	$C_{19}H_{14}F_3NO$	330.1100	330.1100	-0.12
Flutolanil	5.76	$C_{17}H_{16}F_3NO_2$	324.1206	324.1207	0.42
Formetanate	1.43	$C_{11}H_{15}N_3O_2$	222.1237	222.1238	0.48
Halosulfuron methyl	5.96	$C_{13}H_{15}ClN_6O_7S$	435.0484	435.0490	1.28
Hexaconazole	6.98	$C_{14}H_{17}Cl_2N_3O$	314.0821	314.0823	0.42
Hexythiazox	8.18	$C_{17}H_{21}ClN_2O_2S$	353.1085	353.1087	0.65
Imazalil	4.49	$C_{14}H_{14}Cl_2N_2O$	297.0556	297.0559	0.95
Imidacloprid	1.99	$C_9H_{10}ClN_5O_2$	256.0596	256.0596	-0.03
Indoxacarb	7.54	$C_{22}H_{17}ClF_3N_3O_7$	528.0780	528.0785	0.95
Isoprocarb	4.39	$C_{11}H_{15}NO_2$	194.1174	194.1178	1.33
Linuron	5.34	$C_9H_{10}Cl_2N_2O_2$	249.0192	249.0194	0.92
Metalaxyl	4.59	$C_{15}H_{21}NO_4$	280.1543	280.1546	0.85
Methidathion OA	2.70	$C_6H_{11}N_2O_5PS_2$	286.9920	286.9919	-0.43
Methiocarb	5.38	$C_{11}H_{15}NO_2S$	226.0896	226.0898	0.76
Methomyl	1.45	$C_5H_{10}N_2O_2S$	163.0536	163.0537	0.74

Table 1 (continued). Targeted pesticides and their associated retention times (RT), actual and theoretical *m/z*, and calculated mass errors

Compound	RT	Formula	Theoretical *m/z*	Detected *m/z*	Delta (ppm)
Methoxyfenozide	5.86	$C_{22}H_{28}N_2O_3$	369.2173	369.2176	0.79
Metribuzin	3.35	$C_8H_{14}N_4OS$	215.0961	215.0963	0.67
Monocrotophos	1.74	$C_7H_{14}NO_5P$	224.0682	224.0684	0.83
Myclobutanil	5.92	$C_{15}H_{17}ClN_4$	289.1215	289.1218	1.09
Norflurazon	4.78	$C_{12}H_9ClF_3N_3O$	304.0459	304.0461	0.80
Norflurazon desmethyl	4.27	$C_{11}H_7ClF_3N_3O$	290.0303	290.0305	0.92
Oxamyl	1.57	$C_7H_{13}N_3O_3S$	237.1016	237.1017	0.34
Oxamyl oxide	1.66	$C_5H_{10}N_2O_2S$	163.0536	163.0537	0.74
Oxydemeton methyl sulfone	4.41	$C_6H_{15}O_4PS_2$	247.0222	247.0224	0.82
Phorate sulfone	4.41	$C_7H_{17}O_4PS_3$	293.0099	293.0101	0.57
Phorate sulfoxide	4.25	$C_7H_{17}O_3PS_3$	277.0150	277.0153	0.97
Pirimicarb	2.80	$C_{11}H_{18}N_4O_2$	239.1503	239.1503	0.07
Promecarb	5.57	$C_{12}H_{17}NO_2$	208.1332	208.1335	1.26
Propamocarb	1.51	$C_9H_{20}N_2O_2$	189.1598	189.1599	0.75
Propargite	8.38	$C_{19}H_{26}O_4S$	368.1890	368.1893	0.88
Propiconazole	6.89	$C_{15}H_{17}Cl_2N_3O_2$	342.0771	342.0775	1.15
Propoxur	3.46	$C_{11}H_{15}NO_3$	210.1125	210.1125	0.23
Pyraclostrobin	7.08	$C_{19}H_{18}ClN_3O_4$	388.1059	388.1062	0.80
Pyridaben	8.90	$C_{19}H_{25}ClN_2OS$	365.1449	365.1452	0.78
Pyrimethanil	4.72	$C_{12}H_{13}N_3$	200.1182	200.1184	0.62
Pyriproxyfen	8.05	$C_{20}H_{19}NO_3$	322.1438	322.1439	0.37
Quinoxyfen	8.20	$C_{15}H_8Cl_2FNO$	308.0040	308.0042	0.67
Sethoxydim	7.72	$C_{17}H_{29}NO_3S$	328.1941	328.1942	0.28
Simazine	3.48	$C_7H_{12}ClN_5$	202.0854	202.0855	0.40
Spinosad A	7.27	$C_{41}H_{65}NO_{10}$	732.4681	732.4687	0.77
Spinosad D	7.66	$C_{42}H_{67}NO_{10}$	746.4838	746.4838	-0.01
Spiromesifen	8.36	$C_{23}H_{30}O_4$	388.2482	388.2485	0.62
Sulfentrazone	3.81	$C_{11}H_{10}Cl_2F_2N_4O_3S$	404.0157	404.0159	0.57
Tebuconazole	6.75	$C_{16}H_{22}ClN_3O$	308.1524	308.1526	0.65
Tebufenozide	6.58	$C_{22}H_{28}N_2O_2$	353.2224	353.2226	0.59
Tebuthiuron	3.62	$C_9H_{16}N_4OS$	229.1118	229.1119	0.39
Thiabendazole	1.95	$C_{10}H_7N_3S$	202.0433	202.0435	0.71
Thiabendazole, 5-hydroxy	1.65	$C_{10}H_7N_3OS$	218.0383	218.0384	0.61
Thiacloprid	2.60	$C_{10}H_9ClN_4S$	253.0309	253.0310	0.27
Thiobencarb	7.16	$C_{12}H_{16}ClNOS$	258.0714	258.0715	0.56
Triadimefon	5.82	$C_{14}H_{16}ClN_3O_2$	294.1004	294.1005	0.49
Triadimenol	5.96	$C_{14}H_{18}ClN_3O_2$	296.1160	296.1163	0.92
Trifloxystrobin	7.51	$C_{20}H_{19}F_3N_2O_4$	409.1370	409.1373	0.73
Triflumizole	7.56	$C_{15}H_{15}ClF_3N_3O$	346.0929	346.0930	0.39

