An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database

Application Note

Food Safety and Environmental

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Abstract

An application kit for pesticide screening has been developed for the Agilent time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF) mass spectrometers using a database with almost 1600 entries. It can be quickly and easily used for both food and environmental samples where the ability to detect and identify a large number of pesticides is necessary. The system allows the user to create custom databases containing retention times of compounds of interest for targeted analysis. Screening with this database thus provides both targeted and non-targeted pesticide detection. A test mix for both positive ion and negative ion modes is provided to demonstrate the functionality of the kit. An example of a general method for pesticide screening is given along with an example of a spinach extraction using the Agilent SampliQ extraction and dispersive SPE kits for complete food analysis.

Agilent Technologies

Introduction

Because over 1000 pesticides have been in use over the last century and new pesticides are being developed, there is a great need to perform both targeted and non-targeted screening in food and the environment. The Agilent time-of-flight (TOF) mass spectrometers provide both high mass resolution and mass accuracy that allow comparison of the measured mass to the exact mass of an ionized compound. In addition, the tandem hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer provides the capability of both screening and confirming compounds in one instrument.[1] Both liquid chromatography (LC) combined with TOF MS and Q-TOF MS provide a robust and sensitive means to perform this type of screening at levels required by the international community. Because TOF is a pulsing instrument the resulting data is always full spectra, which allows the screening of compounds that are sought (targeted) and those that may not be expected (non-targeted).[2] In contrast LC/MS/MS with a triple quadrupole in its most sensitive mode, multi-reaction monitoring (MRM), provides targeted screening and confirmation only.[3]

Recently Agilent has introduced the Pesticide Personal Compound Database (PCD) consisting of 1600 compounds and pesticides. With PCD the analyst can use the pesticide database as is for non-targeted screening or create custom databases from the read-only supplied database. The custom database can be edited by changing entries, adding, and deleting entries. In addition, a powerful feature of updating retention times allows the users' custom database to be modified with retention times from the users' chromatographic conditions.[4] The analyst can create as many custom databases with LC-dependent retention times as needed. This allows easy targeted (compounds verified with standards run with specific conditions) and non-target analysis (compounds in the database that have not been verified). The ability to detect and identify compounds not being sought in food and environmental samples can be very important. However, this ability must not be confused with affirmation that compounds not detected are not present. This can only be done by validation studies showing that the specific LC/MS method employed on specific matrices can detect the compounds reported as not present at the levels of concern. As an example, the pesticide database contains compounds not amenable to LC/MS such as hexachlorobenzene. These are included for the added information of the user. In addition, confirmation of positives would always require standards run with chromatographic conditions that would provide indicative retention times and additional structural information that can be obtained from fragments generated by MS/MS. Even with these analytical considerations, screening for a large list of pesticides as enabled by the LC/TOF or Q-TOF with the Agilent Pesticide PCD can be very valuable in detecting and identifying compounds that should not be present.

Experimental

Reagents and Chemicals

Pesticide standards were from a variety of sources: Sigma, Ultra-Scientific, ChemService, and Dr. Ehrenstorfer. For trace analysis the highest purity mobile phases are recommended. B&J LC/MS grade acetonitrile and methanol are used here. Buffers should be prepared from the highest quality chemicals such as GFS doubly distilled acetic acid, formic acid and ammonium hydroxide. If solid ammonium acetate and ammonium formate is used it should be prepared in a concentrated solution and then any particulates removed with 0.2-µm filters. Agilent Pesticide Test Mix, p/n 5190-0469 acid and base diluted separately as instructed to 10 ppb in 10% acetonitrile/90% water. An Agilent SampliQ QuEChERS AOAC Extraction kit, p/n 5982-5755. Agilent SampliQ QuEChERS AOAC Dispersive SPE kits for Highly Pigment Fruits and Vegetables, p/n 5982-5321 (2 mL) and p/n 5982-5356 (15 mL).

LC/MS methods are given in the Appendices:

Appendix I, LC/MS/MS Conditions for Test Mix Positive and Negative Ion Samples.

Appendix II. Agilent 1200 Series SL LC Parameters.

Appendix III, Agilent 1290 Infinity LC Parameters.

Spinach sample preparation

- Weigh 15 g (±0.1 g) of homogenized spinach sample.
- Spike standards or IS solution if necessary.
- Vortex 30 s.
- Add 15 mL of 1% acetic acid in acetonitrile.
- Add 1 bag of extraction kit (p/n 5982-5982-5755) buffered QuEChERS extraction tubes, AOAC Method 2007.01 with 6 g $MgSO_4$, 1.5 g NaAcetate.
- Cap and hand shake vigorously for 1 min.
- Centrifuge at 4000 rpm for 5 min.
- Transfer 1 mL or 8 mL of the upper layer to the dispersive SPE kit (p/n 5982-5321 or p/n 5982-5356) for highly pigmented fruits and vegetables.
- Vortex 1 min.
- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15-mL tubes at 4000 rpm for 5 min.

- Transfer 200 µL of the upper layer to the autosampler vial.
- Add 800 µL of water or appropriate standard spiking solution.
- Vortex 1 min, and prepare for LC/MS/MS analysis.

Results and Discussion

Fast and easy startup with Agilent test mix

To facilitate fast startup for pesticide screening, a positive and negative ion compound test mix is included with the Agilent Application Kit. This type of screening depends on obtaining accurate mass results and the TOF or Q-TOF should be operated with appropriate reference ions so that the best results will be obtained. Each of these test mixtures are prepared with a final injection concentration at 10 ppb, the accepted limit for pesticides worldwide. The extracted ion chromatogram (EIC) for each of the pesticides in the positive ion mix is shown in Figure 1. A method is provided with the kit that will allow the user to repeat this analysis. This method is an acquisition only method. Similar results demonstrate that the system is working properly. There are also two methods provided for work list automation data analysis that will generate the summary report of a database search of the Pesticide PCD. One method is the MFE_pesticide and this uses the “find compounds by molecular feature extraction (MFE)” algorithm in MassHunter Qualitative Analysis, a powerful data mining tool. This unique data mining program searches the data for all ions that can be associated with a real chromatographic peak and that may represent a "feature" of a molecule. This excludes reference ions and constant background ions and "spikes" that do not represent real compounds in the data file. MFE will create a compound list of all peaks in the data file that it has determined to represent real molecules. This algorithm is fast and generates good results with appropriate settings. The resulting report is shown in Table 1 for the positive test mix. This mixture contains only the compounds highlighted in the report. Please note that the database search screen does not confirm the presence of compounds and that compounds listed in the results does not indicate that they are conclusively present. Compounds listed could be from the blank, carry over or other sources.

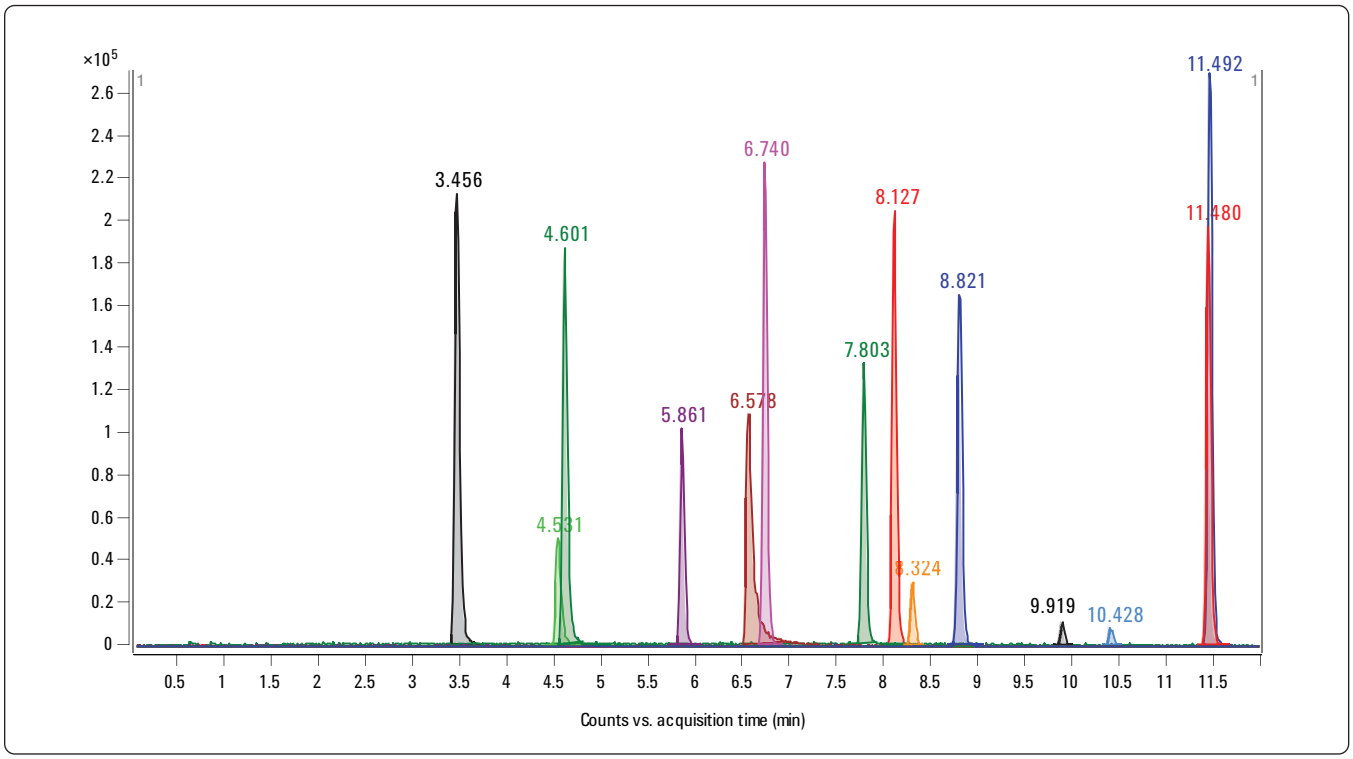


Figure 1. Extracted ion chromatogram of the positive ion test mix .

