Development and Application of an Exact Mass LC/MS/MS Library for the Accurate Mass Screening and Quantitation of Pesticides in Fruits and Vegetables

Application Note

Food, Pesticide Analysis

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Abstract

This application note describes the creation of an exact mass library for pesticides and its application to the screening, quantitation and verification of pesticide residues in fruit and vegetables. An Agilent 1290 Infinity LC System was coupled to an Agilent 6540 Ultra High Definition QTOF LC/MS System which was operated in positive and negative electrospray using Dual Spray Agilent Jet Stream Technology. Target MS/MS acquisition was used for quantitation and verification of pesticide residues. Results of the successful validation of a fast UHPLC-QTOF-MS/MS method for three different commodity groups are shown. The method was appropriate for the analysis of pesticides in food extracts with regards to the required limits of quantitation (LOQs), linearity, and reproducibility. When applied to real-world samples from a routine monitoring program, all pesticides detected by triple quadrupole LC/MS and GC/MS methods were identified by the UHPLC-QTOF/MS method. Quantitation results were also in good agreement.

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Introduction

There is an ever increasing number of pesticides, and although the use of many of them is no longer permitted, they still occur from time to time as contaminants in food products. Accurate-Mass LC/MS screening for pesticides in food is of growing interest since it allows testing of a large number of potential contaminations. This is especially useful for commercial testing labs as a means of increasing the scope of the analysis, increasing the sample throughput, and minimizing cost-per-analysis. This increased interest is due to the recent implementation of guideline SANCO/12495/2011 [1] establishing method validation and quality control procedures for the analysis of pesticide residues in food and feed. For the first time, there are specified criteria for qualitative screening without the use of expensive standards for each pesticide in each batch of samples.

In the EU, maximum residue levels (MRLs) for pesticides are set by European Commission regulation (EC) 396/2005 and its amendments [2]. Appendix II and III specify more than 170,000 MRLs for various matrix pesticide combinations. The equivalent for the US is 40 CFR part 180 which sets tolerances and exemptions for pesticides in food. In addition, it specifies methodologies allowed for the analyses.

Modern LC/QTOF/MS instruments allow the analysis of most LC/MS amenable pesticides well below the MRLs specified by EU and US legislation. A typical workflow includes quantitation of all regulated pesticide residues considered to be a risk for a given country by using MS domain data. A list of less likely contaminants can be found and identified, when comprehensive database searches are applied, using the additional information resident in the full scan accurate mass data that comes from a time-of-flight instrument [3]. In complex samples such as QuEChERS extracts [4], the challenge is to rule out potential false positives. In accurate mass LC/TOF/MS, the retention time, mass accuracy, isotope distribution, and adduct pattern are used to verify positives. The availability of true MS/MS information gives a higher level of confidence in the identification especially if accurate mass fragment information is available.

This application note describes the creation of an exact mass LC/MS/MS library containing CID spectra for three different collision energies for over 300 LC/MS amenable pesticides listed high in the Check-your-scope ranking of the EURL for pesticides. This exact mass MS/MS library was then used as part of a LC/QTOF/MS/MS workflow for the screening and identification of pesticides in fruit and vegetable extracts. Pesticides verified by MS/MS spectral comparison were also quantified using the MS domain data. Results of the successful validation of the workflow, according to SANCO/12495/2011, for more than 50 pesticides and three representative fruit and vegetable commodities belonging to different commodity groups [5] are presented. When the workflow was applied to real world samples which were part of routine pesticide monitoring, all pesticides detected earlier by triple quadrupole LC/MS and GC/MS analysis were identified and quantitation results were also in agreement.

Experimental

Sample preparation

Fruits and vegetables were obtained from a local grocer. Samples were extracted according to the official citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits. Ten grams homogenized fruit and vegetable (cucumber, lemon, and rucola) samples were weighed in 50 mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. The lemon homogenate was neutralized afterwards by adding 600 µL of a 5 M sodium hydroxide solution. After adding an extraction salt packet containing 4 g anhydrous $MgSO_4$, 1 g NaCl, and 1.5 g buffering citrate salts, the mixture was again shaken for 1 minute and then centrifuged at 3,000 rpm for 5 minutes.

After phase separation, a 6-mL aliquot of the upper acetonitrile phase was transferred into an Agilent BondElut QuEChERS EN dispersive SPE tube (p/n 5982-5256) containing 150 mg primary secondary amine (PSA) and 15 mg graphitized carbon black for sample cleanup and 900 mg anhydrous $MgSO_4$ to remove water. The tubes were closed and shaken for another minute. Afterwards, the tubes were centrifuged at 3,000 rpm for 5 minutes. A 4-mL amount of the final extract was transferred into a clean polypropylene vial. To improve the stability of the target pesticides, 40 µL formic acid was added to the final extract. For use in method validation, a pesticide mixture containing 55 target compounds was spiked into an aliquot of the final extracts at four different levels corresponding to 5, 10, 50, and 100 µg/kg.

LC/MS/MS Analyses

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6540A UHD Quadrupole Time-of-Flight LC/MS System equipped with a Dual Spray Agilent Jet Stream electrospray ionization source and operated in the 2 GHz extended dynamic range mode.

Reference mass ions were delivered using an Agilent Infinity 1260 Isocratic pump (G1310B) at a flow rate of 10 µL/min using a 1 in 100 flow splitter (G1607-60000). The importance of a reliable reference mass delivery is described in great detail in [6]. Agilent MassHunter workstation B.05.00 software was used for data acquisition, MassHunter Qualitative Analysis B.06.00 and Quantitative Analysis B.05.02 was used for data analysis. Table 1 shows the UHPLC parameters; Table 2 shows the Agilent Jet Stream parameters.

Exact mass LC/MS/MS library spectra for more than 300 pesticides were acquired at collision energies of 10, 20, and 40 eV by injecting individual standards in acetonitrile with a concentration of 1 ng/µl in flow injection analysis (FIA) into the LC/QTOF/MS/MS system operated in target MS/MS mode. The $[M+H]^+$ and the $[M-H]^-$ ions were specified as the target masses. After curation of the acquired MS/MS spectra for their exact fragment masses, the spectra were included in the pesticide PCDL which was then applied for pesticide discovery and verification.

In this step, final QuEChERS extracts were injected onto the Q-TOF system, operating with an acquisition rate of 5 scans/sec in the MS domain, and 3 scans/sec in the MS/MS domain. Data was collected in positive and negative ion mode in two consecutive analytical runs. In the TOF mode (MS domain), a mass range of *m/z* 100 to 1,100 amu was acquired. In the target MS/MS mode, a mass range of *m/z* 50 to 1,000 amu was acquired for more than 200 target masses rated high in the Check-your-scope list of the EURL for pesticides, using an acquisition window of 0.6 minutes. A collision energy ramp was applied to the target masses using an offset of 4 eV and a slope of 6 eV per 100 amu.

