Application Note: 477

Screening for 250 Pesticides in Orange Oil and Ginseng Extract by LC-MS/MS Using TraceFinder Software

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Key Words

- TraceFinder software
- TSQ Vantage
- Triple Quadrupole
- Food safety
- Pesticides

Introduction

Orange oil is widely used for its fragrance and flavoring in consumer products such as cosmetics, medications, and processed foods. In addition, as consumers demand more environmentally friendly cleaning options, orange oil is used increasingly in household cleaning products. Orange oil is derived from the outermost part of the orange; therefore, pesticide contamination is a concern.

Ginseng, an herb used to stimulate the adrenal gland and increase energy, has been used in various systems of medicine for centuries. The root of the ginseng plant contains active chemical components called ginsenosides, which are believed to be responsible for the medicinal properties of the herb. Therefore, the root is commonly dried and made into tablets, extracts, and teas to be taken internally or made into creams for external use. Pesticide contamination in these products is also a concern because the ginseng plant may carry residuals of environmentally persistent pesticides.

Analyzing orange oil and ginseng for pesticides is challenging in part because of the extensive exporting and importing of produce. Pesticides that are approved in one country may be banned in another, and approved pesticides may have different restrictions on the permissible levels of exposure.

Thermo Scientific TraceFinder, a software program with built-in workflows, has been developed to assist routine analysis in environmental and food residue applications. It includes a methods database pre-loaded with the appropriate *m/z* and optimized parameters of contaminants commonly encountered in environmental and food samples, which can be customized by the user to include unique compounds. An LC-MS/MS library of commonly found contaminants, organized in National Institute of Standards and Technology (NIST) format, helps to confirm the compounds being analyzed. Data collection, analysis, and report generation can be performed using the same software program. To demonstrate the software capabilities, a mixture of 250 pesticides spiked into orange oil samples and ginseng extract samples were analyzed using both negative and positive ionization modes on a Thermo Scientific TSQ Vantage Extended Mass Range (EMR) mass spectrometer.

Goal

To develop a quick and efficient LC-MS/MS method for screening pesticides in orange oil and ginseng extract using TraceFinder™ software.

Experimental Conditions

Method

Orange oil and ginseng extract were spiked with a mixture of 250 pesticides (Table 1) to give solutions containing 1 ppb and 10 ppb of each pesticide. A 5 µL sample of the spiked orange oil or ginseng extract was injected directly onto the HPLC column. A simple gradient was used with a retention time of 18 minutes. Using the TraceFinder software, Timed-Selective Reaction Monitoring (T-SRM) was used to create the instrument method, collect and process the data. In a T-SRM experiment, using prior knowledge of the retention times of the compounds, the method is set to look for specific transitions only during the expected retention-time window. This increases the number of SRM transitions that can be monitored effectively per experiment. It also increases the dwell time and duty cycle for monitoring individual compounds per experiment. The result is more accurate and sensitive quantitation.

Sample Preparation

Samples were prepared by a modified QuEChERS procedure.[1] Mixtures of 250 pesticides were prepared in acetonitrile at concentrations of 20 ppb and 200 ppb. For the 10 ppb experiment, a solvent standard was made by mixing 50 µL of the 200 ppb pesticide mixture, 150 µL of acetonitrile, and 800 µL of buffer. The 10 ppb spiked sample was prepared by adding 50 µL of the 200 ppb pesticide mixture, 50 µL of acetonitrile, and 800 µL of water to orange oil or ginseng that has been extracted with 100 µL of acetonitrile. The sample was filtered with a 0.2 µm nylon membrane to remove any particulates.

Similarly, for the 1 ppb experiment, the solvent standard was prepared by mixing 50 µL of the 20 ppb pesticide mixture, 150 µL of acetonitrile, and 800 µL of buffer. The 1 ppb spiked sample was prepared by adding 50 µL of the 200 ppb pesticide mixture, 50 µL of acetonitrile, and 800 µL of water to orange oil or ginseng that has been extracted with 100 µL of acetonitrile. The sample was filtered to remove any particulates.

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Table 1. 250 pesticides and SRM transitions

