Development of a Method for Multipesticide Analysis Using the Agilent 1260 Infinity Analytical SFC System with Triple Quadrupole MS Detection

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Application Note

Food Testing & Agriculture

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Abstract

This Application Note describes the development of a method for multipesticide analysis by supercritical fluid chromatography (SFC) using the Agilent 1260 Infinity Analytical SFC System in combination with an Agilent 6460 Triple Quadrupole Mass Spectrometer. The final multipesticide method was used for the determination of more than 200 pesticides in a single analysis. Different matrixes from fruits and vegetables were spiked with pesticides at several levels in a relevant concentration range and quantified. Individual calibration and performance data are presented and discussed.

Agilent Technologies

Introduction

Today, several hundreds of pesticide compounds are available on the market, and are in use on a worldwide basis for protection against various pests of plant food products such as vegetables, fruits, corn, and grain. Before plant-based food products enter the market, they have to be tested for possible pesticide residues, and they have to meet the legal limits[1]. The sheer number of possible pesticide-matrix combinations makes it necessary that methods used for the quantitative determination of pesticides in food products cover the widest possible range of compounds. This is typically done by HPLC methods in combination with mass spectrometry, where the compounds are separated by LC, and the selective detection is performed by triple quadrupole mass spectrometry in multiple reaction monitoring (MRM) mode. The optimization of supercritical fluid chromatography (SFC) separations for pesticides, the optimization of their mass spectrometric detection, and the influence of matrix compounds was shown previously[2,3].

Compared to HPLC, SFC offers the ability to use cheaper solvents such as carbon dioxide, less harmful solvents such as methanol or ethanol, lower costs for solvent waste disposal, and shorter run times. Samples of the complete plant food product have to be extracted and transferred into an analyzable form, typically a solution in organic solvent. This extraction is primarily done by the QuEChERS procedure[4], and the final extracts are analyzed by HPLC/triple quadrupole MS. While the extraction of samples in pure solvents such as acetonitrile in HPLC often compromises the peak shapes of the early eluting compounds, they are directly usable for injection in SFC.

This Application Note demonstrates the detection of more than 200 pesticide residues by SFC with triple quadrupole mass spectrometry in complex food matrixes after optimization of the SFC separation of a multiple-pesticide standard. The advantages of using an SFC as a front end for mass spectrometry for the analysis of pesticides in plant food samples are the separation speed, the orthogonal selectivity to LC, and the tolerance to injections with organic solvents as they are obtained from sample preparation. Data about the limits of detection (LODs), limits of quantitation (LOQs), linearity, retention time, and area RSDs of selected individual compounds are presented.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1260 Infinity Analytical SFC System (G4309A) comprising:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1260 Infinity Diode Array Detector with a high pressure SFC flow cell
- Agilent 6460 Triple Quadrupole LC/MS system (G6460C)
- Agilent 1260 Infinity Isocratic Pump (G1310B)
- Splitter Kit (G4309-68715)

Instrument setup

Figure 1 shows the recommended configuration of the Agilent 1260 Infinity Analytical SFC System with the Agilent 6460 Triple Quadrupole LC/MS System. The column is directly connected to a splitter assembly, which contains two combined splitters, an additional check valve to prevent CO_2 flowing back into the make-up pump, and a solvent filter. At the first splitter, the make-up flow coming from the isocratic pump is introduced into the flow path. This splitter is connected to the second splitter by a short 0.12-mm id capillary. Here, the flow is split with one part going to the MS and the other part going to the backpressure regulator (BPR) of the SFC module.
The connection to the MS is made by a special 50-µm id stainless steel capillary of 1-m length, which is included in the splitter kit. The split ratio depends on the backpressure generated by this restriction capillary and the pressure set by the BPR. Generally, an SFC backpressure of 120 bar diverts about 0.45 mL/min of the SFC flow to the ion source, and a 200-bar backpressure diverts about 0.6 mL/min to the ion source. Since electrospray MS is concentration-dependent, this has no influence on signal intensity.