Table 1. UHPLC Method Parameters

UHPLC column	Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 × 150 mm, 1.8 µm at 30 °C	
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol	
Gradient program	Min	% B
	0	10
	0.5	10
	3.5	50
	17.0	100
	20.0	100
	20.1	10
Stop time	22 min	
Flow rate	0.40 mL/min	
Injection volume	3 µL	

Table 2. Agilent Jet Stream Parameters

Parameter	Value	
Gas temperature	200 °C	
Gas flow	8.0 L/min	
Nebulizer	35 psi	
Sheath gas temperature	350 °C	
Sheath gas flow	11.0 L/min	
	Value positive (V)	**Value negative (V)**
Vcap	4,000	3,000
Nozzle voltage	300	0
Fragmentor	120	120
Skimmer 1	65.0	65.0
Octopole RF peak	750	750
Reference mass correction	Enabled	
	Detection window	50 ppm
	Minimum height	500 counts
Reference mass ions	Positive	Negative
	121.050873	119.03632
	922.009798	966.000725

Pesticide discovery was done using the Find by Formula (FBF) data mining algorithm. In the Find by Formula workflow, compounds are searched in the MS domain using the molecular formula information out of the Personal Compound Database and Library (PDCL) along with the user supplied definition of the ion species. Table 3 shows the parameters used in the FBF algorithm which represent good values for the screening of residues and contaminants in samples with high chemical background.

Results were scored based on retention time, mass accuracy, and isotope pattern matching. When a compound was identified in the MS domain, CID spectra were automatically extracted and searched against the exact-mass MS/MS library spectra included in the PCDL. When suspect compounds were verified by a MS/MS library search, pesticides were included in quantitation for an efficient batch review and to assign a concentration value.

Figure 1 illustrates the applied workflow for the screening, identification, and quantitation of pesticides in fruit and vegetable extracts. This approach was used also for real world samples. Identified pesticides as well as quantitation results were compared to results previously collected on triple quadrupole LC/MS and GC/MS systems.

Table 3. Parameter Settings for the FBF Data Mining Algorithm

Parameter	Value	
Extraction data format	Centroid for both, chromatographic and spectral extraction	
Integrator	Agile, no peak thresholds	
Spectra to include	> 10% peak height < 20% saturation (in the *m/z* ranges used in the chromatogram Background subtraction of average spectra at peak start and end No peak thresholds	
Charge state	Limited to # 1	
Isotope model	Common organic molecules; except for fenbutatinoxide (separate evaluation with unbiased)	
PCDL	G3878CA Pesticide PCDL containing 741 compounds with exact mass spectra for both polarities and up to three different collision energies	
Positive ions	$[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$	
Negative ions	$[M-H]^-$, $[M+HCOO]^-$	
Formula matching	Match tolerance (spectra)	± 6 ppm
	Recognition window	± 0.35 minutes
	EIC extraction	± 10 ppm
	Extraction window	± 1.0 minute
	No peak thresholds	
	Extraction of MS/MS spectrum if available with ± 20 ppm precursor tolerance	
Matching criteria	Warning threshold	Score < 80
	Compound matching	Score > 50
Library search criteria	Precursor ion expansion	± 10 ppm
	Product ion expansion	± 20 ppm
	Collision energy spread	± 20 eV
	Minimum reverse score	> 50
	No peak thresholds	

Results and Discussion

Creation of an exact mass MS/MS library

The presence of pesticides in a sample detected by a qualitative screening method according to guideline SANCO/12495/2011 can be verified by the comparison of the accurate mass MS/MS spectrum with an exact mass reference library spectrum. To create a user defined accurate mass library, accurate mass spectra for more than 300 pesticides were acquired with collision energies of 10, 20, and 40 eV. In either positive or negative ionization mode, meaningful MS/MS spectra were acquired for most of the investigated pesticides. For several compounds, MS/MS library spectra were captured in both ionization modes. To eliminate mass assignment errors, fragment masses in the acquired spectra were compared to the theoretical fragment formulas and corrected to their true (empirical) masses. The corrected spectra were included in the Agilent Pesticide Personal Compound Database and Library (p/n G3878CA) which was used for the screening and verification of pesticide residues in fruit and vegetable samples. Retention time information was added to the library by analyzing comprehensive pesticide standards with the given UHPLC method. Figure 2 shows the Personal Compound Database and Library (PCDL) software along with the exact mass spectrum of Omethoate acquired with a collision energy of 10 eV.

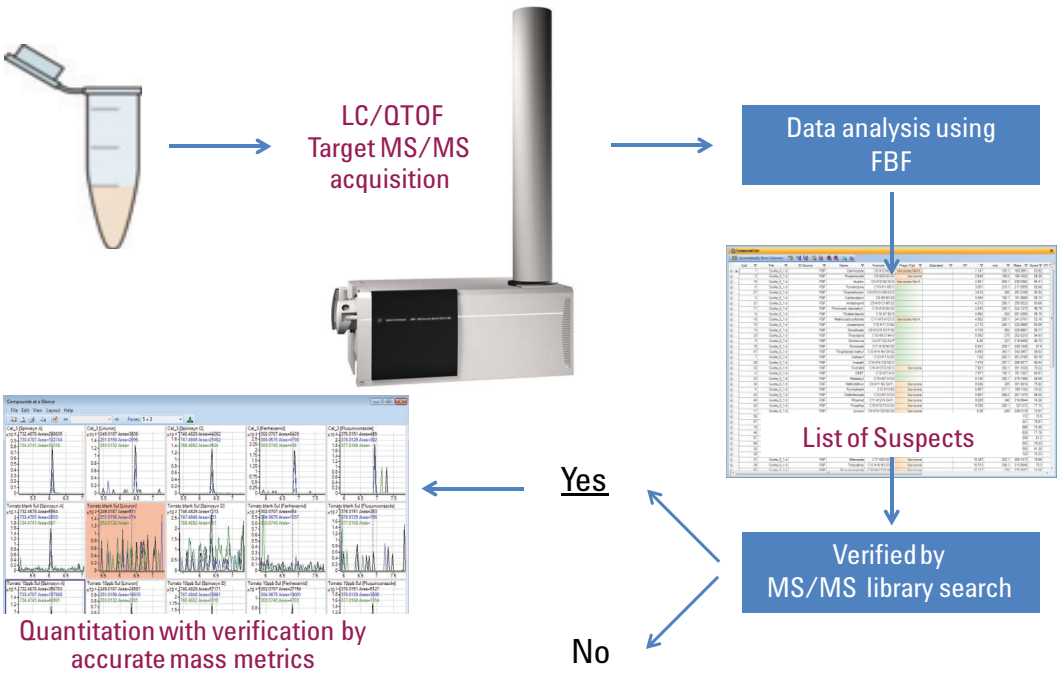

Figure 1. UHPLC-QTOF/MS/MS workflow for discovery, verification and quantitation of pesticides in food extracts.