Pesticide Name	Precursor Ion $[M+H]^+$	Product Ions
Methamidophos	142.0	95.0, 125.0
Naphthol, 1-	143.2	115.1, 143.2
Methomyl	163.1	88.1, 106.1
Fenuron	165.0	46.3, 72.1
o-phenylphenol	169.0	115.3, 141.3
Phropham	180.0	120.0, 138.0
Acephate	184.1	95.2, 143.0
Fuberidazole	185.1	130.1, 157.0
Propamocarb	189.0	102.1, 144.0
Tricyclazole	190.1	136.1, 163.1
Carbendazim	192.1	132.1, 160.1
Isoprocarb	194.1	95.0, 137.0
Cymoxanil	199.1	111.1, 128.1
Cycluron	199.1	72.2, 89.1
Pyrimethanil	200.1	82.0, 107.0
Diamidafos (Nellite)	201.1	82.4, 107.2
Thiabendazole	202.0	131.0, 175.0
Carbaryl	202.1	12.0, 145.0
Dinotefuran	203.2	114.0, 129.0
Aldicarb_Sulfoxide	207.0	89.0, 132.0
Isoproturon	207.1	72.0, 165.2
Promecarb	208.1	109.0, 151.0
Aldicarb+NH_4	208.1	89.2, 116.1
Butocarboxin	208.1	91.4, 109.2
Aminocarb	209.1	137.1, 152.1
Propoxur	210.1	111.1, 168.1
Acibenzolar-S-methyl	211.1	136.0, 140.0
Chlortoluron	213.1	140.0, 168.0
Omethoate	214.1	155.0, 183.0
Simetryne	214.1	96.0, 124.0
Monolinuron	215.1	99.0, 126.0
Metribuzin	215.1	131.0, 187.1
Pymetrozine	218.0	79.0, 105.0
Pyracarbolid	218.2	96.9, 124.9
Thidiazuron	221.1	94.2, 102.1
Formetanate	222.1	120.0, 165.0
Bufencarb	222.1	77.2, 95.2
Methabenzhiazuron	222.1	150.0, 165.0
Carbofuran	222.1	123.1, 165.1
Acetamiprid	223.1	90.2, 126.1
Butoxycarboxin	223.1	86.2, 106.1
Mexacarbate	223.2	151.0, 166.0
Monocrotophos	224.1	127.0, 193.1
Dioxacarb	224.1	123.1, 167.1
Mepanipyrim	224.1	77.0, 106.0
Bendiocarb	224.2	106.0, 109.1
Aldicarb sulfoxide + NH_4	224.2	89.0, 131.7
Mevinphos	225.1	127.1, 192.8
Cyprodinil	226.0	93.0, 108.0
Methiocarb	226.1	121.0, 169.0
Ethiofencarb	226.1	107.0, 106.0
Secbumeton	226.2	99.9, 169.9
Prometon	226.2	141.9, 184.0
Terbumeton	226.2	113.9, 169.9
Ametryn	228.2	96.0, 185.9
Tebuthiuron	229.2	116.1, 172.1
Dimethoate	230.1	125.1, 199.1
Flonicamid	230.1	174.1, 203.1
Fluometuron	233.1	46.3, 72.1
Diuron	233.1	46.3, 72.0
Siduron	233.1	94.0, 137.0
Carboxin	236.0	87.0, 143.0
Thiofanox + NH_4	236.1	57.2, 76.1
Oxamyl + NH_4	237.1	72.1 90.1
Carbetamide	237.1	118.1, 192.0
Carbofuran-3-hydroxy	238.1	181.1, 220.1
Dicrotophos	238.1	112.1, 193.1

Pesticide Name	Precursor Ion $[M+H]^+$	Product Ions
Bentazone_neg	239.1	132.0, 197.0
Pirimicarb	239.1	72.0, 182.0
Butoxycarboxin + NH_4	240.1	86.2, 106.1
Aldicarb sulfone + NH_4	240.1	86.2, 148.0
Prometryn	242.2	157.9, 199.9
Terbutryn	242.2	91.0, 185.9
Ethoprophos	243.1	97.1, 131.1
Cyanophos	244.0	125.1, 212.0
Fonophos	247.0	109.1, 137.1
Fludioxinil	247.1	126.0, 180.0
Forchlorfenuron	248.1	93.0, 129.0
Linuron	249.1	160.0, 182.0
Clothianidin	250.1	132.1, 169.1
Thiacloprid	253.1	90.2, 126.1
Imidacloprid	256.1	175.1, 209.1
Thiobencarb	258.1	100.2, 125.0
Demeton-S	259.0	61.2, 89.2
Metobromuron	259.1	148.0, 170.0
Phorate	261.0	75.1, 143.0
Parathion-methyl	264.0	109.1, 124.9, 232.1
Diethofencarb	268.2	180.1, 226.0
Thiometon + Na	268.9	61.1, 89.1
Mepronil	270.1	119.0, 228.0
Nitenpyram	271.2	225.0, 237.0
Methoprotryne	272.2	198.0, 240.0
Disulfoton	274.9	61.3, 89.3
Neburon	275.1	57.2, 88.0
Bromoxynil	276.1	79.0, 81.0
Fenitrothion	278.0	108.8, 125.1, 246.0
Fenthion	279.0	169.1, 247.0
Oxadixyl	279.0	132.0, 219.0
Metalaxyl	280.1	192.1, 220.1
Propetamphos	282.0	138.1, 156.0
Penconazole	284.1	70.1, 159.0
Ethofumesate	287.0	120.9, 258.9
Vamidothion	288.1	118.1, 146.0
Terbufos	289.0	57.5, 103.1
Iprobenfos	289.0	91.2, 205.0
Myclobutanil	289.1	70.2, 125.0
Chloroxuron	291.1	46.2, 72.2
Parathion	292.0	97.0, 236.0
Uniconazole	292.1	70.2, 125.0
Cyproconazole	292.1	93.2, 125.0
Thiamethoxam	292.2	132.0, 211.1
Amitraz	294.1	122.2, 163.1
Paclobutrazole	294.1	70.0, 125.0
Triadimefon	294.2	197.1, 225.1
Triadimenol	296.1	70.0, 99.0
Imazalil	297.2	159.0, 201.0
Spiroxamine	298.2	100.0, 144.0
Quinalphos	298.9	163.1, 243.0
Mefenacet	299.2	120.1, 148.0
Ditalimfos	300.1	144.2, 145.3
Phenmedipham	301.2	136.0, 168.0
Bifenazate	301.2	152.0, 170.0
Fenhexamid	302.1	55.0, 97.0
Furalaxyl	302.1	95.0, 242.1
Flutriafol	302.2	70.1, 123.0
Fenoxycarb	302.2	88.0, 116.0
Methidathion	302.9	85.2, 144.9
Clofentezine	303.1	102.0, 138.0
Fenamiphos	304.0	217.0, 234.0
Fenpropimorph	304.4	130.1, 147.1
Diazinon	305.0	153.1, 169.1
Pirimiphos-methyl	306.0	108.2, 164.1
Buprofezin	306.2	116.0, 201.0
Fenazaquin	307.2	57.2, 160.9

Table 1. 250 pesticides and SRM transitions (continued)