Column

Agilent ZORBAX NH_2, 4.6 × 150 mm, 5 µm (p/n 883952-708)

Software

- Agilent MassHunter Data Acquisition Software for triple quadruple mass spectrometer, version 06.00. including SFC software add-on
- Agilent MassHunter Qualitative Software, version 07.00
- Agilent MassHunter Quantitative Software, version 07.00

Standards

The Agilent LC/MS Pesticides Comprehensive Test Mix (p/n 5190-0551) was used as standard mixture. This mix comprises eight submixtures, with a total of 254 pesticide compounds. The stock solutions contain the pesticides at a concentration of 100 ppm each. This stock solution was diluted to a working stock solution of 1 ppm in acetonitrile.

Chemicals

All solvents were LC/MS grade. Ethanol was purchased from J.T. Baker, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

Sample preparation

Fruits and vegetables were obtained from a local greengrocer. Samples were extracted according to the official citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). A 10-g amount of homogenized sample was weighed in a 50-mL polypropylene tube, and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. After the addition of an extraction salt packet containing 4 g anhydrous $MgSO_4$, 1 g of NaCl, and 1.5 g buffering citrate salts, the mixture was again shaken for 1 minute, then centrifuged at 4,000 rpm for 5 minutes.

After phase separation, a 6-mL aliquot of the upper acetonitrile phase was transferred to an Agilent BondElut QuEChERS EN Dispersive SPE Tube (p/n 5982-5056) containing 150 mg of primary secondary amine (PSA) for sample cleanup, and 900 mg of anhydrous $MgSO_4$ to remove water. The tubes were closed and shaken for another minute. Afterwards, the tubes were centrifuged at 4,000 rpm for 5 minutes. A 4-mL aliquot of the final extract was transferred to a clean polypropylene vial. To improve the stability of the target pesticides, 40 µL of formic acid was added to the final extract.

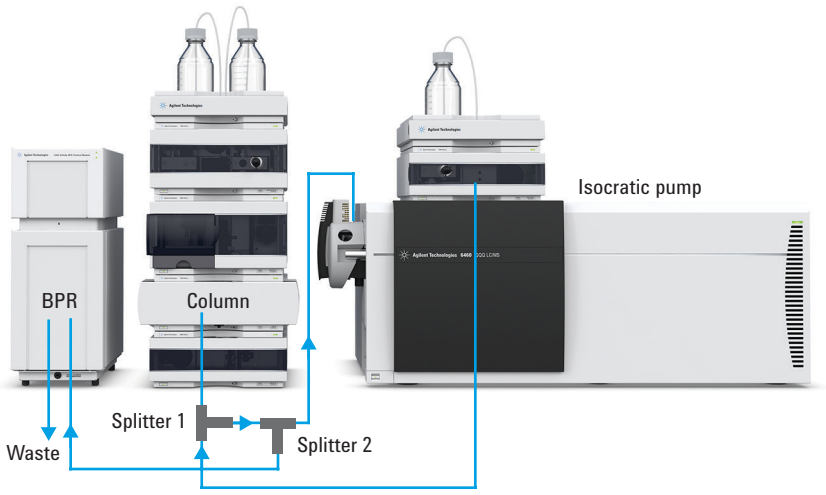

Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC System with the Agilent 6460 Triple Quadrupole LC/MS System. The column is directly connected to splitter 1 in the splitter assembly (BPR = backpressure regulator, UV detector not used, splitter Kit p/n G4309-68715).

SFC method

Parameter	Value
SFC flow	3 mL/min
SFC gradient	0 minutes, 2 %B 10 minutes, 10 %B 14 minutes, 26 %B 14.1 minutes, 50 %B Stop time 20 minutes Post time 2 minutes
Modifier	Methanol
BPR temperature	60 °C
BPR pressure	120 bar
Column temperature	40 °C
Injection volume	5 µL, 3-times loop overfill

Connection of SFC to MS by splitting and make-up flow

Parameter	Value
Make up composition	Methanol/water (95/5), 0.5 mM ammonium formate, + 0.2 % formic acid
Make-up flow	0.5 mL/min

MS method

Parameter	Value
Ionization mode	Positive
Capillary voltage	2,500 V
Nozzle voltage	2,000 V
Gas flow	8 L/min
Gas temperature	220 °C
Sheath gas flow	12 L/min
Sheath gas temperature	380 °C
Nebulizer pressure	25 psi
DMRM conditions	See Appendix Table 1, showing detailed retention time, retention time window, fragmentor, and collision energy details.