Table 1. Find Compounds by Molecular Feature Extractor with Pesticide Database Search Report for Positive Ion Test Mix

Data File	TestMix_pos_1.d	**Sample Name**	Test_Mix_pos_1
Sample Type	Sample	**Position**	P1-F2
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_Mix_Pos.m	**Acquired Time**	6/1/2009 3:28:51 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 19:	Aminocarb	3.472	208.1213	Aminocarb	$C_{11}H_{16}N_2O_2$	–0.44
Cpd 40:	Imazapyr	4.543	261.1113	Imazapyr	$C_{13}H_{15}N_3O_3$	–0.03
Cpd 41:	Thiabendazole	4.612	201.036	Thiabendazole	$C_{10}H_7N_3S$	0.2
Cpd 52:	Ethiofencarb sulfoxide	5.176	241.0777	Ethiofencarb sulfoxide	$C_{11}H_{15}NO_3S$	–1.91
Cpd 62:	Dimethoate	5.866	228.9998	Dimethoate	$C_5H_{12}NO_3PS_2$	–0.75
Cpd 65:	Imazalil	6.549	296.0488	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–1.58
Cpd 66:	Imazalil	6.579	296.0485	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–0.65
Cpd 68:	Metoxuron	6.746	228.0666	Metoxuron	$C_{10}H_{13}ClN_2O_2$	–0.09
Cpd 85:	Carbofuran	7.805	221.1054	Carbofuran	$C_{12}H_{15}NO_3$	–1.05
Cpd 88:	Atrazine	8.138	215.094	Atrazine	$C_8H_{14}ClN_5$	–0.92
Cpd 89:	DEET	8.2	191.1309	DEET	$C_{12}H_{17}NO$	0.53
Cpd 90:	Tibenzate	8.323	228.0607	Tibenzate	$C_{14}H_{12}OS$	1
Cpd 91:	Metosulam	8.33	417.0069	Metosulam	$C_{14}H_{13}Cl_2N_5O_4S$	–0.98
Cpd 92:	Fluoroglycofen	8.33	419.0033	Fluoroglycofen	$C_{16}H_9ClF_3NO_7$	–3.28
Cpd 93:	Tibenzate	8.433	228.0608	Tibenzate	$C_{14}H_{12}OS$	0.39
Cpd 97:	Tibenzate	8.527	228.0609	Tibenzate	$C_{14}H_{12}OS$	–0.12
Cpd 99:	Metazachlor	8.837	277.0983	Metazachlor	$C_{14}H_{16}ClN_{3O}$	–0.53
Cpd 107:	Molinate	9.927	187.1027	Molinate	$C_9H_{17}NOS$	2.02
Cpd 111:	Malathion	10.448	330.036	Malathion	$C_{10}H_{19}O_6PS_2$	0.2
Cpd 113:	Phenylacrylicacid	10.558	148.0522	Phenylacrylicacid	$C_9H_8O_2$	1.59
Cpd 121:	Tri-n-butyl phosphate	11.177	266.1645	Tri-n-butyl phosphate	$C_{12}H_{27}O_4P$	0.58
Cpd 123:	Tri-n-butyl phosphate	11.272	266.1646	Tri-n-butyl phosphate	$C_{12}H_{27}O_4P$	0.32
Cpd 125:	Pyraclostrobin	11.477	387.0989	Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	–0.9
Cpd 127:	Diazinon	11.497	304.1012	Diazinon	$C_{12}H_{21}N_2O_3PS$	–0.56

Database Search Results

Compound	Hits
Aminocarb	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Aminocarb	TRUE	$C_{11}H_{16}N_2O_2$	208.1213	208.1212	–0.44	3.472

Database Search Results

Compound	Hits
Imazapyr	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazapyr	TRUE	$C_{13}H_{15}N_3O_3$	261.1113	261.1113	–0.03	4.543

Database Search Results

Compound	Hits
Thiabendazole	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Thiabendazole	TRUE	$C_{10}H_7N_3S$	201.036	201.0361	0.2	4.612

Database Search Results

Compound	Hits
Ethiofencarb sulfoxide	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Thiabendazole	TRUE	$C_{10}H_7N_3S$	201.036	201.0361	0.2	4.612

Database Search Results

Compound	Hits
Ethiofencarb sulfoxide	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Ethiofencarb sulfoxide	TRUE	$C_{11}H_{15}NO_3S$	241.0777	241.0773	–1.91	5.176
Methiocarb sulfoxide	FALSE	$C_{11}H_{15}NO_3S$	241.0777	241.0773	–1.91	5.176

Database Search Results

Compound	Hits
Dimethoate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Dimethoate	TRUE	$C_5H_{12}N0_3PS_2$	228.9996	228.9996	–0.75	5.866

Database Search Results

Compound	Hits
Imazalil	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazalil	TRUE	$C_{14}H_{14}Cl_2N_2O$	296.0488	296.0483	–1.58	6.549

Database Search Results

Compound	Hits
Imazalil	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Imazalil	TRUE	$C_{14}H_{14}Cl_2N_2O$	296.0485	296.0483	–0.65	6.579

Database Search Results

Compound	Hits
Metoxuron	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metoxuron	TRUE	$C_{10}H_{13}ClN_2O_2$	228.0666	228.0666	–0.09	6.746

Database Search Results

Compound	Hits
Carbofuran	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Carbofuran	TRUE	$C_{12}H_{15}NO_3$	221.1054	221.1052	–1.05	7.805

Database Search Results

Compound	Hits
Atrazine	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Atrazine	TRUE	$C_8H_{14}ClN_5$	215.094	215.0938	–0.92	8.138

Database Search Results

Compound	Hits
DEET	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
DEET	TRUE	$C_{12}H_{17}NO$	191.1309	191.131	0.53	8.2

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0607	228.0609	1	8.323

Database Search Results

Compound	Hits
Metosulam	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metosulam	TRUE	$C_{14}H_{13}Cl_2N_5O_4S$	417.0069	417.0065	–0.98	8.33

Database Search Results

Compound	Hits
Fluoroglycofen	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Fluoroglycofen	TRUE	$C_{16}H_9ClF_3NO_7$	419.0033	419.002	–3.28	8.33

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0608	228.0609	0.39	8.433

Database Search Results

Compound	Hits
Tibenzate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tibenzate	TRUE	$C_{14}H_{12}OS$	228.0609	228.0609	–0.12	8.527

Database Search Results

Compound	Hits
Metazachlor	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Metazachlor	TRUE	$C_{14}H_{16}ClN_3O$	277.0983	277.0982	-0.53	8.837

Database Search Results

Compound	Hits
Molinate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Molinate	TRUE	$C_9H_{17}NOS$	187.1027	187.1031	2.02	9.927

Database Search Results

Compound	Hits
Malathion	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Malathion	TRUE	$C_{10}H_{19}O_6PS_2$	330.036	330.0361	0.2	10.448

Database Search Results

Compound	Hits
Phenylacrylicacid	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Phenylacrylicacid	TRUE	$C_9H_8O_2$	148.0522	148.0524	1.59	10.558

Database Search Results

Compound	Hits
Tri-n-butyl phosphate	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tri-n-butyl phosphate	TRUE	$C_{12}H_{27}O_4P$	266.1645	266.1647	0.58	11.177
Tri-iso-butyl phosphate		$C_{12}H_{27}O_4P$	266.1645	266.1647	0.58	11.177

Database Search Results

Compound	Hits
Tri-n-butyl phosphate	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Tri-n-butyl phosphate	TRUE	$C_{12}H_{27}O_4P$	266.1646	266.1647	0.32	11.272
Tri-iso-butyl phosphate		$C_{12}H_{27}O_4P$	266.1646	266.1647	0.32	11.272

Database Search Results

Compound	Hits
Pyraclostrobin	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Pyraclostrobin	TRUE	$C_{19}H_{18}ClN_3O_4$	387.0989	387.0986	–0.9	11.477

Database Search Results

Compound	Hits
Diazinon	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Diazinon	TRUE	$C_{12}H_{21}N_2O_3PS$	304.1012	304.1011	–0.56	11.497

The second method is Find_formula_pesticide. This method uses the “find by formula” algorithm of MassHunter Qualitative Analysis. This algorithm searches the data for the ions specified for each molecule in the database. For the supplied database this would entail generating extracted ion chromato-grams for each entry times each adduct (1600 for H^+, 1600 for Na^+, etc.). This is thorough but slower. However, if these searches are done automatically in a worklist, the processing time is reasonable. The analyst must determine what is the best fit-for-purpose procedure. Note that automatic database searching can be done during the worklist acquisition or after. Using the “worklist run” parameter of MassHunter acquisition, acquisition and data analysis can be selected, or data analysis only after the data has been collected. The data analysis methods can be added to the worklist by adding the column "Override DAMethod" to the MassHunter worklist and inserting the method to be used. (The qualitative analysis methods can be saved to the name of the acquisition method eliminating the need to add the "Override" column. (However, keeping acquisition methods and data analysis methods separate provides more flexibility.) All methods can be customized to meet the needs of a particular analysis.

Figure 2 shows the chromatogram of the negative ion compound test mix. Table 2 shows the results automatically generated for the negative ion mixture using the MFE_pesticide method. The report is generated using "Find and Identify" selection of compound automation and the 1600-compound pesticide database, pesticides.mtl, is searched. The worklist automation uses the "Compound automation and report" selection. To obtain the report shown, the "CompoundReportwithIdentificationHits.xltx" template of the "Common Reporting Options" in the General Navigation bar of MassHunter Qualitative analysis must be selected. This is important because as shown in the compound list of Table 2, the wrong isomer, dinoprop, is listed. This is the first isomer found in the database. The selected report template then lists the results of each database hit and the three isomers in the database are shown under this heading in the report. If the data were analyzed with the "Find by Formula" algorithm, the report would include all the isomers in the database in the main body of the report. If a retention time that matched the compound were in a custom database, only that isomer would be reported (targeted analysis). (Note that for the find by formula method to work within a worklist the Worklist Actions of the method should separately list "Compound Automation without report" and then "Generate Compound Report.")