Pesticide Name	Precursor Ion [M+H]+	Product Ions
Quinoxyfen	307.9	161.9, 196.8
Tebuconazole	308.2	70.2, 125.0
Diflubenzuron	308.9	156.0, 289.0
Fensulfothion	309.2	163.0, 251.0
Edifenphos	311.0	109.1, 283.0
Fenamidone	312.2	236.2, 264.2
Triazophos	314.0	119.2, 162.1
Kresoxim-methyl	314.1	222.1, 267.1
Hexaconazole	314.1	70.2, 159.0
DEF	315.0	169.0, 259.1
Nuarimol	315.1	81.0, 251.9
Flusiazole	316.2	165.0, 247.1
Bupirimate	317.3	108.1, 166.1
Phosmet	317.9	133.1, 160.1
Azinphos-methyl	317.9	125.0, 261.0
Triticonazole	318.1	70.0, 125.0
Desmedipham + NH_4	318.2	136.0, 182.0
Tebupirimfos	319.1	166.1, 210.2
Metconazole	320.2	70.1, 124.9
Phenthoate	320.9	79.3, 247.0
Iprovalicarb	321.2	119.0, 203.0
Pyriproxyfen	322.2	96.0, 185.3
Sulprofos	322.9	218.9, 247.0
Sulfotep-ethyl	323.2	219.0, 247.1
EPN	324.0	157.0, 296.0
Flutolanil	324.2	242.0, 262.0
Cyazofamid	325.2	108.0, 261.0
Famphur	326.0	217.0, 281.0
Diniconazole	326.2	70.2, 148.2
Benalaxyl	326.2	148.0, 208.0
Dimoxystrobin	327.1	116.0, 205.0
Diclobutrazol	328.1	70.2, 159.0
Etaconazole	328.2	123.0, 159.0
Epoxiconazole	330.2	121.0, 123.0
Malathion	330.9	99.2, 285.0
Fenarimol	331.1	81.0, 268.0
Pirimiphos ethyl	334.1	182.1, 198.1
Ipconazole	334.1	70.2, 125.0
Tebufenpyrad	334.2	117.0, 145.2
Zoxamide	336.2	159.0, 187.0
Fenbuconazole	337.0	70.4, 125.1
Bitertanol	338.1	99.0, 269.0
Mesotrione	340.2	185.9, 228.0
Pyridaphenthion	341.1	189.0, 205.0
Prothioconazole	342.0	100.0, 306.0
Propiconazole	342.2	69.2, 159.0
Thiophanate-methyl	343.2	151.1, 311.2
Boscalid	343.2	271.0, 307.0
Azinphos-ethyl	346.0	132.1, 160.1
Isofenfos	346.0	217.0, 245.0
Triflumizole	346.1	73.0, 278.1
Tebufenozide	353.1	133.0, 297.0
Hexythiazax	353.2	168.1, 228.2
Piperonyl butoxide	356.2	119.0, 177.0
Triflumuron	359.1	139.0, 156.0
Clethodim	360.2	164.0, 268.0
Etoxazole	360.2	141.0, 177.1
Isoxaflutole	360.2	220.0, 251.0
Topramezone	364.2	124.9, 333.9
Flufenacet	364.2	152.0, 194.0
Benzoximate	364.4	105.2, 199.2
Pyridaben	365.2	147.0, 309.1
Methoxyfenozide	367.3	105.0, 149.0
Propargite	368.2	174.9, 231.0
Picoxystrobin	368.2	145.0, 205.1
Loxynil	369.9	127.0, 215.0, 242.9
Spiromefesin	371.3	255.3, 273.3
Tetraconazole	372.2	70.0, 159.0
Famoxadone	373.1	282.4, 329.6
Pyrazophos	374.0	194.0, 222.1
Fluquinconazole	376.2	307.0, 349.2
Prochloraz	376.2	266.0, 308.0
Bromuconazole 46	378.0	70.2, 159.0
Teflubenzuron	379.2	196.0, 339.0
Benthiavalicarb	382.1	116.0, 180.0
Furathiocarb	383.2	195.0, 252.0
Ethion	384.9	97.1, 143.0
Dimethomorph	388.1	165.0, 301.0
Pyraclostrobin	388.2	163.0, 194.0
Famoxadone + NH_4	392.1	238.0, 331.2
Rotenone	395.3	192.1, 213.2
Ethiprole	397.1	255.0, 351.0
Flucarbazone	397.1	115.0, 129.9
Alanycarb	400.3	91.0, 238.0
Pinoxaden	401.2	57.1, 317.0
Sulfentrazone	404.0	307.0, 387.0
Azoxystrobin	404.1	329.1, 372.1
Difenoconazole	406.2	111.0, 251.0
Trifloxystrobin	409.3	186.0, 206.1
Spirodiclofen	411.0	213.1, 313.1
Benfuracarb	411.1	195.1, 252.0
Mandipropamid	412.1	327.9, 355.9
Carfentrazone-ethyl	412.2	366.2, 384.0
Fenpyroximate	422.2	214.0, 366.0
Fipronil	437.2	330.2, 368.0
Hexaflumuron	458.9	175.0, 439.0
Fluoxastrobin	459.2	188.0, 427.1
Fluazinam	463.2	398.0, 416.0
Temephos	466.9	405.1, 419.1
Dioxathion	474.0	153.0, 271.1
Flufenoxuron	487.2	156.0, 304.0
Novaluron	491.2	305.0, 471.0
Butafenacil + NH_4	492.3	180.0, 331.0
Novaluron	493.3	141.0, 158.0
HydramethylNon	495.3	150.9, 323.0
Lufenuron_neg	509.2	175.0, 326.0
Lufenuron	511.3	141.0, 158.0
Milbemycin A3	511.4	475.2, 493.2
Milbemycin A4 - H_2O	525.4	489.2, 507.2
Noviflumuron	527.0	193.0, 344.0
Indoxacarb	528.3	203.0, 293.0
Chlorfluazuron	539.7, 541.9	383.0, 385.0
Milbemycin A4 + NH_4	560.4	507.2, 525.2
Moxidectin	640.2	498.5, 528.5
Spinosyn A	732.5	98.0, 142.0
Spinosyn D	746.5	98.0, 142.0
Emamectin B1b	872.4	158.2, 302.3
Avermectin B1b + NH_4	876.5	145.0, 291.0
Emamectin	886.7	158.0, 302.0
Avermectin B1a + NH_4	890.4	305.3, 307.0, 567.4
Ivermectin B1a + NH_4	892.5	307.0, 569.0
Avermectin B1a + Na	895.4	183.1, 751.5
Doramectin	916.4	331.4, 593.5
Eprinomectin B1a	936.5	352.1, 490.2