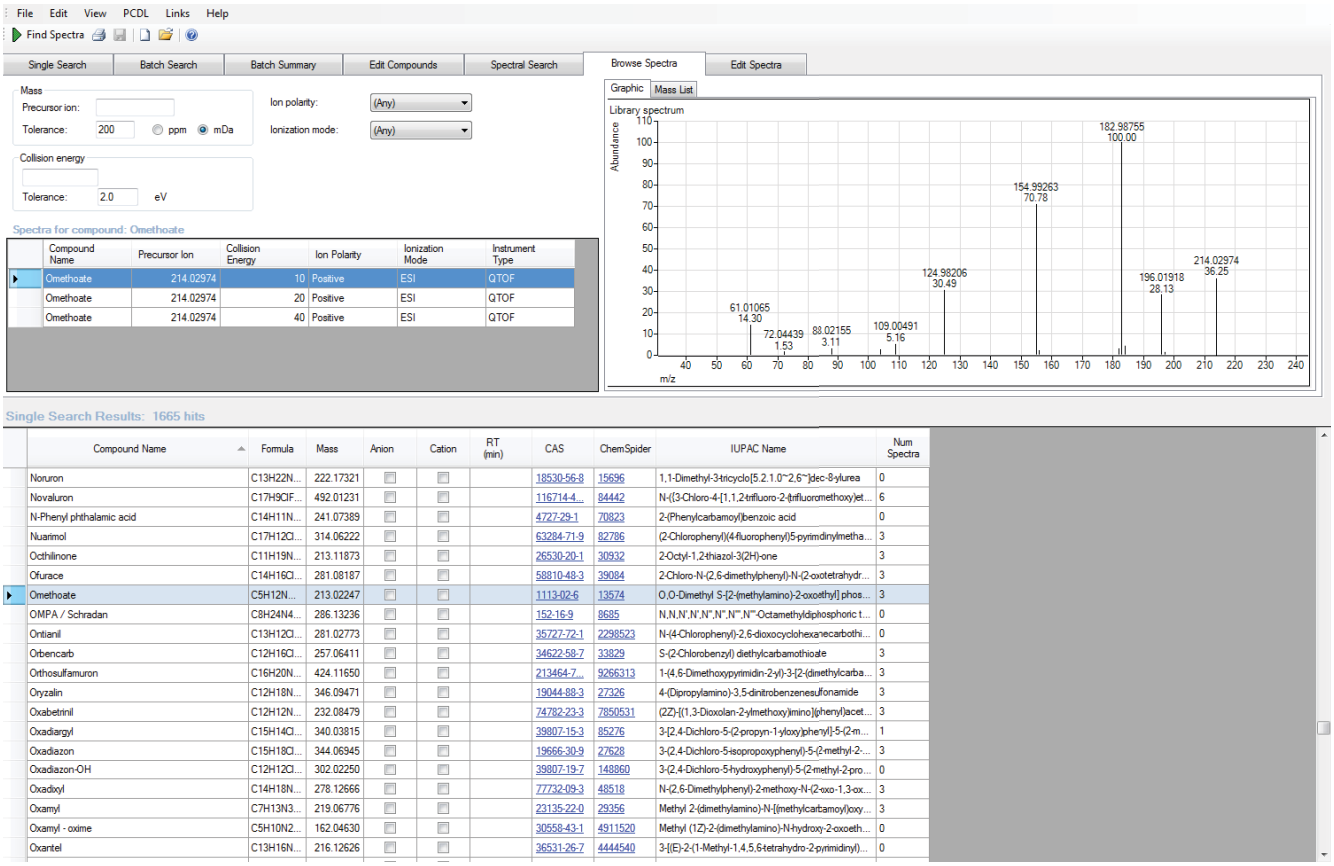

Figure 2. PCDL software showing the pesticide library and the exact mass spectrum of omethoate.

Validation of the screening and verification workflow for the identification of pesticides in fruit and vegetable matrices

Cucumber, lemon, and rucola extracts spiked with 55 relevant pesticides were analyzed using UHPLC separation and target MS/MS acquisition. The masses of the precursor ions ($[M+H]^+$) of the spiked pesticides were included in the target list along with the precursor masses of 150 other relevant pesticides. The FBF algorithm was used for compound searching. It automatically generates an extracted ion chromatogram for the expected ion species for all target compounds in the accurate mass database. Peak spectra are extracted and the experimentally measured results are compared against the calculated results for the database entries. The results are scored depending on the agreement of the accurate monoisotopic mass, the isotope ratio, the isotope spacing, and the retention time. Figure 3 shows, as an example, the compound chromatogram and peak spectrum for methidathion spiked into a QuEChERS extract of rucola and obtained by the FBF algorithm.

The automatically generated extracted ion chromatogram (EIC) summarizes the signals for all selected adduct species of methidathion (A). Even for a low spiking concentration corresponding to 10 µg/kg and the most complex matrix, a very good signal-to-noise (S/N) ratio of 238.2 (peak-to-peak noise algorithm) was observed. Figure 3B shows the compound spectrum of methidathion (green centroided signals) in comparison to the theoretical isotope pattern (red boxes) for all detected adducts. Figure 3C shows the same picture zoomed in for the major $[M+H]^+$ species. The software could allocate 11 ions to the different adducts of methidathion including their isotope signals. In addition, the signal intensities were in good agreement with the expected isotope ratios even with coeluting background signals from the matrix with intensities of up to 3 x 10^6 counts. Consequently, a high overall score of 95.7 (out of 100) was observed for methidathion which reflects a combination of the mass accuracy score (97.6 out of 100), the isotope abundance score (93.5 out of 100), and the isotope spacing score (97.8 out of 100).

Table 4 shows the compound table for all 53 pesticides measured in positive mode and spiked in cucumber extract.