The compound actually present is dinoseb and if this were a non-targeted analysis the analyst would need to confirm which one was present.

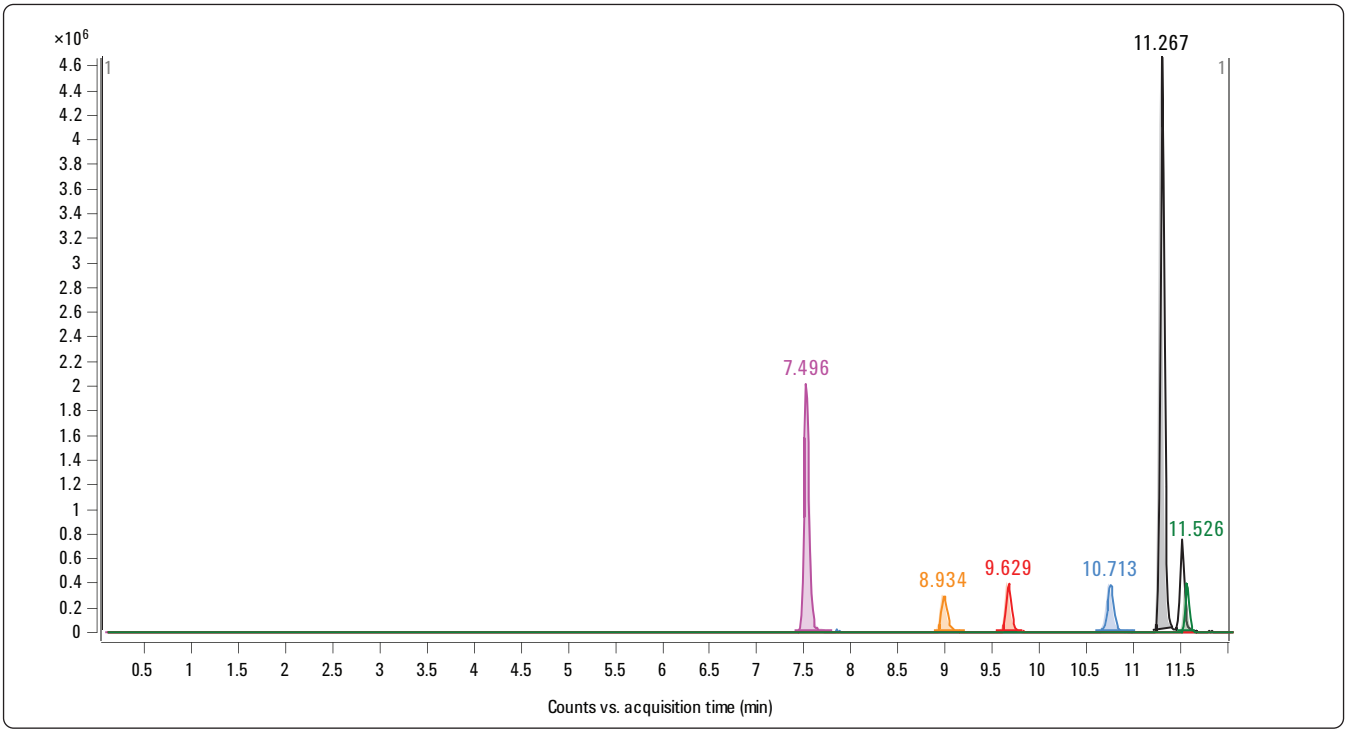


Figure 2. Extracted ion chromatogram of the negative ion test mix.

Table 2. Find compounds by Molecular Feature Extractor with Pesticide Database Search Report for Negative Ion Test Mix

Data File	Test_mix_neg_01.d	**Sample Name**	Test Mix Neg 1
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_mix_neg.m	**Acquired Time**	6/1/2009 1:33:54 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 12:	Bentazone	7.491	240.0573	Bentazone	$C_{10}H_{12}N_2O_3S$	–1.69
Cpd 15:	Dibutyl succinate	7.904	230.1517	Dibutyl succinate	$C_{12}H_{22}O_4$	0.5
Cpd 24:	2,4-D Methyl ester	8.768	233.9847	2,4-D Methyl ester	$C_9H_8Cl_2O_3$	1.7
Cpd 26:	2,4,5-T	8.934	253.9306	2,4,5-T	$C_8H_5Cl_3O_3$	–0.72
Cpd 32:	Silvex	9.623	267.9465	Silvex	$C_9H_7Cl_3O_3$	–1.6
Cpd 37:	Citronellal hydrate	10.219	172.1465	Citronellal hydrate	$C_{10}H_{20}O_2$	–1.25
Cpd 39:	Citronellal hydrate	10.37	172.1464	Citronellal hydrate	$C_{10}H_{20}O_2$	–0.47
Cpd 41:	Acifluorfen	10.716	360.9967	Acifluorfen	$C_{14}H_7ClF_3NO_5$	–0.55
Cpd 42:	Citronellal hydrate	10.736	172.1466	Citronellal hydrate	$C_{10}H_{20}O_2$	–1.37
Cpd 51:	Alantolactone	11.249	232.1462	Alantolactone	$C_{15}H_{20}O_2$	0.35
Cpd 52:	Dinoprop (see below)	11.267	240.075	Dinoprop	$C_{10}H_{12}N_2O_5$	–1.72
Cpd 56:	Hexaflumuron	11.53	459.982	Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	–0.76

Database Search Results

Compound	Hits
Bentazone	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Bentazone	TRUE	$C_{10}H_{12}N_2O_3S$	240.0573	240.0569	–1.69	7.491

Database Search Results

Compound	Hits
Dibutyl succinate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Dibutyl succinate	TRUE	$C_{12}H_{22}O_4$	230.1517	230.1518	0.5	7.904

Database Search Results

Compound	Hits
2,4,5-T	2

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
2,4,5-T	TRUE	$C_8H_5Cl_3O_3$	253.9306	253.9304	–0.72	8.934
Tricamba		$C_8H_5Cl_3O_3$	253.9306	253.9304	–0.72	8.934

Database Search Results

Compound	Hits
Silvex	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Silvex	TRUE	$C_9H_7Cl_3O_3$	267.9465	267.9461	–1.6	9.623

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1465	172.1463	–1.25	10.219

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1464	172.1463	–0.47	10.37

Database Search Results

Compound	Hits
Acifluorfen	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Acifluorfen	TRUE	$C_{14}H_7ClF_3NO_5$	360.9967	360.9965	–0.55	10.716

Database Search Results

Compound	Hits
Citronellal hydrate	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Citronellal hydrate	TRUE	$C_{10}H_{20}O_2$	172.1466	172.1463	–1.37	10.736

Database Search Results

Compound	Hits
Alantolactone	1

Compound	Best	Formula	Mass	Tgt Mass	Diff (ppm)	RT
Alantolactone	TRUE	$C_{15}H_{20}O_2$	232.1462	232.1463	0.35	11.249

Database Search Results (Note that the following are isomers with the same formula even though the compound present is listed as "FALSE.")

Compound	Hits					
Dinoprop	3					
Compound	**Best**	**Formula**	**Mass**	**Tgt Mass**	**Diff (ppm)**	**RT**
Dinoprop	TRUE	$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267
Dinoseb	FALSE	$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267
Dinoterb		$C_{10}H_{12}N_2O_5$	240.075	240.0746	–1.72	11.267

Database Search Results

Compound	Hits					
Hexaflumuron	1					
Compound	**Best**	**Formula**	**Mass**	**Tgt Mass**	**Diff (ppm)**	**RT**
Hexaflumuron	TRUE	$C_{16}H_8Cl_2F_6N_2O_3$	459.982	459.9816	–0.76	11.53

Customized databases with user added retention times

One of the powerful benefits of the supplied database is that it can be saved to a user customized database. To create a read-write customizable database, the user selects the "File" menu item and the "New Database." The software then allows selection of an existing database and then the naming of a new database. A description can also be given. When "Create" is selected the database with the new name contains all the entries of the selected database. In this way multiple custom databases can be created. The technical note on the Pesticide PCD [4] shows how the user can run standards with unique chromatographic conditions and easily update retention times in their custom database. This is shown in Figures 3 and 4 for the positive and negative ion test mix respectively.