HPLC

Chromatographic analysis was performed using the Thermo Scientific Accela HPLC pump and Accela™ autosampler. The chromatographic conditions were as follows:

Column:	Thermo Scientific Hypersil GOLD PFP (100 mm × 2.1 mm, 1.9 µm)		
Injection volume:	5 µL		
Column temperature:	45 °C		
Mobile phase A:	5 mM ammonium formate in water		
Mobile phase B:	5 mM ammonium formate in methanol		
Flow rate:	0.3 mL/min		
Gradient:	Time (min)	A%	B%
	0	95	5
	2	75	25
	30	0	100
	35	0	100

MS

MS analysis was carried out on a TSQ Vantage EMR™ triple stage quadrupole mass spectrometer with a HESI-II heated electrospray ionization source.

The MS conditions were as follows:

Ion source polarity:	Positive and negative ion mode
Spray voltage:	3500 V
Vaporizer temperature:	400 °C
Ion sweep gas:	2.0 units
Ion transfer tube temperature:	200 °C
Sheath gas pressure (N_2):	55 units
Auxiliary gas pressure (N_2):	15 units
Resolution:	0.7 amu (FWHM) on Q1 and Q3
Scan Width:	0.002 Da
Chrom Filter:	10.0 ms
Collision Gas Pressure:	1.5 mTorr
Scan Type:	Timed SRM (T-SRM)
Cycle Time:	0.4 s

Two SRM transitions per pesticide were monitored for confirmation (Table 1).

Software

Data collection and processing was handled by TraceFinder environmental and food safety software. TraceFinder includes several methods applicable to the environmental and food safety markets, as well as a comprehensive Compound Datastore (CDS). The CDS includes SRM transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds that are of interest to the environmental and food safety industries. A user can select one of the included methods in TraceFinder or quickly develop new or modified methods by using the pre-existing SRM transition information in the CDS, thus eliminating time-consuming compound optimizations.

Results and Discussion

Method Development

The method development section of the software allows the user to choose the compounds that will be analyzed. In this experiment, the appropriate SRMs for the 250 pesticides were chosen from the CDS (Figure 1) and inserted into the instrument method for detection (Figure 2). No compound optimization is necessary for compounds already in the CDS.

Additionally, the calibration levels, QC levels, and peak detection settings are defined in the method development section. Results can be flagged based on user-defined criteria. For example, a flag can be set for a compound whose calculated concentration is beyond the upper limit of linearity, above a defined reporting limit, or below a limit of detection. This allows for faster reviewing of the data after collection; positive samples can be quickly identified.

Acquisition

The Acquisition section provides a step-by-step process to acquire data. The overall progress is followed in an overview section on the left side of the screen (Figure 3). A green check box indicates that the step has been completed and that there are no errors. The steps include template selection (pre-defined sample lists, which are helpful in routine analysis), method selection, sample list definition, report selection, and instrument status.

A final status page summarizes the method and all of the samples to be run. In addition, it gives an overall summary of the status of the instrument (Figure 4). Three colored dots are shown: green indicates an “ok” status; yellow indicates that the attached device is in standby; and red indicates that the attached device is not ready. From the final status page, the batch can be acquired or saved to be run at a later date. A previously saved calibration curve can be used, so that a calibration need not be run every day.

Data Review

The targeted screening analysis of 250 pesticides in a ginseng extract sample was reviewed in the Data Review section of TraceFinder software. In this section, calibration lines, ion ratios, peak integration, and MS spectra (if applicable) can all be viewed (Figure 5). In addition, the Data Review section can flag samples that meet certain user-set criteria. For example, if a tolerance is specified for the ion ratio, a green flag means that the criteria has been met, while a red or yellow flag indicates that it has not. As another example, flags can be used to alert for the presence of carry-over in a blank sample. A red flag indicates that there is a significant issue with the blank sample. In this experiment, the two-point calibration was sufficient to show the calculated amount of the different pesticides found in ginseng extract.

The Data Review section allows user adjustments, such as peak reintegration. The effects of the changes on the results are instantly updated in the results grid.