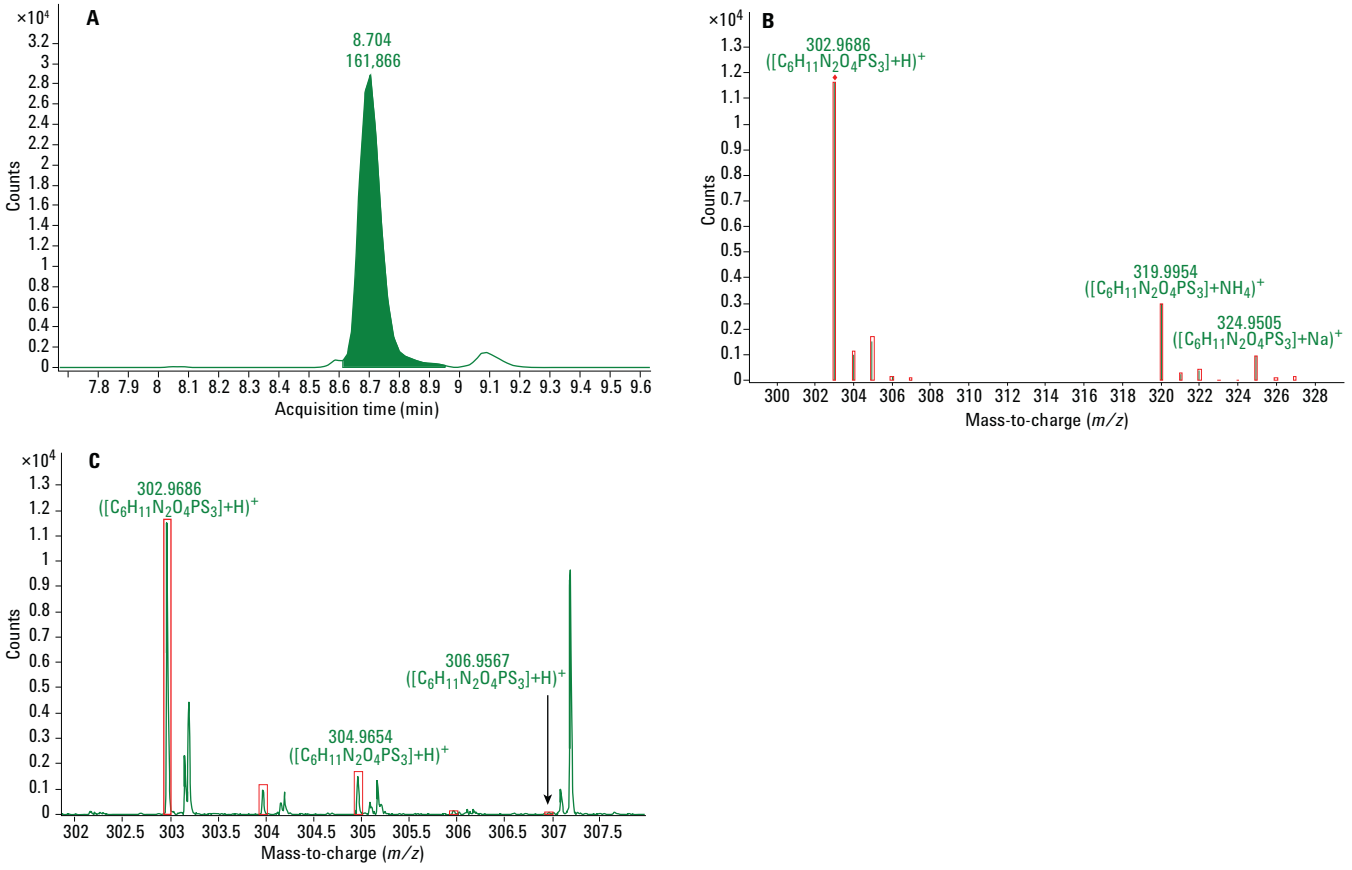

Figure 3. Compound chromatogram and peak spectrum obtained by the Find by Formula algorithm for methidathion spiked into a QuEChERS extract of rucola equivalent to a concentration of 10 µg/kg (50% of the MRL for rucola).

Table 4. Compound Table for 53 Pesticides Measured in Positive Mode and Spiked in QuEChERS Extract of Cucumber Equivalent to a Concentration of 10 µg/kg

Compound name	RT	Mass	Formula	Mass deviation (ppm)	Total score	Mass score	Isotope abundance score	Isotope spacing score
Acetamiprid	4.704	222.0672	$C_{10}H_{11}ClN_4$	–0.12	97.88	99.94	97.37	98.85
Azoxystrobin	9.384	403.1171	$C_{22}H_{17}N_3O_5$	0.77	97.48	99.38	99.33	99.92
Bifenazate	10.48	300.1474	$C_{17}H_{20}N_2O_3$	–2.89	81.58	94.96	76.84	48.77
Boscalid	9.85	342.0323	$C_{18}H_{12}C_{12}N_2O$	–1.15	95.49	98.18	82.18	97.04
Buprofezin	13.956	305.1563	$C_{16}H_{23}N_3OS$	0.37	98.44	99.92	92.61	99.37
Carbaryl	7.012	201.0785	$C_{12}H_{11}NO_2$	–1.58	98.63	98.92	97.08	99.72
Carbendazim	4.047	191.0694	$C_9H_9N_3O_2$	–0.4	93.35	99.94	88.37	73.67
Chlorpyrifos	14.529	348.9259	$C_9H_{11}Cl_3NO_3PS$	–0.97	95.03	99.27	76.61	98.7
Chlorpyrifos-methyl	12.905	320.895	$C_7H_7Cl_3NO_3PS$	–0.82	83.91	95.55	78.24	67.44
Cyhexatin	14.711	360.1564	$C_{18}H_{32}Sn$	–0.66	79.62	99.67	n.a.	94.31
Cyprodinil	11.385	225.1267	$C_{14}H_{15}N_3$	0.4	99.52	99.92	99.44	97.83
DEET (Diethyltoluamide)	7.989	191.1304	$C_{12}H_{17}NO$	–0.46	87.17	99.92	97.26	49.5
Dichlorvos	6.272	219.9448	$C_4H_7Cl_2O_4P$	–2.83	94.04	96.36	79.63	95.54
Difenconazole(I)	12.935	405.0648	$C_{19}H_{17}Cl_2N_3O_3$	0.35	94.31	99.73	88.64	92.25
Diflubenzuron	11.404	310.0321	$C_{14}H_9ClF_2N_2O_2$	0.17	95.61	99.03	87.03	99.0
Dimethoate	4.734	228.9993	$C_5H_{12}NO_3PS_2$	–1.49	98.18	99.01	99.56	91.78
Dimethomorph	10.122	387.1236	$C_{21}H_{22}ClNO_4$	–0.29	97.73	99.86	96.81	90.05
Famoxadon	12.282	374.1288	$C_{22}H_{18}N_2O_4$	–0.89	79.47	99.4	75.86	41.24
Fenhexamid	10.798	301.0641	$C_{14}H_{17}Cl_2NO_2$	–1.46	95.37	99	84.52	91.85
Fluazifop	9.353	327.071	$C_{15}H1_2F_3NO_4$	–2.56	86.13	95.34	87.34	52.34
Fludioxonil	9.771	248.0392	$C_{12}H_6F_2N_2O_2$	–2.01	84.76	97.95	58.57	48.92
Fluquinconazole	10.738	375.0095	$C_{16}H_8Cl_2FN_5O$	–1.36	93.7	98.5	84.06	96.44
Flutriafol	7.915	301.1025	$C_{16}H_{13}F_2N_3O$	0.64	93.15	99.71	76.03	96.21
Imazalil	7.5	296.0481	$C_{14}H_{14}Cl_2N_2O$	0.77	98.46	99.66	95.78	98.73
Imidacloprid	4.377	255.0522	$C_9H_{10}ClN_5O_2$	0.41	97.53	99.99	91.34	97.02
Iprodione	11.29	329.0335	$C_{13}H_{13}Cl2N_3O_3$	0.95	94.0	99.06	84.76	88.52
Kresoxim-methyl	11.721	313.1312	$C_{18}H_{19}NO_4$	0.64	99.13	99.94	98.53	96.47
Linuron	9.414	248.0117	$C_9H_{10}Cl_2N_2O_2$	1.02	94.33	99.41	75.86	95
Mandipropamid	9.881	411.1236	$C_{23}H_{22}ClNO_4$	0.26	98.21	100	91.13	99.52
Metalaxyl	8.162	279.1471	$C_{15}H_{21}NO_4$	–0.17	98.03	99.89	99.21	98.3
Methidathion	8.64	301.9614	$C_6H_{11}N_2O_4PS_3$	1.54	97.46	98.34	98.73	99.92
Myclobutanil	10.313	288.1147	$C_{15}H_{17}ClN_4$	–1.9	89.78	97.46	79.86	90.79
Penconazole	11.825	283.064	$C_{13}H_{15}Cl_2N_3$	1.15	97.95	99.22	91.67	98.87
Pendimethalin	14.675	281.1376	$C_{13}H_{19}N_3O_4$	–2.19	75.62	97.09	26.23	50.0
Phosmet	9.032	316.9943	$C_{11}H_{12}NO_4PS_2$	0.72	98.79	98.83	96.57	98.95
Phoxim	12.39	298.0544	$C_{12}H_{15}N_2O_3PS$	–0.86	99.23	99.95	98.95	96.6
Piperonyl butoxide	14.252	338.2097	$C_{19}H_{30}O_5$	–1.06	97.33	99.44	99.55	98.41
Pirimicarb	6.346	238.1429	$C_{11}H_{18}N_4O_2$	0.36	96.46	99.96	99.04	79.31
Pirimicarb, desmethyl-	4.529	224.128	$C_{10}H_{16}N_4O_2$	–2.13	81.3	96.34	89.45	13.11
Propamocarb	2.885	188.1521	$C_9H_{20}N_2O_2$	1.89	98.72	98.46	99.66	97.59
Propargite	14.94	350.1553	$C_{19}H_{26}O_4S$	–0.47	98.41	99.52	93.17	99.3
Propiconazole(I)	12.132	341.0688	$C_{15}H_{17}Cl_2N_3O_2$	3.02	94.19	99.2	95.45	95.38
Prosulfocarb	13.45	251.1343	$C_{14}H_{21}NOS$	0.43	97.6	99.99	95.94	92.63
Pyraclostrobin	12.451	387.0987	$C_{19}H_{18}ClN_3O_4$	–0.28	97.65	99.92	99.8	98.95
Pyridaben	15.757	364.1379	$C_{19}H_{25}ClN_2OS$	–0.74	96.98	99.46	88.62	98.56
Teflubenzuron	14.216	379.9735	$C_{14}H_6Cl_2F_4N_2O_2$	1.9	83.77	97.16	25.64	94.31
Thiabendazole	4.565	201.0359	$C_{10}H_7N_3S$	1.01	97.01	99.58	96.38	95.7
Thiacloprid	5.114	252.0235	$C_{10}H_9ClN_4S$	0.49	98.67	99.77	97.28	97.94
Thiamethoxam	3.818	291.0191	$C_8H_{10}ClN_5O_3S$	0.76	98.79	99.35	97.35	97.87
Thiophanate-methyl	6.519	342.0452	$C_{12}H_{14}N_4O_4S_2$	1.18	95.87	99.09	89.6	97.49
Triadimefon	10.199	293.093	$C_{14}H_{16}ClN_3O_2$	0.52	93.03	99.78	68.44	95.1
Triazophos	10.522	313.0649	$C_{12}H_{16}N_3O_3PS$	0.33	94.09	99.94	94.34	94.62
Trifloxystrobin	13.148	408.1298	$C_{20}H_{19}F_3N_2O_4$	–0.16	99.43	99.98	98.57	98.19