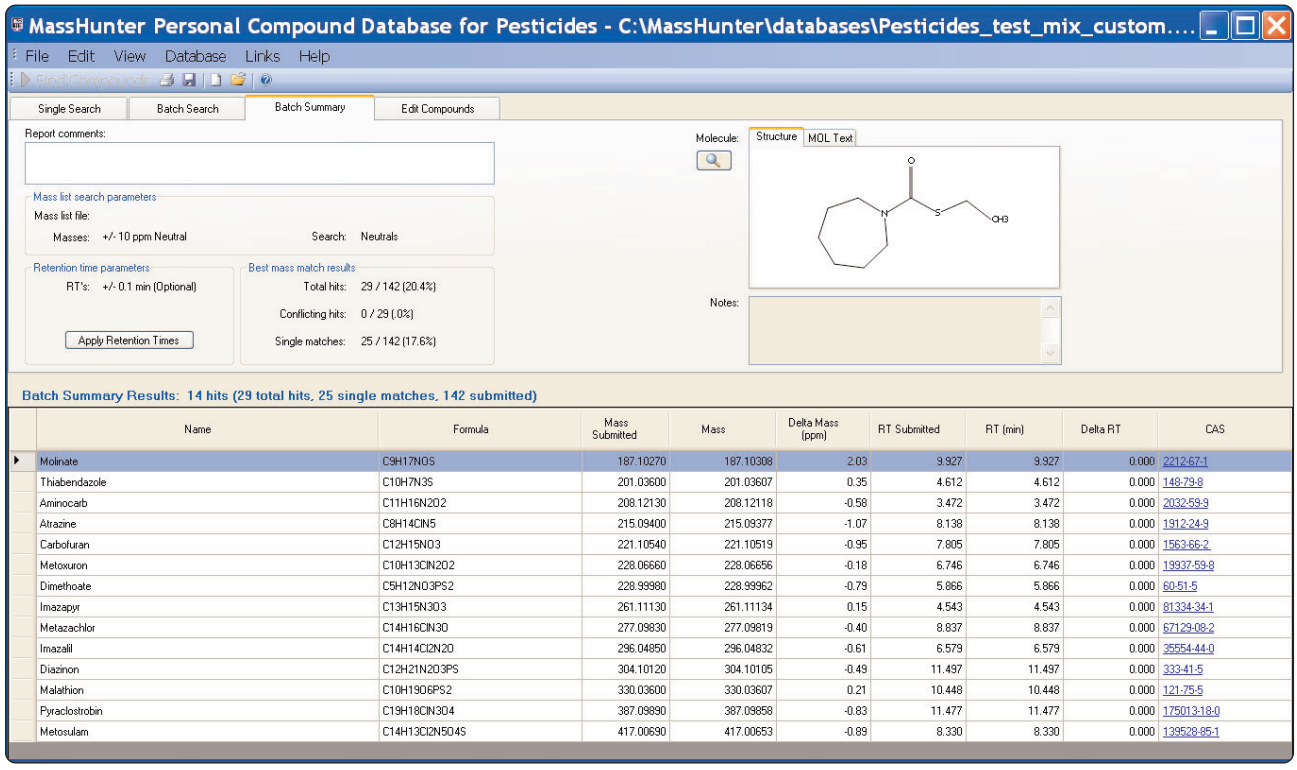
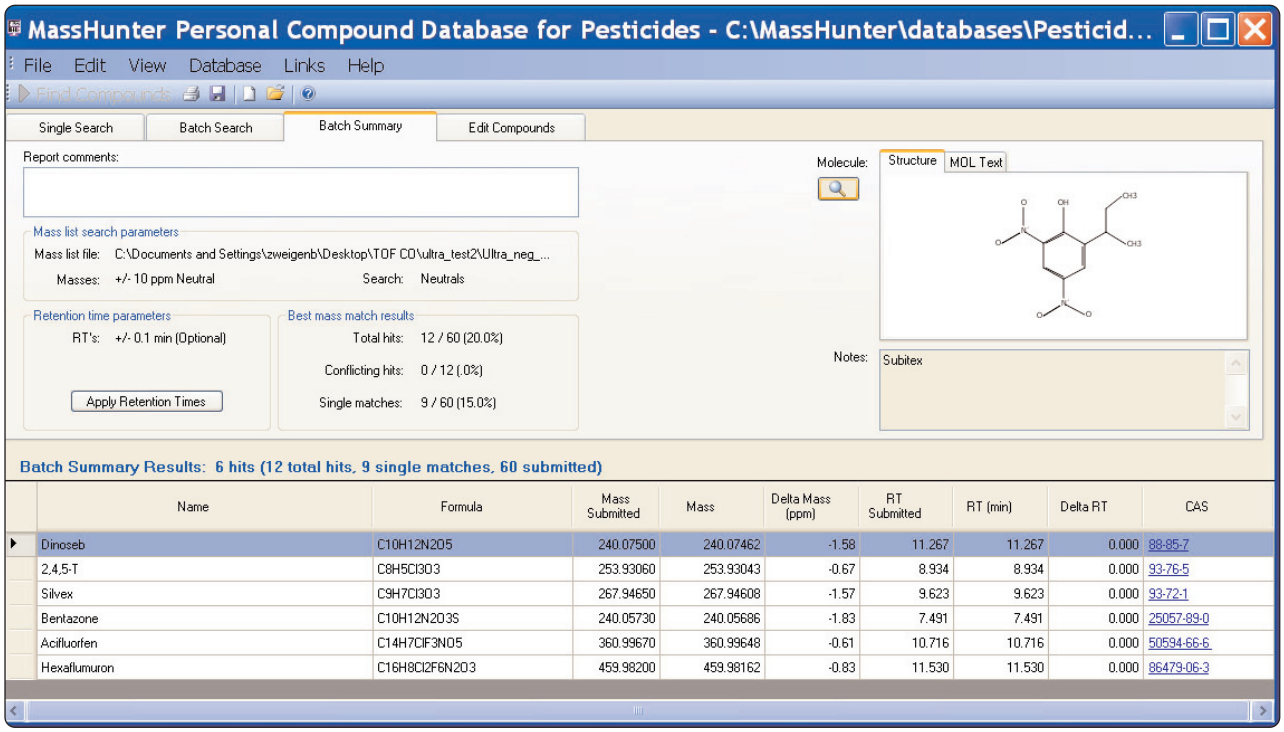


Figure 3. Pesticide Personal Compound Database (now with Library –PCDL, not shown) customized with retention times from positive ion test mix.



Name	Formula	Mass Submitted	Mass	Delta Mass (ppm)	RT Submitted	RT (min)	Delta RT	CAS
Dinoseb	C10H12N2O5	240.07500	240.07462	-1.58	11.267	11.267	0.000	88-85-7
2,4,5-T	C8H5Cl3O3	253.93060	253.93043	-0.67	8.934	8.934	0.000	93-76-5
Silvex	C9H7Cl3O3	267.94650	267.94608	-1.57	9.623	9.623	0.000	93-72-1
Bentazone	C10H12N2O3S	240.05730	240.05686	-1.83	7.491	7.491	0.000	25057-89-0
Acifluorfen	C14H7ClF3NO5	360.99670	360.99648	-0.61	10.716	10.716	0.000	50594-66-6
Hexaflumuron	C16H8Cl2F6N2O3	459.98200	459.98162	-0.83	11.530	11.530	0.000	86479-06-3

Figure 4. Pesticide Personal Compound Database (PCDL) customized with retention times for negative ion test mix.

If the analysis were for targeted compounds where retention times are known, dinoseb would be chromatographically separated from the other isomers. It is a simple exercise to take the results of the test mix, create a custom database from the provided pesticide database and update retention times. This would now create a targeted analysis. Either data analysis method can be modified for a targeted and non-targeted analysis by selecting "mass and retention time (optional)" for the search criteria. Targeted only analysis would be performed if "mass and retention time (required)" was checked. A report for a targeted and non-targeted analysis of the negative test mix with the method Find_by Formula and a custom database with the retention time for dinoseb would only list that compound. In this result, only dinoseb is reported because it is the compound in the custom database that matches the retention time. Even with retention times, identified compounds in the database must be confirmed. Both screening and confirmation can be done with the LC/Q-TOF.

Table 3. Find compounds by Formula with Pesticide Database Search Report for Negative ion Test Mix

Data File	Test_mix_neg_01.d	**Sample Name**	Test Mix Neg 1
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	
Acq Method	Test_mix_neg.m	**Acquired Time**	6/1/2009 1:33:54 PM
IRM Calibration Status	Success	**DA Method**	find_by_formula_pesticids.m
Comment			

Compound Table

Compound Label	RT	Mass	Abund	Name	Formula	Tgt Mass	DB Diff (ppm)
Cpd 1: Dichloromethoxybenzene	5.583	175.9796	9712	Dichloromethoxybenzene	$C_7H_6Cl_2O$	175.9796	0.11
Cpd 2: Bentazone	7.492	240.0573	108523	Bentazone	$C_{10}H_{12}N_2O_3S$	240.0569	1.69
Cpd 3: Dibutyl succinate	7.904	230.1517	7790	Dibutyl succinate	$C_{12}H_{22}O_4$	230.1518	–0.5
Cpd 6: Dichloroprop	8.764	233.9845	33463	Dichloroprop	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 7: Disugran	8.764	233.9845	33463	Disugran	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 4: Dichlorophenol 2,4-	8.764	161.9633	6051	Dichlorophenol 2,4-	$C_6H_4Cl_2O$	161.9639	–3.59
Cpd 5: 2,4-D Methyl ester	8.764	233.9845	33463	2,4-D Methyl ester	$C_9H_8Cl_2O_3$	233.985	–2.39
Cpd 9: Tricamba	8.941	253.9306	15646	Tricamba	$C_8H_5Cl_3O_3$	253.9304	0.75
Cpd 8: 2,4,5-T	8.941	253.9306	15646	2,4,5-T	$C_8H_5Cl_3O_3$	253.9304	0.75
Cpd 10: Trichlorophenol, 2,4,6-	9.613	195.9245	5877	Trichlorophenol, 2,4,6-	$C_6H_3Cl_3O$	195.9249	–2.12
Cpd 11: Silvex	9.624	267.9468	18804	Silvex	$C_9H_7Cl_3O_3$	267.9461	2.64
Cpd 14: Acifluorfen	10.708	360.9966	18261	Acifluorfen	$C_{14}H_7ClF_3NO_5$	360.9965	0.43
Cpd 13: Nitrofluorfen	10.708	317.0062	10928	Nitrofluorfen	$C_{13}H_7ClF_3NO_3$	317.0067	–1.41
Cpd 12: Azinphos-methyl	10.708	317.0061	9536	Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	317.0058	1
Cpd 15: Citronellal hydrate	10.732	172.1466	132453	Citronellal hydrate	$C_{10}H_{20}O_2$	172.1463	1.39
Cpd 16: Alantolactone	11.251	232.1462	10414	Alantolactone	$C_{15}H_{20}O_2$	232.1463	–0.54
Cpd 20: Ethiofencarb sulfoxide	11.262	241.078	23056	Ethiofencarb sulfoxide	$C_{11}H_{15}NO_3S$	241.0773	2.86
Cpd 21: Methiocarb sulfoxide	11.262	241.078	23056	Methiocarb sulfoxide	$C_{11}H_{15}NO_3S$	241.0773	2.86
Cpd 19: Dinoprop	11.262	240.0752	249379	Dinoprop	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 17: Dinoseb	11.262	240.0752	249379	Dinoseb	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 18: Dinoterb	11.262	240.0752	249379	Dinoterb	$C_{10}H_{12}N_2O_5$	240.0746	2.22
Cpd 22: Hexaflumuron	11.533	459.9823	19824	Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	459.9816	1.44