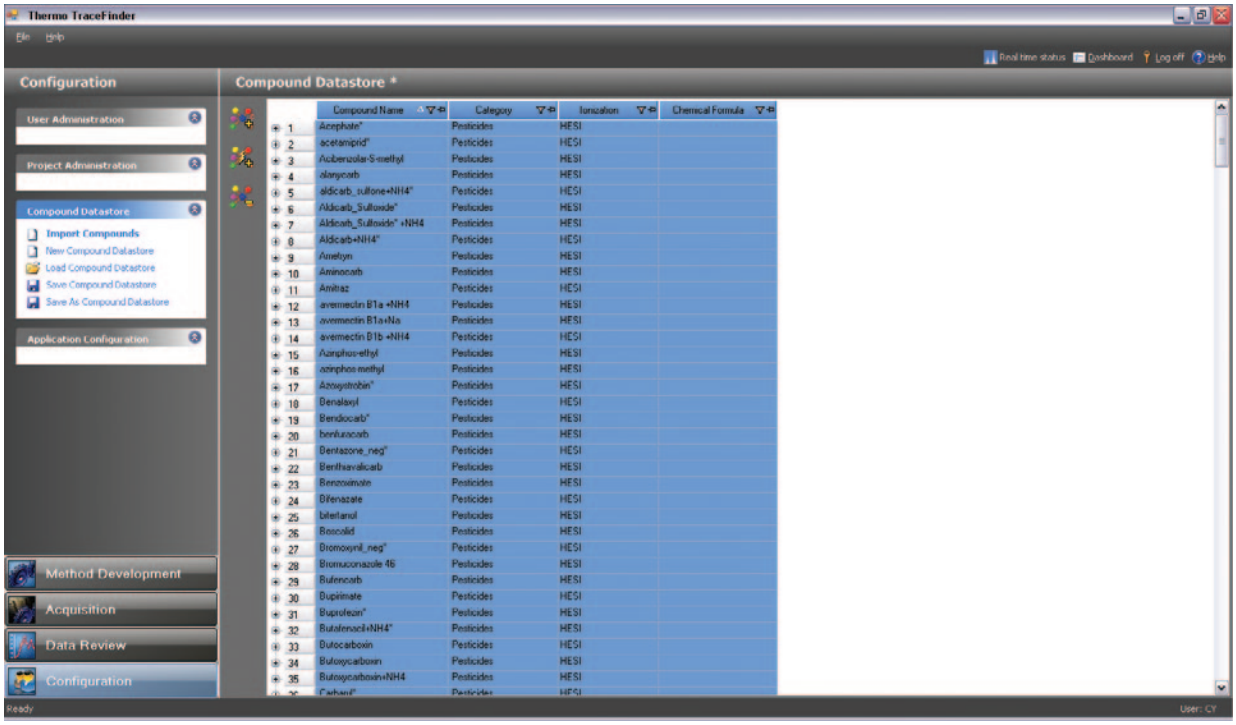

Figure 1. TraceFinder Compound Datastore (CDS)

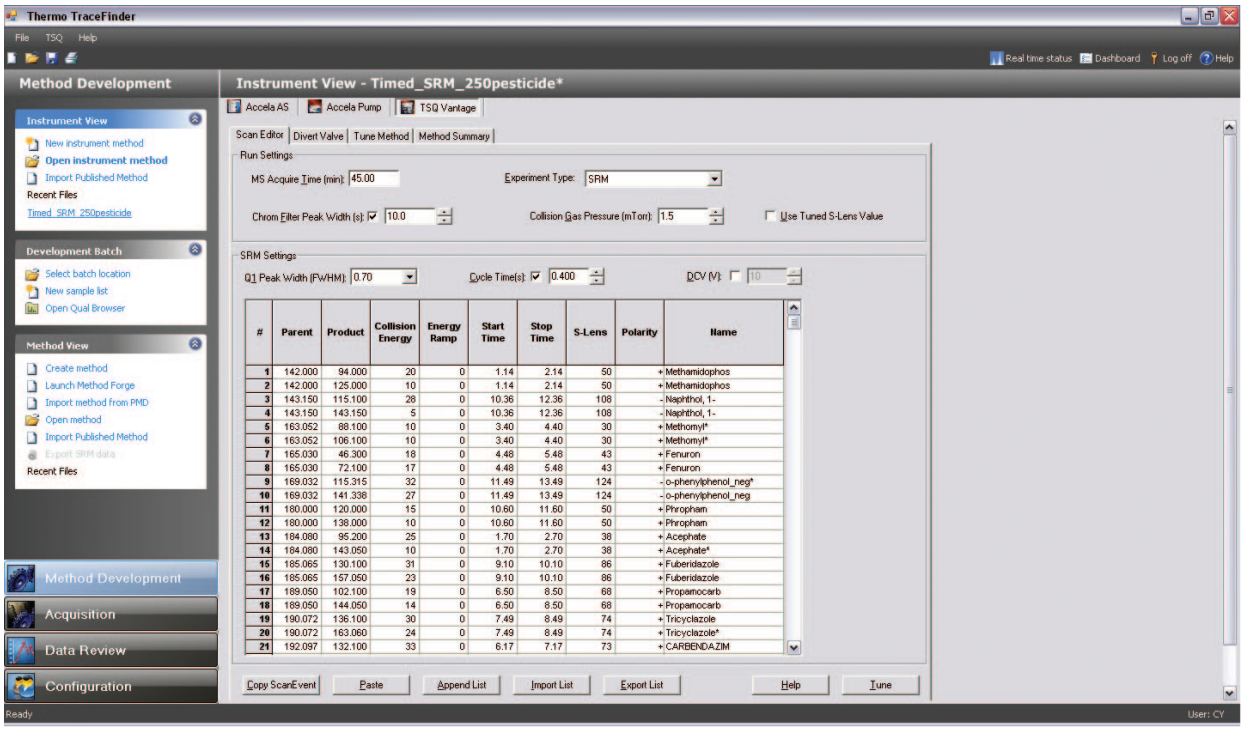

Figure 2. The Compound Datastore is easily inserted into the Instrument Method page.