The mass error of the major ion species, and the resulting scores for all compounds are given. At a concentration corresponding to 10 µg/kg, all compounds were found by the FBF algorithm with the specified settings. The mass deviations of the measured masses compared to the theoretical masses generally were below 1 ppm, and for only seven compounds, which were detected with lower peak intensities, a mass deviation between 2 and 3 ppm, was observed. Most of the pesticides got a score of 90 (out of 100) or above. The quality filter used in this data processing method requires at least two or more individual ions for the compound. In combination with the retention time, this typically is sufficient for the identification. Three compounds had an overall score below 80 and were flagged for inspection. In all cases, one of the specified adducts showed up with low abundance and, thus, had either a bigger mass deviation or missing isotope signals.

MS/MS spectra were extracted automatically over the peak window and were matched against the library spectra contained in the Agilent Pesticide Personal Compound Database and Library. Since a precursor mass dependent collision energy ramping was used in the MSMS experiments, a search filter on collision energy of ± 20 eV was applied to focus comparisons of library spectra to those library entries of similar collision energy decent. Figure 4 shows the MS/MS spectrum for methidathion acquired in the rucola extract spiked at a concentration corresponding to 10 µg/kg in comparison to the library spectrum from the Agilent Pesticide PCDL. All major fragment ions listed in the library spectrum of methidathion were found in the measured spectrum within a narrow mass extraction window and in a similar ratio to the reference spectrum for a collision energy of 20 eV. The forward search against the exact mass pesticide library resulted in a score of 95.9 out of 100 and verified the presence of methidathion in the sample. Additional signals in the acquired mass spectrum belong to matrix components with similar precursor masses.