The power of Q-TOF for screening and confirmation

As an example of the power of this technique, a strawberry extract was spiked and analyzed using an Agilent 1200 Series SL LC with an Agilent 6520 Q-TOF. The extracted ion chromatogram of the over 100 pesticides spiked into this sample is shown in Figure 5. A pesticide screen with a Q-TOF is the same as with a TOF. However, LC/Q-TOF MS offers the highly selective MS/MS with accurate mass measurement that provides a workflow for both screening and confirmation. [1]

Screening hundreds of target and non-target pesticides using the Agilent 1200 Series SL with 6230 TOF

A standard of over 200 pesticides is run in a similar fashion and the EIC generated from the pesticides detected in a "find compounds by molecular feature" extractor with database search is shown in Figure 4. This method employs the Agilent 1200 Series SL and the Agilent 6230 TOF with Jet Stream Technology. This is the preferred configuration as it provides additional sensitivity to meet the demanding needs of multi-residue analysis. The method for this analysis is also provided with the Agilent Pesticide Screen Application Kit for TOF and Q-TOF.

The highest quality results are obtained with good chromatographic and mass spectral resolution. The ability to detect and identify thousands of compounds lies in both these parameters and accurate mass measurement. However, for any given real food sample only a few pesticides will be found. This may not be the case for environmental samples but the possibility of no more than 10 to 20 per site would be realistic. Given this reality, the need to be able to validate that hundreds of compounds can be detected in a fast analysis would provide this capability. Figure 6 shows a 3-minute run of over 100 pesticides using the new Agilent 1290 Infinity LC connected to the new Agilent 6540 Q-TOF. Given the chromatographic resolution achieved and the mass spectral resolution obtained, this analysis is reasonable for screening pesticides in food and environmental samples. The quality of the mass spectral data is shown in Figure 7 and this was collected at rate of 10 spectra per second.

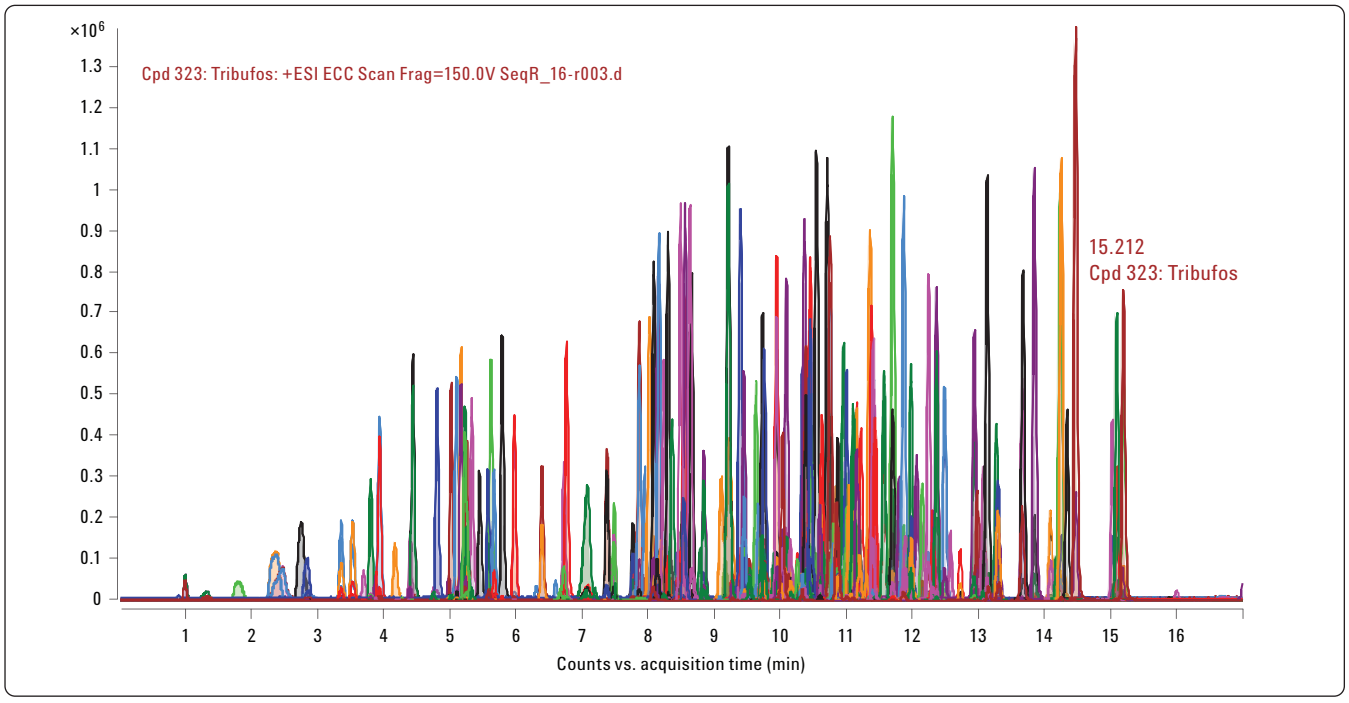


Figure 5. Extracted compound chromatogram (from compounds found by MFE) of 200 pesticides using the Agilent 1200 Series SL LC with the Agilent 6230 TOF.

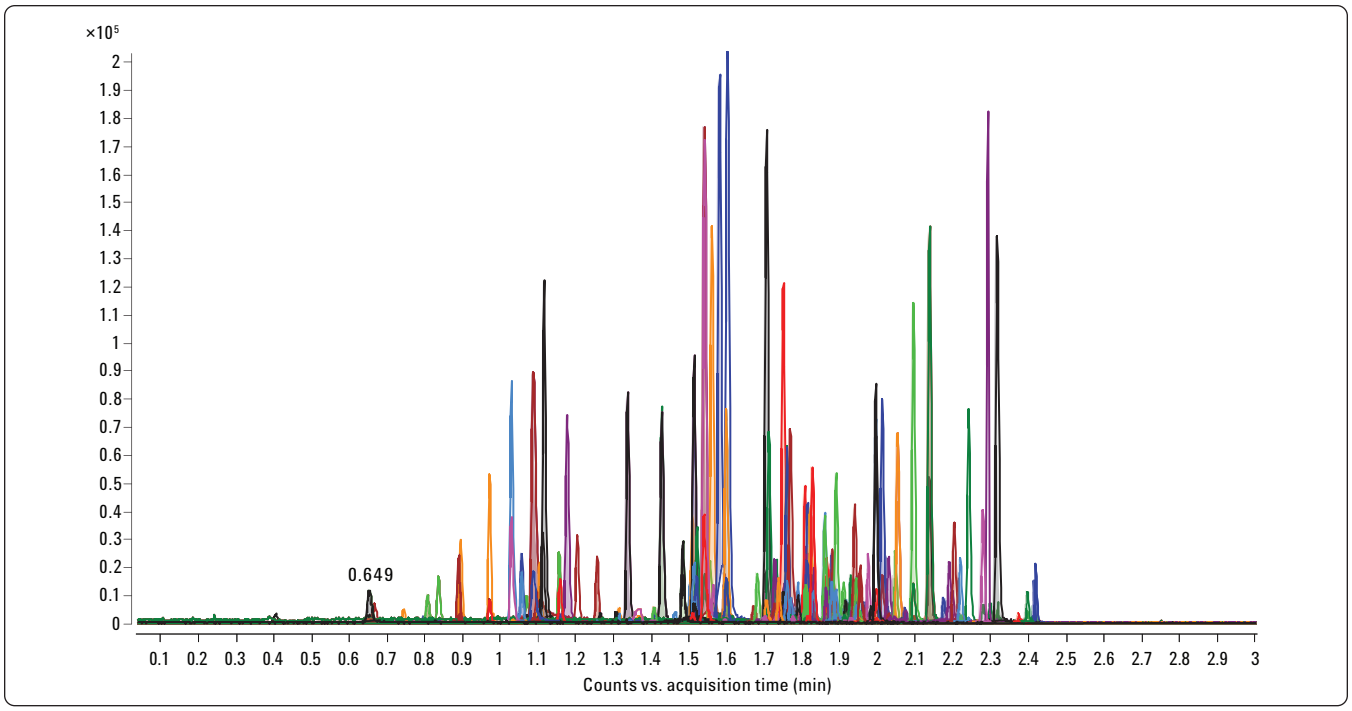
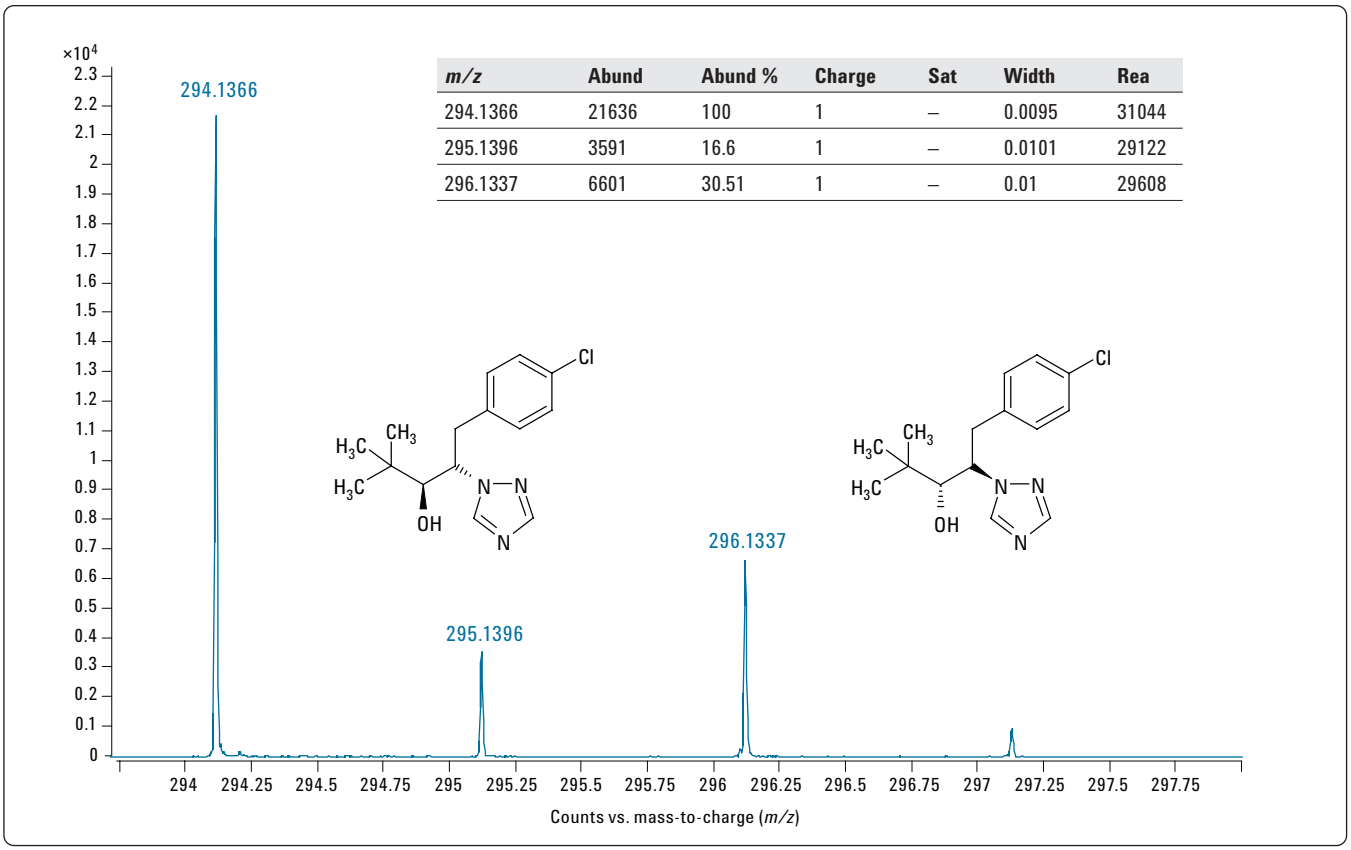