Reporting

A large number of report templates are available in TraceFinder software. The user has the option of creating PDF reports, printing reports directly to the printer, or saving reports in an XML format, which is useful with laboratory information management systems (LIMS). The user can decide which reports are most applicable to each particular method. In this manner, a supervisor or lab director can set up methods and reports, lock the method, and make it non-editable by technicians. In this way, the integrity of a method is preserved, which is especially useful in controlled environments.

Two examples of the reports generated by TraceFinder software are shown in Figures 6 and 7. This view shows

the on-screen preview function. Figure 6 shows the Calibration Density Report, which displays calibration curves for each compound on one page. Figure 7 shows the Quantitation Report for 1 ppb level in ginseng extract. In this report, the sample summary is provided at the top of the page, and the quantified results follow beneath the chromatogram. TraceFinder can generate results for the entire batch with one click, or the user can view reports individually and print only those of interest.

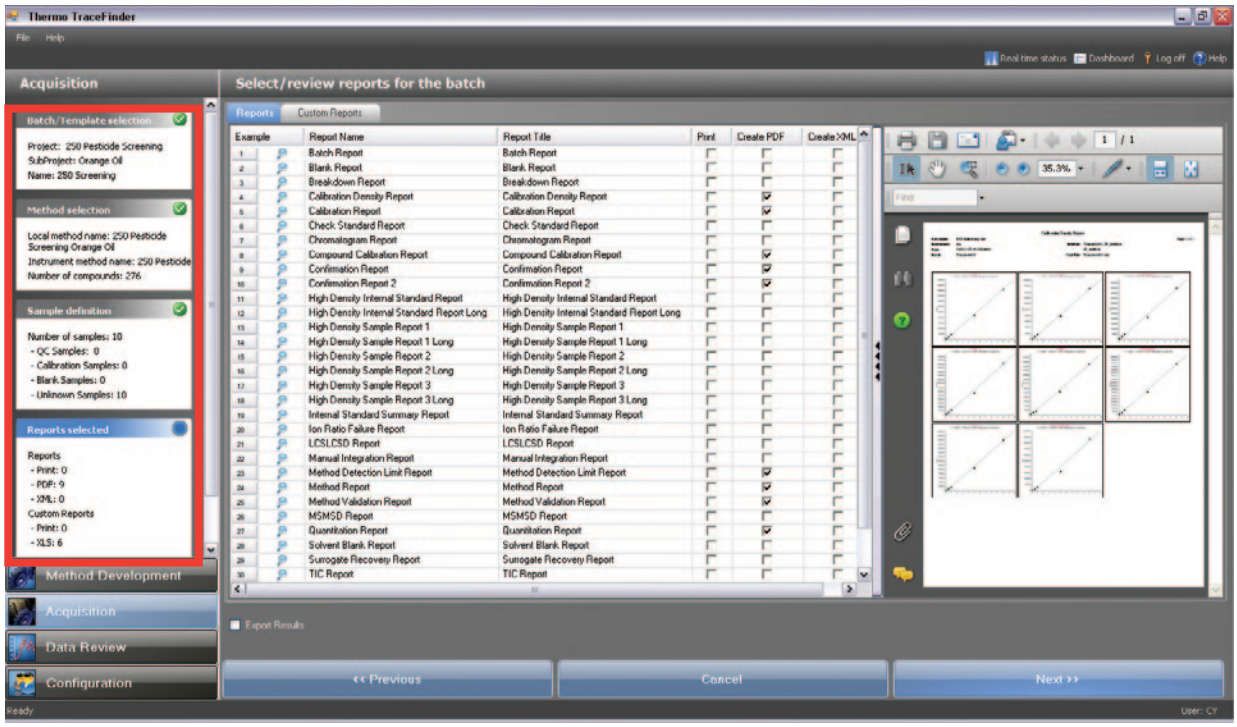

Figure 3. Acquisition section, showing the reporting templates and report preview. The red box at the left outlines the overall progress.