Results and Discussion

The extracted ion chromatograms shown in Figure 1 illustrate the quality of the UHPLC separation at 1 ppb in onion matrix. All analytes gave very good linear response in the calibration range of 1.35–1280 ppb depending on the starting concentration in the mixture. The quantification data showed good reproducibility and recovery rates.

Table 2 shows the retention time, R^2, and LOQ for the pesticides analyzed in onion matrix. The mass accuracy of the LOQ (less than 2 ppm), as well as the retention times and curve fits, increase the confidence level for the analyst.

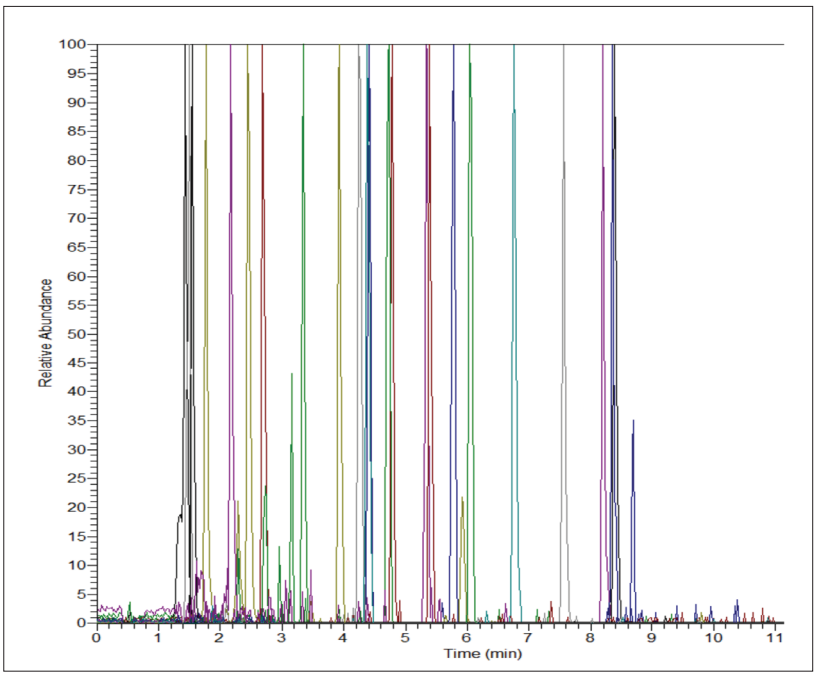

Figure 1. Extracted ion chromatograms showing peak shape and elution time at 1 ppb level in onion matrix

Table 2. Tabulated results of LOQs for each targeted compound, with retention times and curve fit R^2