Figure 6. Extracted compound chromatogram of 100 pesticides in 3 min using the new Agilent 1290 Infinity LC with the new Agilent 6540 Q-TOF.



m/z	Abund	Abund %	Charge	Sat	Width	Rea
294.1366	21636	100	1	–	0.0095	31044
295.1396	3591	16.6	1	–	0.0101	29122
296.1337	6601	30.51	1	–	0.01	29608

Figure 7. Example mass spectrum from data on 3 min run with Agilent 1290 Infinity LC and Agilent 6540 Q-TOF. Note the mass resolution at 10 spectra per second.

Extraction to analysis with SampliQ extraction and SPE Kits

Finally, as an example of a complete analysis a spinach sample was spiked with pesticides at the 10-ppb level and extracted using the SampliQ QuEChERS Kit p/n 5982-5755. Then the Agilent SampliQ QuEChERS AOAC Dispersive SPE kit for highly pigmented fruits and vegetables, p/n 5982-5356 (15 mL), was used for clean-up. In addition, a reagent blank was prepared and run using an Agilent 1200 Series SL/6530 LC/Q-TOF and the standard screened with “find by molecular feature extractor” and the Pesticide database (not customized). The resulting mass list from the reagent blank was placed in the MFE settings by exporting the mass list to a .csv file, selecting "exclude these masses" under "Filter Mass", and using the exported .csv file as the database. In this way all the ions in the reagent blank will be removed from standards and samples processed with this method. The spiking solution (neat standard) was analyzed using the same acquisition method and the Worklist Automation. The results are given in Table 4 and represent the pesticides in the standard. It should be noted that if background removal is performed, the mass list should be searched by the database to make sure that compounds of concern will not be excluded. The .csv file is editable in Excel and masses can be removed from the exclusion list if necessary (for example, if pesticides are found in the blank).

Table 4. Neat Pesticide Standard for Spinach Extract

Data File	2ppb neat std .d	**Sample Name**	2ppb neat in 20:80 ACN/H2O
Sample Type	Sample	**Position**	P1-F1
Instrument Name	CAS6530_1	**User Name**	Jaz
Acq Method	MFE_Compound_report.m	**Acquired Time**	7/10/2009 12:43:56 PM
IRM Calibration Status	Success	**DA Method**	MFE_Pesticide_report.m
Comment			

Compound Table

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 12: Methamidophos	2.053	141.0012	Methamidophos	$C_2H_8NO_2PS$	0.96	1
Cpd 15: Acephate	2.467	183.0115	Acephate	$C_4H_{10}NO_3PS$	2.24	1
Cpd 18: Acephate	2.632	183.0119	Acephate	$C_4H_{10}NO_3PS$	–0.02	1
Cpd 24: Pymetrozine	3.242	217.0967	Pymetrozine	$C_{10}H_{11}N_5O$	–1.54	1
Cpd 25: Pymetrozine	3.361	217.0965	Pymetrozine	$C_{10}H_{11}N_5O$	–0.5	
Cpd 29: Carbendazim	4.259	191.0695	Carbendazim	$C_9H_9N_3O_2$	0.06	1
Cpd 35: Thiabendazole	4.633	201.0359	Thiabendazole	$C_{10}H_7N_3S$	0.81	1
Cpd 44: Imidacloprid	5.564	255.0527	Imidacloprid	$C_9H_{10}ClN_5O_2$	–1.43	1
Cpd 51: Imazalil	6.587	296.0489	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–1.92	1
Cpd 54: Dicyclanil	7.172	190.0968	Dicyclanil	$C_8H_{10}N_6$	–0.77	1
Cpd 55: Thiophanate-methyl	7.426	342.0463	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	–1.97	1
Cpd 86: Propoxur	7.621	209.1053	Propoxur	$C_{11}H_{15}NO_3$	–0.35	1
Cpd 87: Pyrocatechol	7.621	110.0368	Pyrocatechol	$C_6H_6O_2$	–0.59	1
Cpd 89: Norethynodrel	7.631	298.192	Norethynodrel	$C_{20}H_{26}O_2$	4.2	1
Cpd 91: Carbaryl	7.994	201.079	Carbaryl	$C_{12}H_{11}NO_2$	–0.07	7
Cpd 92: Naphthol, 1-	7.995	144.0575	Naphthol, 1-	$C_{10}H_8O$	0.01	1
Cpd 97: Ethoprop	9.908	242.0569	Ethoprop	$C_8H_{19}O_2PS_2$	–2	1
Cpd 99: Penconazole	10.219	283.0649	Penconazole	$C_{13}H_{15}Cl_2N_3$	–2.26	1
Cpd 101: Cyprodinil	10.482	225.1268	Cyprodinil	$C_{14}H_{15}N_3$	–0.68	1
Cpd 105: Kresoxim methyl	10.924	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.93	1

Figure 8 shows the extracted compound chromatogram from the "Molecular Feature Extractor" of the spinach extract. Even with the clean-up procedure and background ions removed, this is a complex sample. Table 5 shows the database search result for the spinach extract and all compounds detected in the standards were detected in the extract. If this were an analysis done for targeted and non-targeted analysis, all non-target positives (those without matching retention times) should be examined in MassHunter Qualitative Analysis before further analysis.