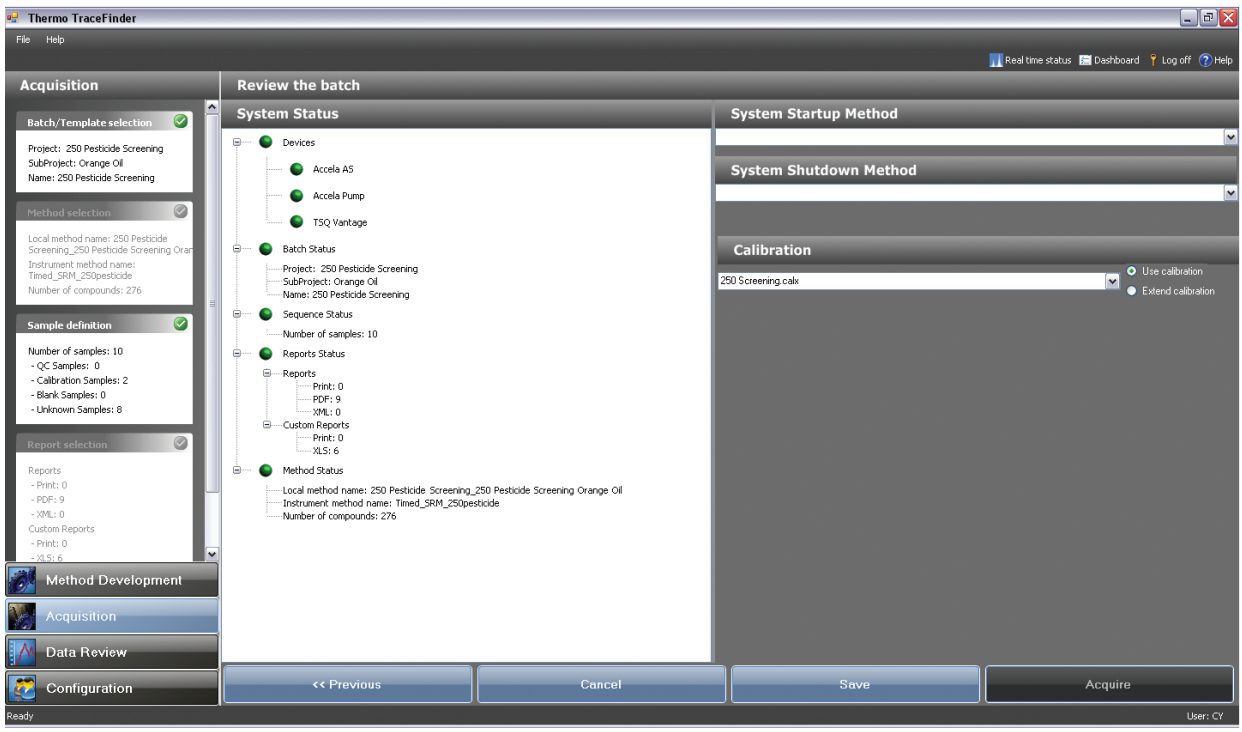

Figure 4. TraceFinder Acquisition status page. This is the final view before submitting a batch for analysis, providing the user instant instrument and method feedback.

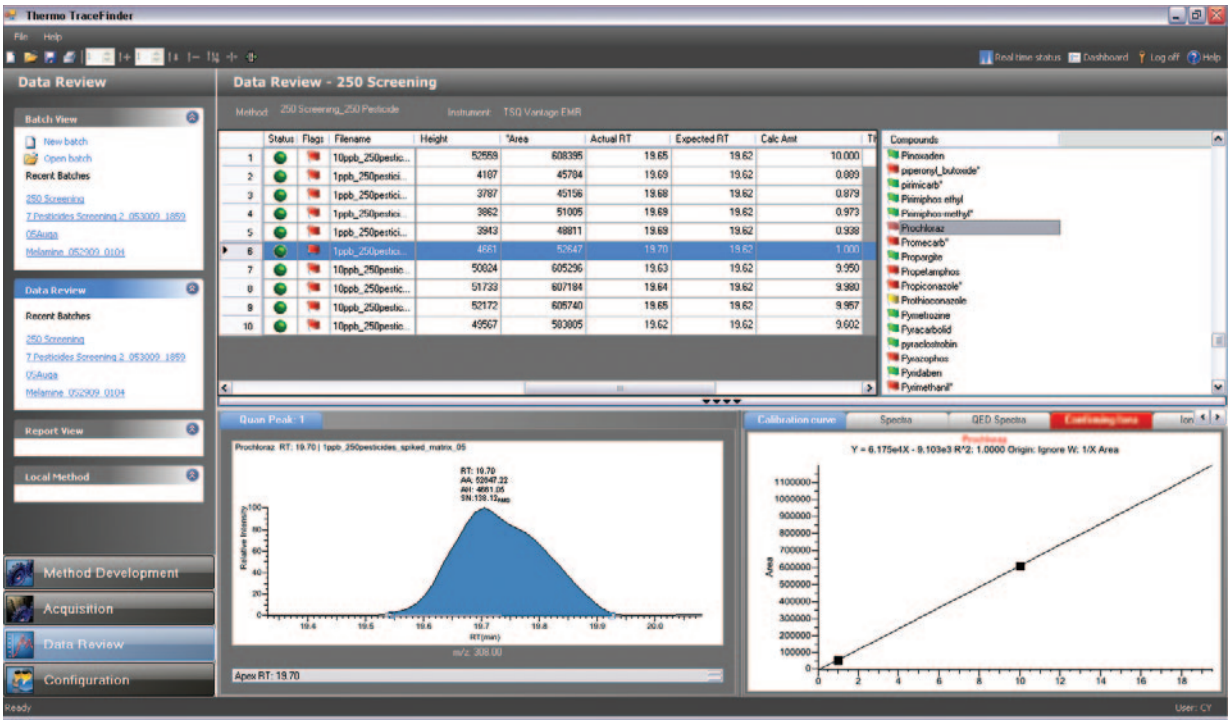

Figure 5. TraceFinder Data Review section. The red and yellow flags indicate that there are certain issues with the compound. For example, the ion ratio may be off or the value may be below the specified limit of detection.