Compound	RT	R^2	LOQ (ppb)
Acetamiprid	2.27	0.9990	0.125
Aldicarb	2.80	0.9956	2.000
Aldicarb sulfone	1.49	0.9986	0.500
Aldicarb sulfoxide	1.55	0.9979	0.500
Atrazine	4.38	0.9994	0.500
Azinphos methyl	5.01	0.9959	2.500
Azinphos methyl OA	2.90	0.9992	0.500
Azoxystrobin	5.40	0.9992	0.125
Bendiocarb	3.52	0.9991	0.250
Benoxacor	4.97	0.9986	0.500
Bifenazate	6.04	0.9706	0.500
Boscalid	5.61	0.9991	0.500
Buprofezin	7.70	0.9989	0.025
Carbaryl	3.88	0.9989	0.250
Carbofuran	3.52	0.9988	0.250
Carbofuran, 3-hydroxy	2.19	0.9985	0.500
Carboxin	3.77	0.9990	0.250
Carfentrazone ethyl	6.62	0.9991	0.250
Chlorpyrifos OA	6.37	0.9994	0.050
Clofentezine	7.27	0.9937	1.000
Clothianidin	2.03	0.9978	0.250
Cymoxanil	2.53	0.9029	0.500
Difenoconazole	7.40	0.9994	0.250
Diflubenzuron	6.50	0.9996	1.000
Dimethomorph	5.80	0.9993	0.250
Dinotefuran	1.49	0.9974	0.050
Diuron	4.68	0.9990	1.000
Famoxadone	7.04	0.9992	0.250
Fenamidone	5.46	0.9990	0.025
Fenamiphos sulfone	3.77	0.9992	0.250
Fenamiphos sulfoxide	3.93	0.9992	0.025
Fenbuconazole	6.46	0.9993	0.500
Fludioxonil	5.71	0.9991	0.500
Fluridone	5.19	0.9987	0.250
Flutolanil	5.76	0.9990	0.125
Formetanate	1.43	0.9983	0.050
Halosulfuron methyl	5.96	0.9866	0.500
Hexaconazole	6.98	0.9986	1.000
Hexythiazox	8.18	0.9990	0.250
Imazalil	4.49	0.9995	0.250
Imidacloprid	1.99	0.9965	0.050
Indoxacarb	7.54	0.9989	0.500
Isoprocarb	4.39	0.9971	0.500
Linuron	5.34	0.9989	0.500
Metalaxyl	4.59	0.9992	0.125

Table 2 (continued). Tabulated results of LOQs for each targeted compound, with retention times and curve fit R^2

Compound	RT	R^2	LOQ (ppb)
Methidathion OA	2.70	0.9991	2.000
Methiocarb	5.38	0.9990	0.500
Methomyl	1.45	0.9968	0.100
Methoxyfenozide	5.86	0.9996	0.250
Metribuzin	3.35	0.9992	0.250
Monocrotophos	1.74	0.9989	0.025
Myclobutanil	5.92	0.9988	0.500
Norflurazon	4.78	0.9992	0.500
Norflurazon desmethyl	4.27	0.9988	0.050
Oxamyl	1.57	0.9992	0.500
Oxamyl oxide	1.66	0.9966	1.000
Oxydemeton methyl sulfone	4.41	0.9979	0.250
Phorate sulfone	4.41	0.9984	0.025
Phorate sulfoxide	4.25	0.9990	0.050
Pirimicarb	2.80	0.9988	0.100
Promecarb	5.57	0.9990	0.250
Propamocarb	1.51	0.9981	0.500
Propargite	8.38	0.9993	0.025
Propiconazole	6.89	0.9992	0.500
Propoxur	3.46	0.9993	0.500
Pyraclostrobin	7.08	0.9990	0.125
Pyridaben	8.90	0.9991	0.125
Pyrimethanil	4.72	0.9995	0.250
Pyriproxyfen	8.05	0.9990	0.125
Quinoxyfen	8.20	0.9993	0.125
Sethoxydim	7.72	0.9964	0.250
Simazine	3.48	0.9999	0.250
Spinosad A	7.27	0.9995	0.420
Spinosad D	7.66	0.9994	0.080
Spiromesifen	8.36	0.9987	0.250
Sulfentrazone	3.81	0.9986	0.500
Tebuconazole	6.75	0.9994	0.050
Tebufenozide	6.58	0.9989	0.500
Tebuthiuron	3.62	0.9990	0.125
Thiabendazole	1.95	0.9986	0.250
Thiabendazole, 5-hydroxy	1.65	0.9993	0.250
Thiacloprid	2.60	0.9993	0.125
Thiodicarb	4.23	0.9953	20.000
Triadimefon	5.82	0.9990	0.500
Triadimenol	5.96	0.9981	1.500
Trifloxystrobin	7.51	0.9989	0.125
Triflumizole	7.56	0.9994	0.251