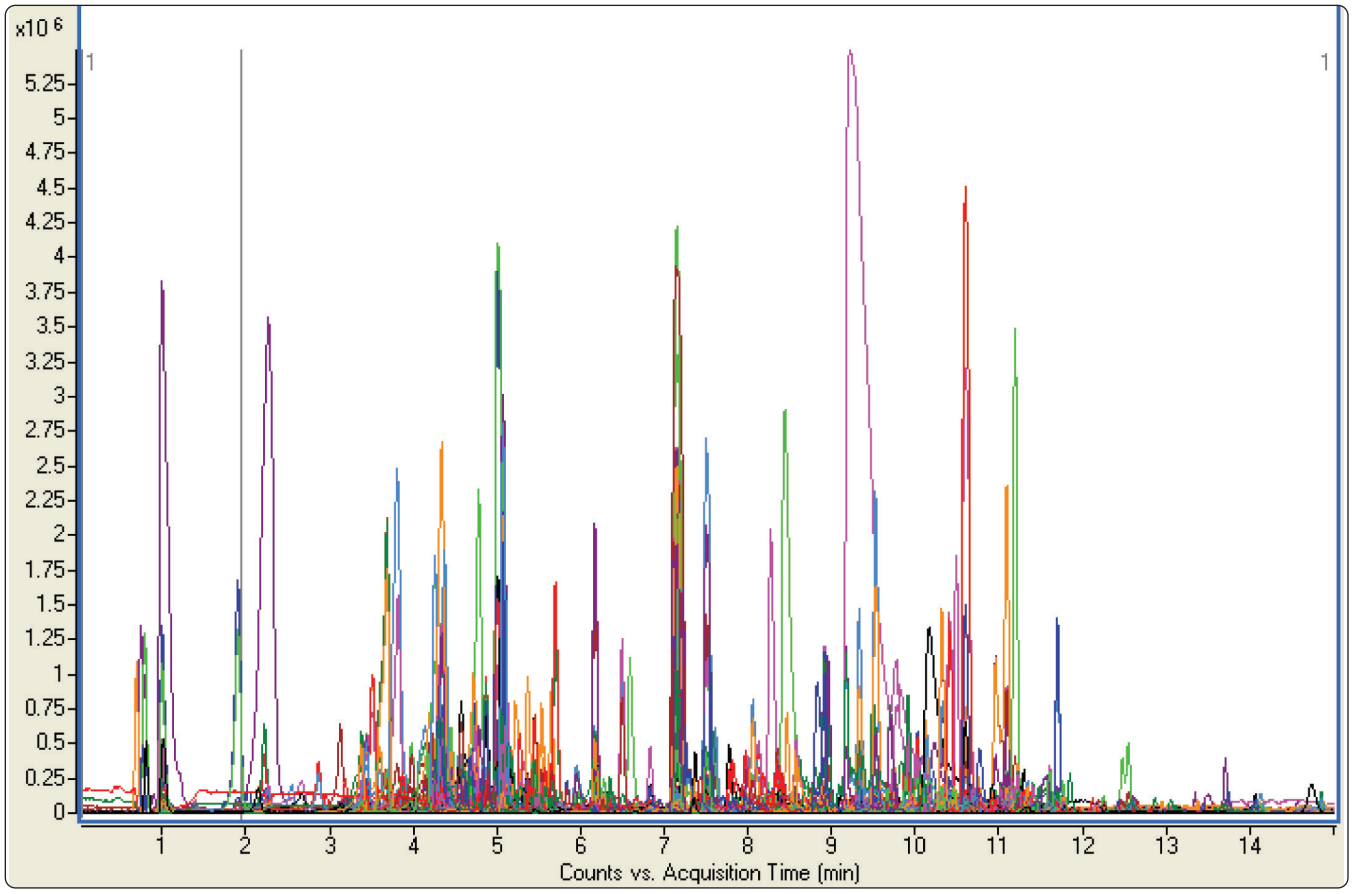


Figure 8. Extracted compound chromatogram of spinach sample with over 1200 compound features found .

Table 5. Results of Spinach Screen using Molecular Feature Extractor

Data File	Spinach AOAC 10ppb.d	**Sample Name**	Spinach AOAC 10 ppb (2 ppb in sample)
Sample Type	Sample	**Position**	P1-A4
Instrument Name	CAS6530_1	**User Name**	Jaz
Acq Method	MFE_Compound_report.m	**Acquired Time**	7/10/2009 12:40:59 PM
IRM Calibration Status	Some Ions Missed	**DA Method**	MFE_Pesticide_report.m
Comment			

Compound Table

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 36:	Methamidophos	2.042	141.001	Methamidophos	$C_2H_8NO_2PS$	2.37
Cpd 42:	Carbofuran-3-OH-7-phenol	2.226	180.0786	Carbofuran-3-OH-7-phenol	$C_{10}H_{12}O_3$	0.12
Cpd 44:	Metolcarb	2.233	165.0789	Metolcarb	$C_9H_{11}NO_2$	0.43
Cpd 74:	Acephate	2.614	183.0116	Acephate	$C_4H_{10}NO_3PS$	1.72
Cpd 94:	Decarbofuran	3.127	207.0896	Decarbofuran	$C_{11}H_{13}NO_3$	–0.45
Cpd 97:	Quinacetol sulfate	3.14	187.0631	Quinacetol sulfate	$C_{11}H_9NO_2$	1.39
Cpd 103:	3,5-Xylyl methylcarbamate	3.197	179.0945	3,5-Xylyl methylcarbamate	$C_{10}H_{13}NO_2$	0.78
Cpd 119:	Carbofuran, - 3 hydroxy	3.323	237.1003	Carbofuran, - 3 hydroxy	$C_{12}H_{15}NO_4$	–0.88
Cpd 121:	Pymetrozine	3.36	217.0963	Pymetrozine	$C_{10}H_{11}N_5O$	0.44
Cpd 136:	Propoxur	3.445	209.1053	Propoxur	$C_{11}H_{15}NO_3$	–0.42
Cpd 140:	3,5-Xylyl methylcarbamate	3.471	179.0953	3,5-Xylyl methylcarbamate	$C_{10}H_{13}NO_2$	–3.52
Cpd 161:	8-Hydroxyquinoline	3.59	145.0526	8-Hydroxyquinoline	C_9H_7NO	0.82
Cpd 198:	Metalaxyl	3.805	279.1478	Metalaxyl	$C_{15}H_{21}NO_4$	–2.52
Cpd 207:	Phenyl isocyanate	3.817	119.0373	Phenyl isocyanate	C_7H_5NO	–1.32
Cpd 221:	Aspidinol	3.946	224.1049	Aspidinol	$C_{12}H_{16}O_4$	–0.24
Cpd 284:	Phenoxyacetic acid	4.155	152.0472	Phenoxyacetic acid	$C_8H_8O_3$	1.13
Cpd 303:	Dimethyl phthalate	4.188	194.0579	Dimethyl phthalate	$C_{10}H_{10}O_4$	0.28
Cpd 310:	Trinexapac	4.21	224.0685	Trinexapac	$C_{11}H_{12}O_5$	0.08
Cpd 316:	Carbendazim	4.254	191.0695	Carbendazim	$C_9H_9N_3O_2$	–0.21
Cpd 323:	Geraniol	4.282	154.1357	Geraniol	$C_{10}H_{18}O$	0.55
Cpd 338:	Dimethyl phthalate	4.316	194.0579	Dimethyl phthalate	$C_{10}H_{10}O_4$	–0.1
Cpd 368:	Propoxur	4.372	209.1054	Propoxur	$C_{11}H_{15}NO_3$	–1.02
Cpd 386:	Aldicarb	4.443	190.0776	Aldicarb	$C_7H_{14}N_2O_2S$	–0.09
Cpd 455:	Phenoxyacetic acid	4.617	152.0474	Phenoxyacetic acid	$C_8H_8O_3$	–0.33
Cpd 461:	Thiabendazole	4.628	201.0362	Thiabendazole	$C_{10}H_7N_3S$	–0.66
Cpd 492:	Butopyronoxyl	4.723	226.1207	Butopyronoxyl	$C_{12}H_{18}O_4$	–0.65
Cpd 584:	Tiocarbazil	4.904	279.167	Tiocarbazil	$C_{16}H_{25}NOS$	–4.82

Compound Label		RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 587:	Kresoxim methyl	4.905	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.89
Cpd 641:	Pyrethrin I	5.022	328.2041	Pyrethrin I	$C_{21}H_{28}O_3$	–0.8
Cpd 642:	Allethrin	5.022	302.1884	Allethrin	$C_{19}H_{26}O_3$	–0.79
Cpd 644:	Spiromesifen	5.022	370.2148	Spiromesifen	$C_{23}H_{30}O_4$	–1.01
Cpd 720:	Phosfon	5.19	396.1312	Phosfon	$C_{19}H_{32}Cl_3P$	–1.33
Cpd 721:	Santonin	5.197	246.1259	Santonin	$C_{15}H_{18}O_3$	–1.43
Cpd 740:	Dimethyl phthalate	5.255	194.0576	Dimethyl phthalate	$C_{10}H_{10}O_4$	1.68
Cpd 743:	Metaldehyde	5.265	176.1044	Metaldehyde	$C_8H_{16}O_4$	2.75
Cpd 804:	Phosfon	5.439	396.1301	Phosfon	$C_{19}H_{32}Cl_3P$	1.47
Cpd 816:	Allethrin	5.454	302.1882	Allethrin	$C_{19}H_{26}O_3$	–0.08
Cpd 830:	Buthiobate	5.498	372.1696	Buthiobate	$C_{21}H_{28}N_2S_2$	–0.67
Cpd 858:	Imidacloprid	5.57	255.0527	Imidacloprid	$C_9H_{10}ClN_5O_2$	–1.47
Cpd 976:	Kresoxim methyl	6.15	313.1321	Kresoxim methyl	$C_{18}H_{19}NO_4$	–2.14
Cpd 1047:	Kresoxim methyl	6.366	313.132	Kresoxim methyl	$C_{18}H_{19}NO_4$	–1.78
Cpd 1063:	Alantolactone	6.463	232.1463	Alantolactone	$C_{15}H_{20}O_2$	0.23
Cpd 1075:	Santonin	6.521	246.1258	Santonin	$C_{15}H_{18}O_3$	–0.98
Cpd 1089:	Imazalil	6.595	296.049	Imazalil	$C_{14}H_{14}Cl_2N_2O$	–2.29
Cpd 1124:	Salbuterol	6.838	239.1522	Salbuterol	$C_{13}H_{21}NO_3$	–0.34
Cpd 1144:	Butopyronoxyl	6.93	226.1206	Butopyronoxyl	$C_{12}H_{18}O_4$	–0.52
Cpd 1212:	Cinmethylin	7.152	274.1937	Cinmethylin	$C_{18}H_{26}O_2$	–1.56
Cpd 1242:	Dicyclanil	7.22	190.0968	Dicyclanil	$C_8H_{10}N_6$	–0.54
Cpd 1274:	Thiophanate-methyl	7.419	342.0457	Thiophanate-methyl	$C_{12}H_{14}N_4O_4S_2$	–0.24
Cpd 1331:	Bisphenol A	7.596	228.1141	Bisphenol A	$C_{15}H_{16}O_2$	4.06
Cpd 1335:	Propoxur	7.614	209.1052	Propoxur	$C_{11}H_{15}NO_3$	–0.1
Cpd 1337:	Pyrocatechol	7.615	110.0369	Pyrocatechol	$C_6H_6O_2$	–1.11
Cpd 1348:	Cinmethylin	7.7	274.1933	Cinmethylin	$C_{18}H_{26}O_2$	–0.16
Cpd 1357:	Bisphenol A	7.778	228.1147	Bisphenol A	$C_{15}H_{16}O_2$	1.28
Cpd 1394:	Naphthol, 1-	7.996	144.0574	Naphthol, 1-	$C_{10}H_8O$	0.69
Cpd 1395:	Carbaryl	7.996	201.0791	Carbaryl	$C_{12}H_{11}NO_2$	–0.39
Cpd 1410:	Spinosyn B	8.128	717.4462	Spinosyn B	$C_{40}H_{63}NO_{10}$	–1.38
Cpd 1429:	Spinosyn A	8.278	731.4619	Spinosyn A	$C_{41}H_{65}NO_{10}$	–1.43
Cpd 1438:	Spiroxamine	8.364	297.2675	Spiroxamine	$C_{18}H_{35}NO_2$	–2.27
Cpd 1477:	Spinosyn D	8.621	745.4775	Spinosyn D	$C_{42}H_{67}NO_{10}$	–1.29
Cpd 1494:	Embelin	8.752	294.1838	Embelin	$C_{17}H_{26}O_4$	–2.25
Cpd 1501:	Cinmethylin	8.797	274.1935	Cinmethylin	$C_{18}H_{26}O_2$	–0.9
Cpd 1506:	Santonin	8.81	246.1261	Santonin	$C_{15}H_{18}O_3$	–2.01
Cpd 1526:	Cinmethylin	8.908	274.1935	Cinmethylin	$C_{18}H_{26}O_2$	–0.82
Cpd 1574:	Cinmethylin	9.184	274.1936	Cinmethylin	$C_{18}H_{26}O_2$	–1.21
Cpd 1578:	Bromophos	9.22	363.8501	Bromophos	$C_8H_8BrCl_2O_3PS$	–2.55
Cpd 1604:	Bromophos	9.279	363.8502	Bromophos	$C_8H_8BrCl_2O_3PS$	–2.57
Cpd 1715:	Imazethapyr	9.883	289.1417	Imazethapyr	$C_{15}H_{19}N_3O_3$	3.08