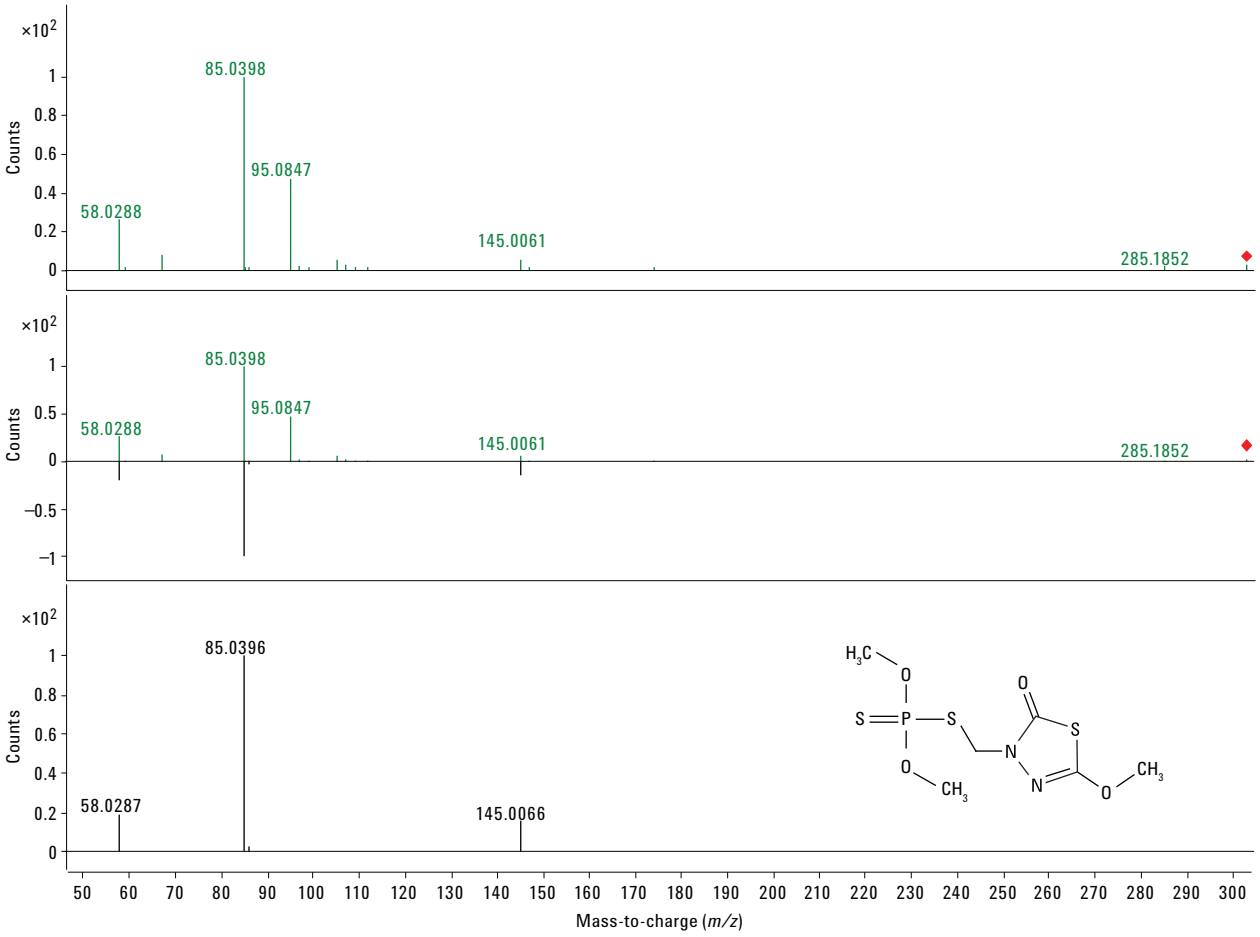

Figure 4. Comparison of the measured spectrum of methidathion in the spiked rucola extract (corresponding to a concentration of 10 µg/kg) with the reference spectra of methidathion from the Agilent Pesticide PCDL.

Accurate mass screening for pesticides, combined with confirmation of identified contaminants by MS/MS library searching, was validated for solvent standards as well as for spiked QuEChERS extracts of cucumber, lemon, and rucola. Pesticide concentrations in solvent and in the spiked QuEChERS extracts were 5, 10, 50, and 100 ng/mL. Table 5 summarizes the results which were obtained by the automatic data analysis using the Find by Formula algorithm and the parameters described in Table 3. Most of the compounds were detected in positive mode, 2,4-D and MCPA were only detected in negative mode, and several compounds were detected in both modes.

The majority of the spiked pesticides was successfully detected by the FBF algorithm even at a concentration equivalent to 5 µg/kg and in complex matrices. Moreover, for most of the pesticides meaningful MS/MS spectra were generated at this concentration which allowed the verification of the compound by library searching against the MSMS spectra in the PCDL.

Under the conditions used, the predominant adduct species for some of the compounds were not the [M+H] ion and no library spectra were available for the corresponding sodium or ammonium adduct. Those compounds are marked with an asterisk. The cucumber sample used for the spiking experiment was most probably contaminated with low levels of iprodione which is used as fungicide for the cultivation of cucumbers. All other positive findings in the blank samples represent very low concentrations and are most probably caused by a carry-over effect.

By adding more compounds to the database and library, the scope of the data analysis was extended to more than 570 of the most important pesticides. For approximately 300 of these pesticides, retention time information was available. Applying this database as formula source to the FBF algorithm, the screening of pesticides in matrix samples yielded several additional contaminant suspects which are summarized in Table 6. For the rucola extract, applying a 300 compound database with retention time information, resulted in the detection of 55 additional suspect pesticides. When applying a 570 compound database without retention time information, 166 additional suspect pesticides were detected. The precursor masses for all suspect pesticides were included as precursor masses in the target MS/MS method. None of the suspect pesticides present could be verified by comparing the target MS/MS spectra against the pesticide reference library. Similar results were obtained for the less complex matrices cucumber and lemon. In the lemon extract, 13 additional suspect pesticides were observed when using the 300 compound database, and 119 additional suspect pesticides were observed when using the 570 compound database without retention time information. In the cucumber extract, 6 and 79 suspects were detected when using the 300 compound database with, and the 570 compound database (without retention time information), respectively. For both matrices, library searching against the Agilent Pesticide PCDL successfully helped to eliminate potential false positives.

Quantitative review

The MS domain data from this method was also used to obtain (semi-)quantitative information for the spiked extracts as well as for several official control samples. The best ions to select for the quantitative method were derived from the compound results extracted from the qualitative software using the 50 ng/mL solvent standard data file. These were exported to a compound exchange file (.cef) which was used in the MassHunter Quantitative software for the automatic creation of a quantitation method. In this way, quantifier and qualifier ions were automatically selected from the observed adduct species and isotope signals, based on their relative abundance.

The limit of quantification for most of the 55 targeted pesticides in the TOF mode was below 5 ng/g in all tested matrices with a linear range of up to four orders of magnitude. Figures 6E and 6F show the linear calibration curves for pirimicarb and boscalid obtained from the MS domain data.

*Table 5. Results Summary for the Screening and Verification of Pesticides in Fruits and Vegetables by Target MS/MS Acquisition and Library Searching. (Green: Compound Automatically Found and Presence Verified by MS/MS Library Confirmation; Yellow: Compound Automatically Found but no Qualified MS/MS Spectrum Available; *No Spectrum Available for Predominant Adduct of Compound; ***Compound Results Acquired in Negative Ion Mode*

	Cucumber					Lemon					Rucola				
	Blank	5 ppb	10 ppb	50 ppb	100 ppb	Blank	5 ppb	10 ppb	50 ppb	100 ppb	Blank	5 ppb	10 ppb	50 ppb	100 ppb
2,4-D***															
Acetamiprid															
Azoxystrobin															
Bifenazate															
Boscalid															
Buprofezin															
Carbaryl															
Carbendazim															
Chlorpyrifos															
Chlorpyrifos-methyl															
Cyhexatin dehydrate*															
Cyprodinil															
DEET															
Dichlorvos*															
Difenconazole															
Diflubenzuron															
Dimethoate															
Dimethomorph															
Famoxadon*															
Fenhexamid															
Fluazifop															
Fludioxonil***															
Fluquinconazole															
Flutriafol															
Imazalil															
Imidacloprid															
Iprodione															
Kresoxim-methyl															
Linuron															
Mandipropamid															
MCPA***															
Metalaxyl															
Methidathion															
Myclobutanil															
Penconazole															
Pendimethalin															
Phosmet															
Phoxim															
Piperonyl butoxide*															
Pirimicarb															
Pirimicarb, desmethyl-															
Propamocarb															
Propargite*															
Propiconazole															
Prosulfocarb															
Pyraclostrobin															
Pyridaben															
Teflubenzuron															
Thiabendazole															
Thiacloprid															
Thiamethoxam															
Thiophanate-methyl															
Triadimefon															
Triazophos															
Trifloxystrobin															