TraceFinder software comes with many features including user-customizable flagging. A green flag next to the name of the compound (Figure 2) indicates the compound was found in the unknown sample, whereas a yellow flag indicates the compound was not found. A red flag indicates the compound has an issue with the calibration curve and that it exceeded the flagging threshold (Figures 3 and 4). A yellow triangle caution sign indicates there is an above-threshold quantitation error with a single or multiple compounds in the sample that needs to be checked.

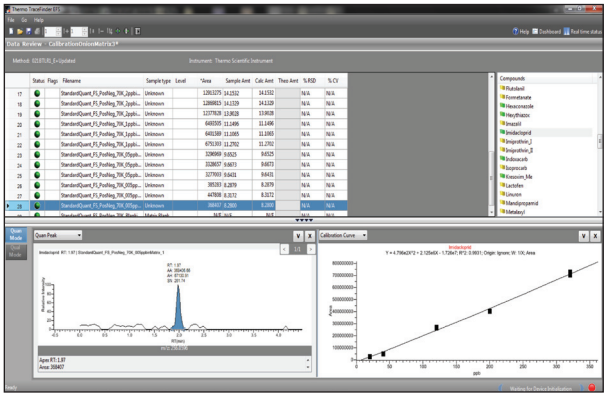

Figure 2. TraceFinder software displays imidacloprid calibration curve plot of matrix, R^2, list of compounds, and chromatogram. A green flag in the compound list indicates the compound was found in the unknown sample, whereas a yellow flag indicates the compound was not found.

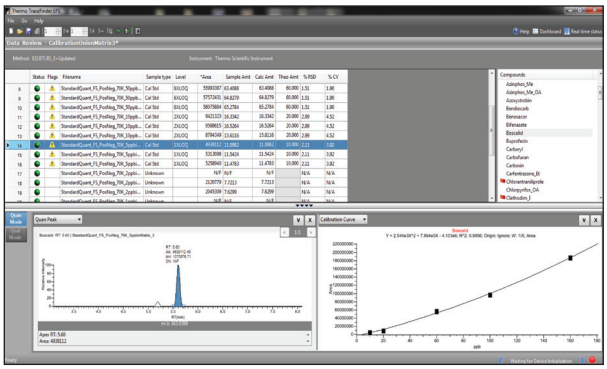

Figure 3. TraceFinder software displays boscalid calibration curve plot of matrix, R^2, list of compounds, and chromatogram. The red flag indicates the compound has an issue with the calibration curve and that it did not meet the flagging requirement. The yellow triangle caution sign indicates there is an issue with a single or multiple compounds in the sample that needs to be checked.

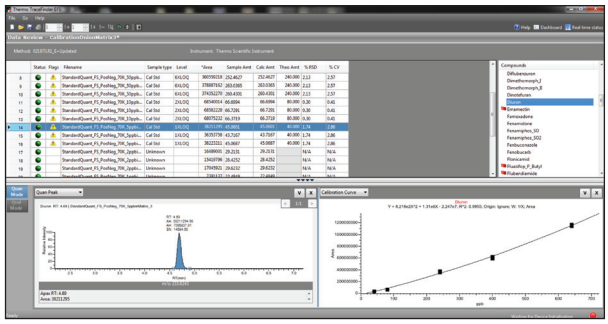

Figure 4. TraceFinder software displays diuron calibration curve plot of matrix, R^2, list of compounds, and chromatogram. The highlighting of diuron in the upper right section indicates that the compound was found within the calibration curve. Therefore, there are no flags present next to the name.

Conclusion

The Exactive Plus benchtop mass spectrometer paired with TraceFinder software provided easy access to full quantitative and targeted screening data in one package. The results showed good linearity with excellent sensitivity at very low LOQs, which will assist in detecting pesticides. The Exactive Plus instrument's exceptionally high mass resolution helped resolve matrix compounds that would otherwise interfere with detection of low-level analytes. The measured mass errors showed high confidence in the data acquired with regard to mass accuracy.

Acknowledgements

The authors wish to thank to Roland Carlson at the California Department of Food and Agriculture for providing the QuEChERS extracts, the pesticide mixture, and the methodology for this study.

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