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)
Cpd 1728: Ethoprop	9.911	242.0566	Ethoprop	$C_8H_{19}O_2PS_2$	–0.93
Cpd 1746: Imazethapyr	10.027	289.1419	Imazethapyr	$C_{15}H_{19}N_3O_3$	2.59
Cpd 1782: Penconazole	10.224	283.0649	Penconazole	$C_{13}H_{15}Cl_2N_3$	–1.99
Cpd 1797: Cinmethylin	10.325	274.1931	Cinmethylin	$C_{18}H_{26}O_2$	0.68
Cpd 1829: Cyprodinil	10.49	225.1271	Cyprodinil	$C_{14}H_{15}N_3$	–2.07
Cpd 1833: Chenodeoxycholic acid	10.526	392.291	Chenodeoxycholic acid	$C_{24}H_{40}O_4$	4.36
Cpd 1838: Embelin	10.541	294.1837	Embelin	$C_{17}H_{26}O_4$	–2.12
Cpd 1885: Chenodeoxycholic acid	10.749	392.2909	Chenodeoxycholic acid	$C_{24}H_{40}O_4$	4.45
Cpd 1908: Cinmethylin	10.852	274.1936	Cinmethylin	$C_{18}H_{26}O_2$	–1.24
Cpd 1927: Kresoxim methyl	10.933	313.1321	Kresoxim methyl	$C_{18}H_{19}NO_4$	–2.18
Cpd 1933: Cinmethylin	10.967	274.1934	Cinmethylin	$C_{18}H_{26}O_2$	–0.53
Cpd 1951: Cinmethylin	11.089	274.1939	Cinmethylin	$C_{18}H_{26}O_2$	–2.13
Cpd 1978: Carbofuranphenol	11.235	164.0841	Carbofuranphenol	$C_{10}H_{12}O_2$	–2.38
Cpd 2017: Spiroxamine	11.536	297.2666	Spiroxamine	$C_{18}H_{35}NO_2$	0.5
Cpd 2044: Spiroxamine	11.731	297.2663	Spiroxamine	$C_{18}H_{35}NO_2$	1.49
Cpd 2054: Spiroxamine	11.846	297.2673	Spiroxamine	$C_{18}H_{35}NO_2$	–1.65
Cpd 2062: Spiroxamine	11.955	297.2672	Spiroxamine	$C_{18}H_{35}NO_2$	–1.26
Cpd 2096: Etacelasil	12.24	316.1098	Etacelasil	$C_{11}H_{25}ClO_6Si$	3.42
Cpd 2205: Spiroxamine	12.797	297.267	Spiroxamine	$C_{18}H_{35}NO_2$	–0.58
Cpd 2206: Ivermectin B1b	12.806	860.488	Ivermectin B1b	$C_{47}H_{72}O_{14}$	4.9
Cpd 2253: Ivermectin B1b	13.128	860.4891	Ivermectin B1b	$C_{47}H_{72}O_{14}$	3.61
Cpd 2314: Ivermectin B1b	13.372	860.4896	Ivermectin B1b	$C_{47}H_{72}O_{14}$	3.04
Cpd 2474: Ivermectin B1b	14.122	860.4906	Ivermectin B1b	$C_{47}H_{72}O_{14}$	1.85

Conclusions

The Agilent TOF and Q-TOF Pesticide Application Kit has been developed to provide comprehensive screening of pesticides for both targeted and non-targeted compounds. The database includes almost 1600 compounds and gives the user great flexibility in its use.

The kit offers:

- Fast and easy startup of complex analyses
- A comprehensive pesticide database of almost 1600 compounds including:
    - Chemical structures, formulas and exact masses
    - Direct Chemical Internet links to PUBCHEM and Chemspider
    - IUPAC Names
    - The ability to create spectral libraries
    - Completely customizable additions/deletions and retention time additions for chromatographic conditions developed by the user
- Results can be searched directly from the PCDL software
- Results can be data-mined with powerful searching tools such as, the Molecular Feature Extractor and Find by Formula
- Searches of the database can be partially or completely automated using MassHunter Qualitative Analysis and the MassHunter Acquisition Worklist

References

1. Agilent Technologies publication 5990-3935EN, "Q-TOF LC/MS Screening and Confirming of Non-Targeted Pesticides in a Strawberry Extract."
2. Agilent Technologies publication 5989-5496EN, "Automated Screening of 600 Pesticides in Food by LC/TOF MS Using a Molecular-Feature Database Search."
3. Agilent Technologies publication 5990-4253EN, "Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS."
4. Agilent Technologies publication 5990-3976EN, "Pesticide Personal Compound Database for Screening and Identification."

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Appendix I

LC/MS/MS Conditions for Test mix Positive and Negative Ion Samples

Agilent 1200 Series SL LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	35 °C
Injection volume:	5
Autosampler temperature:	Ambient
Needle wash:	5 s with methanol
Mobile phase:	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow rate:	0.3 mL/min
Gradient:	5% B at t = 0 to 95% B at t = 12 min
Stop time:	12 min
Post time:	3 min

Agilent 6530 Q-TOF Parameters

Jet Stream Conditions

Gas temperature:	250 °C
Gas flow:	7 L/min
Nebulizer:	40 psi
Sheath gas temperature:	325 °C
Sheath gas flow:	11 L/min
Capillary + ion:	3500 V
Nozzle voltage:	0 V
Capillary – ion:	2500 V
Nozzle voltage:	1500 V
Acquisition Mode:	MS1
Min Range	100 *m/z*
Max Range	1100 *m/z*
Scan Rate	1.4 per s
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)
Reference Masses:	Negative ion 119.0362 ($M-H^-$ for purine) 980.016375 ($M+C_2H_3O_2^-$ for HP-921 acetate adduct)

Appendix II

Agilent 1200 Series SL LC Parameters

Agilent 1200 Series LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature:	55 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25), 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate:	0.3 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.5	No change	600	6
14	No change	600	95
17	No change	600	95

Stop time	17 min
Post time	3 min

Agilent 6230 TOF Parameters

Jet Stream Conditions

Drying gas temperature:	225 °C
Drying gas flow (nitrogen):	9 L/min
Nebulizer gas pressure (nitrogen):	25 psig
Capillary voltage:	4500 V
Sheath gas temperature:	350 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	500 V
Acquisition Mode MS1	
Min Range	25 m/z
Max Range	3200 m/z
Scan Rate	3
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)

Appendix III

Agilent 1290 Infinity LC Parameters

Column:	Agilent ZORBAX Eclipse Plus C18 HD, 2.1 mm × 100 mm, 1.8 µm Agilent p/n
Column temperature:	60 °C
Injection volume:	5.0 µL
Autosampler temperature:	6 °C
Needle wash:	Flushport (MeOH:H_2O 75:25) 5 s
Mobile phase:	A = H_2O w/5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
LC flow rate:	1.0 mL/min

Gradient pump time table

Time	Flow	Pressure	Solv ratio B
0.15	No change	600	6
2.1	No change	600	95
3	No change	600	95

Stop time	3 min
Post time	1 min

6540 Q-TOF Parameters

Jet stream conditions

Drying gas temperature:	325 °C
Drying gas flow (nitrogen):	8 L/min
Nebulizer gas pressure (nitrogen):	60 psig
Capillary voltage:	4000 V
Sheath gas temperature:	350 °C
Sheath gas flow:	12 L/min
Nozzle voltage:	500
Acquisition Mode:	MS1
Min Range	100 *m/z*
Max Range	1000 *m/z*
Scan Rate	10 per s
Reference Masses:	Positive ion 121.050873 ($M+H^+$ for purine) 922.009798 ($M+H^+$ for HP-921)

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Printed in the USA
August 5, 2009
5990-4251EN

Agilent Technologies