Table 6. Number of Suspects Detected and Verified by Accurate Mass Library Searching in the Blank Quechers Matrices (N = 5) When Applying Large Compound Databases With and Without Retention Time Information as Formula Source for the Find-By-Formula Data Mining Algorithm

	Cucumber	Lemon	Rucola
Pesticide suspects identified with find by formula			
RT required (300 target compounds)	6 ± 2	13 ± 6	55 ± 20
Verified by MS/MS library searching	0	0	0
RT optional (570 target compounds)	73 ± 2	119 ± 6	166 ± 24
Verified by MS/MS library searching	0	0	0

Figure 5 shows a screenshot of the compounds at a glance view for the review of multiple samples (organized in rows) and multiple pesticides (organized in columns) in MassHunter Quantitative software.

As illustrated in Table 4, it is possible to compare the isotope pattern of a peak's spectrum with that which is predicted by theory, and then to assign score based on equivalency. This approach can also be implemented in MassHunter Quantitative software (pattern matching). The quantifier-qualifier concept and the accurate mass metrics, increases the confidence in the results and allows an efficient data review in batch processing. Figure 5 shows that a minimum isotope match score has been specified as outlier and has been used to flag samples (in red) for which the mass accuracy and the isotope pattern match was not sufficient for a positive identification of the compound.

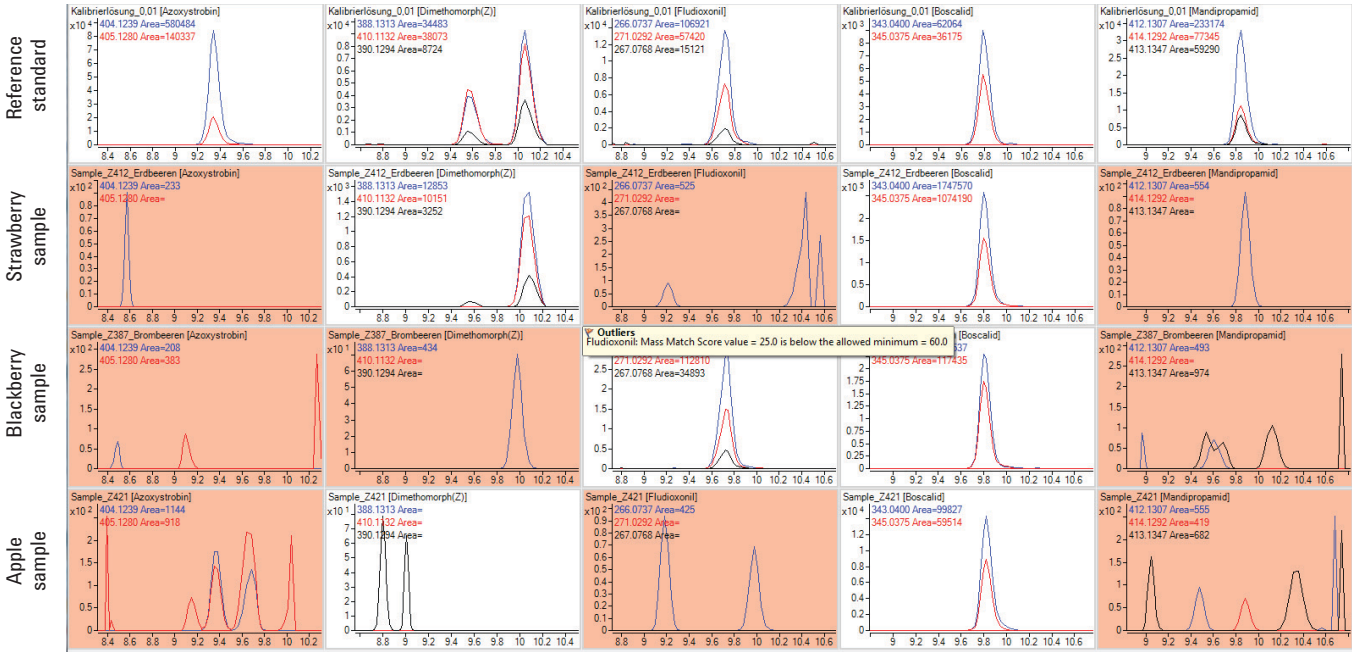

Figure 5. Screenshot of the compounds at a glance view of the MassHunter quantitative software showing multiple compounds for multiple samples. The accurate mass metrics has been used for the flagging of outliers.

Analysis of real samples

In addition to the spiked matrices used to validate the workflow, samples obtained from a routine monitoring laboratory were analyzed using the UHPLC-QTOF method. These samples were also analyzed by LC/MS/MS or GC/MS and several pesticide residues were detected.

Figures 6A and 6B show the chromatograms and spectra for pirimicarb and boscalid, which were found as residues in an apple sample. Figures 6C and 6D show the comparison of the acquired spectra with the associated library spectra (pattern matching). For pirimicarb, the mass deviation of the molecular ion was –0.54 ppm which resulted in a mass score of 99.2. The isotope pattern match score was 97.0. For boscalid, a mass deviation of –0.15 ppm was observed which led to a mass score of 97.5. The isotope pattern match score was 93.0.

Ten pesticides were found in this apple sample which gave good mass match scores and pattern match scores. These scores were consolidated with their associated retention time score which gave values of over 85 for all 10. Seven of these were further verified by target MS/MS acquisition and accurate mass library searching. The other three compounds had no MS/MS spectra in the library for comparison.

Five additional pesticides were detected in the target MS/MS data and identified by library searching with high library match scores. Concentrations of these pesticides were all below 5 µg/kg and, therefore, below the reporting limit.

Table 7 summarizes the comparison of the results of the official control measurements with the results obtained for the UHPLC-QTOF/MS/MS method for four different samples. All of the pesticides found previously with triple quadrupole LC/MS/MS or GC/MS were found by the accurate mass screening method and concentrations of the identified compounds were in good agreement with the concentrations previously determined. Only the parsley sample had concentrations of some compounds significantly lower than the results obtained during the control analysis. This is due to the fact that calibration was based on a solvent calibration, and matrix suppression in the real samples would reduce the apparent recovery of the pesticide compound. By using matrix matched calibrations or other methods to compensate for matrix effects, it can be expected that concentrations would be in better agreement with the results from the official control.