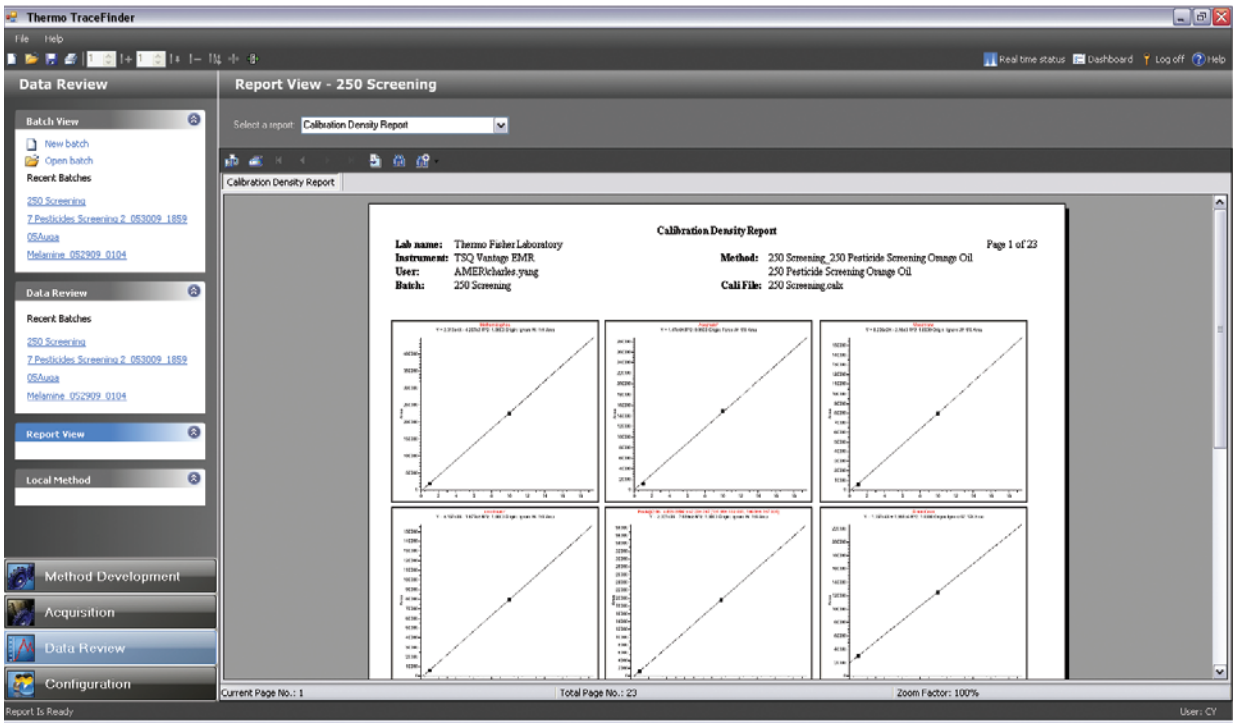

Figure 6. Report View section of TraceFinder, showing calibration curves.

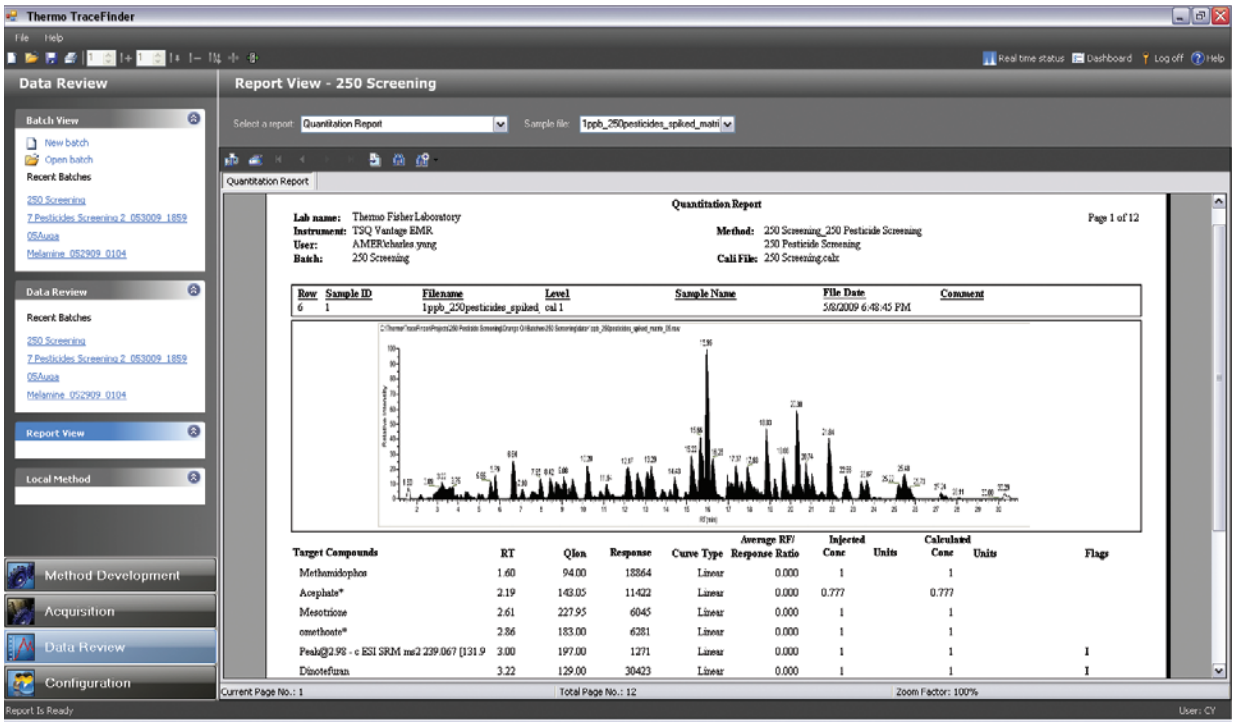

Figure 7. Report View section of TraceFinder showing quantitation results of ginseng.

Conclusion

A new software package, TraceFinder, with an extensive menu of preconfigured methods and report formats, was used to simplify method development for the screening of 250 pesticides in orange oil and ginseng extract. The results from this experiment show positive confirmation of approximately 220 pesticides in orange oil and 250 pesticides in ginseng extract based on the tolerances set in the method for quantitation and confirmation. The method development capabilities and Compound Datastore of TraceFinder software allowed for the quick creation of a method for the analysis of these compounds. In addition, the ability to flag problematic samples in the data review section helped to reduce the overall analysis time by filtering out samples that did not meet predefined criteria.

References

1. Wong, J.W., Hennessy, M.K., Hayward, D.G., Krynitsky, A.J., Cassias, I., Schenck, F.J. (2007) J. Agric. Food Chem. 55, 1117-1128.

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