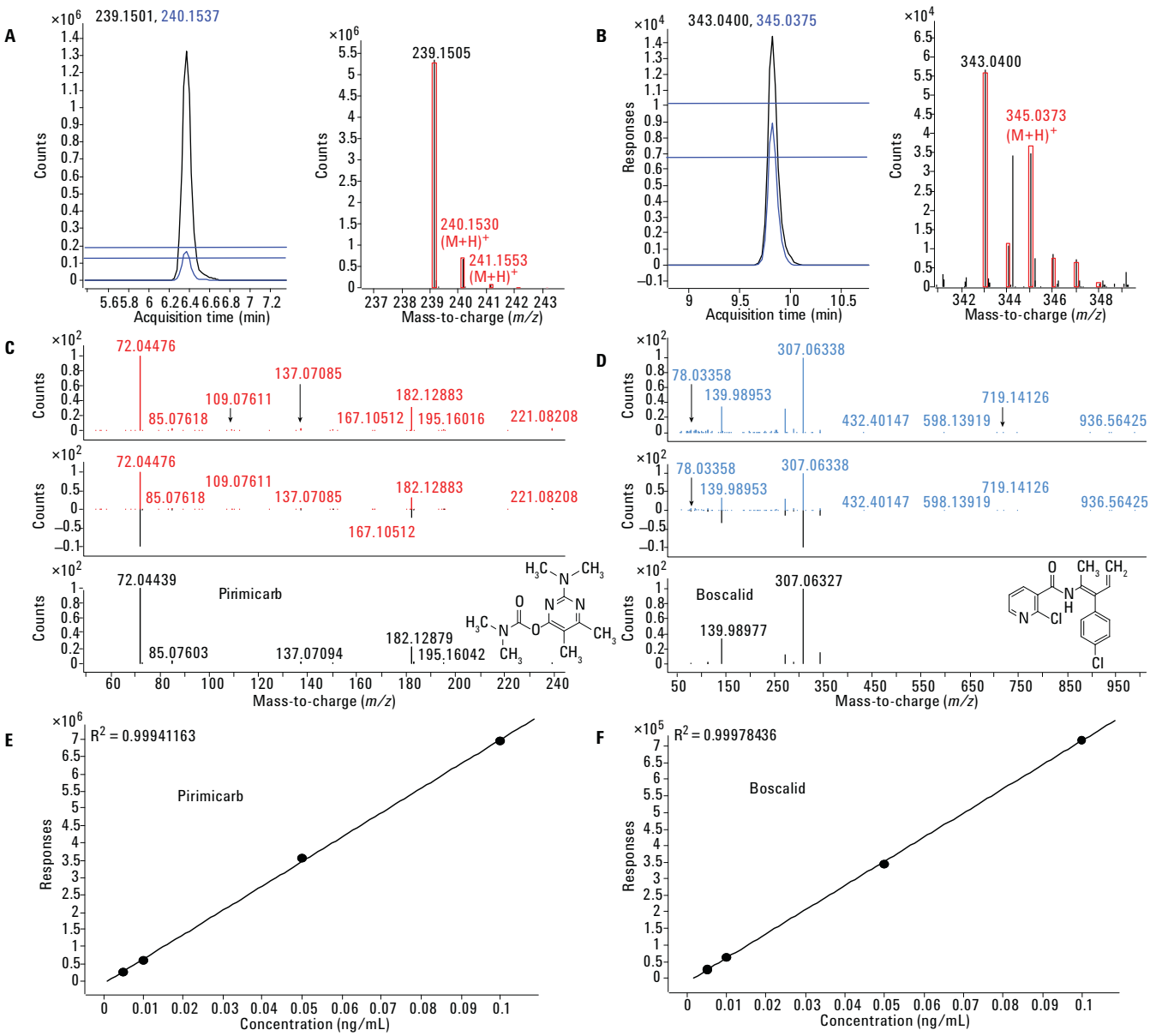

Figure 6. Results of the LC/QTOF/MS screening, confirmation and quantitation of pesticides in an apple sample obtained from a pesticide monitoring laboratory. Pirimicarb and Boscalid are shown with their associated compound chromatogram, TOF and MS/MS spectrum in comparison to the library spectrum, and their calibration curves.

Table 7. Comparison of the Results of the Official Control Measurements (Triple Quadrupole LCMS and GCMS) With the Results Obtained for the UHPLC-QTOF/MS/MS Method for Four Different Samples

	Apple Germany			Strawberry Netherlands			Grapes Brazil			Parsley Germany		
Compounds	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**	**QTOF**		**LC-QQQ and GCMS**
Acetamiprid	YES	0.01	0.01									
Azoxystrobin							YES	0.06	0.09	YES	0.15	0.34
Boscalid	YES	0.02	0.02	YES	0.24	0.02				YES	0.03	0.14
Carbendazim	YES	0.02	0.02				YES	<0.01				
Difenoconazole	YES	<0.01					YES	<0.01	0.02	YES	0.03	0.04
Dimethomorph				YES	<0.01					YES	0.38	0.74
Imidacloprid	YES	<0.01					YES	0.01	0.01			
Linuron										YES	0.01	0.01
Mandipropamid							YES	<0.01	0.01			
Penconazole	YES	<0.01		YES	0.05	0.1						
Pirimicarb	YES	0.01	0.18									
Pirimicarb-desmethyl	YES	0.01	0.01									
Prosulfocarb										YES	0.01	0.01
Pyraclostrobin	YES	<0.01	0.01	YES	0.05	0.09	YES	<0.01		YES	0.02	0.03
Thiacloprid				YES	0.03							
Thiophanate methyl	YES	<0.01					YES	<0.01	0.01			
Trifloxystrobin	YES	0.01	0.01							YES	<0.01	

Conclusions

An exact mass MS/MS library for pesticides was created and applied to the LC/QTOF/MS/MS screening of pesticides in fruit and vegetable extracts. The method was successfully validated for the screening and identification of more than 50 pesticides in three different commodity groups. When applied to real world samples obtained from a routine monitoring program, all pesticides detected previously by triple quadrupole LC/MS and GC/MS were identified. The quantitation results were in good agreement with quantitative results obtained previously.

The Agilent Pesticide PCDL mentioned in this application note is available as (p/n G3878CA) or p/n (G3878AA) which also contains a chromatography column, a comprehensive pesticide standard and application support.

References

1. Method validation and quality control procedures for pesticide residue analysis in food and feed, Document N° SANCO/12495/2011. Implemented by 01/01/2012. http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdf
2. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (including amendments as of 18 March 2008) and complying with regulation (EC) 1107/2009.
3. Agilent TOF LC/MS and Q-TOF LC/MS Screening of Pesticides - Workflow Guide, Agilent publication 5990-7072EN, June 2012
4. Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection, Agilent application note 5990-4248EN, January 2012
5. Codex Classification of Foods and Animal Feeds (Section 2 of Codex Alimentarius, Second Edition, Volume 2 (1993)).
6. Glauner, T. and Zavitsanos, P.: Electrospray Operational Parameters in TOF-MS, in Amadeo R. Fernandez-Alba, editor: Comprehensive Analytical Chemistry, Vol. 58, Elsevier, 2012, pp. 273-305. ISBN: 978-0-444-53810-9

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