Results and Discussion

The Agilent LC/MS Pesticides Comprehensive Test Mix contains eight submixtures, each with approximately 33 compounds. These mixtures were used to develop and optimize the SFC separation method. The amino phase column was chosen due to experience based on an earlier method development work for a multipesticide sample. Ethanol was chosen as a modifier due to its lower elution strength compared to methanol, to enable a broader elution range[2].

In the first experiment, the pesticides from the different submixtures were eluted in a steep gradient, to 50 % modifier in 10 minutes, to see which pesticides could be eluted from the chosen combination of column phase and modifier. Because the elution behavior of most of the compounds under SFC conditions is susceptible to minor changes in the organic modifier even at low values, the submixtures were also tested in a gradient from 2 to 10 % in 10 minutes. Under these conditions, 195 compounds were eluted. An additional 28 compounds were eluted when the modifier was increased to 26 % in 14 minutes, then to 50 % at 14.01 minutes, then held there to 20 minutes. Overall, 223 compounds of the 254 compounds inherent in the mixtures were eluted and detected by MRM. In the remaining group of 31 compounds, some ionized only under negative ionization mode conditions, and others were not eluted with good peak shapes because they did not seem to fit well with the chosen combination of column phase and modifier. Several compounds of the group of sulfunyolurea herbicides were present in this group. To improve the sensitivity of the final method, the MRM method was transferred to a dynamic MRM (DMRM) mode method where each compound was measured at its retention time with a window of twice the peak width. Figure 2 shows the DMRM chromatogram of the separation of 223 compounds within 20 minutes. Figure 3 explains the distribution of the compounds over the complete runtime.

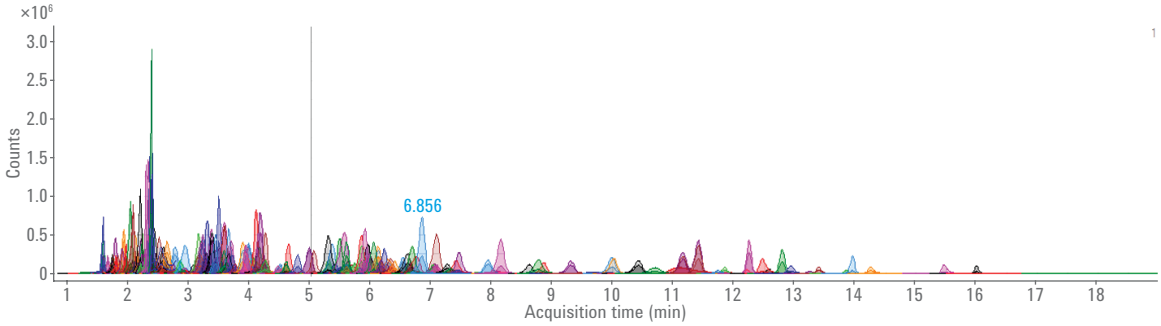

Figure 2. Measurement of 223 pesticides in the Agilent LC/MS Pesticides Comprehensive Test Mix by DMRM. There were 195 compounds eluted within 10 minutes from an amino phase column with 2 to 10 % ethanol as organic modifier, and 28 additional compounds eluted with up to 50 % organic modifier in 20 minutes.

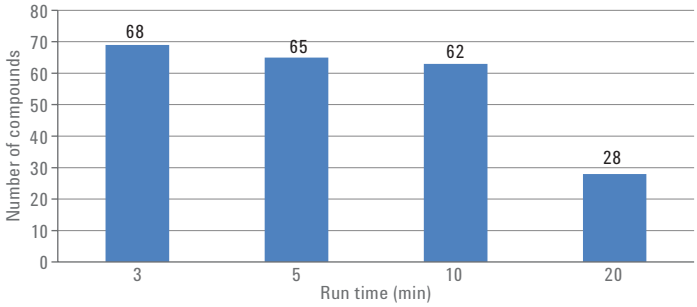

Figure 3. Distribution of pesticide elution over total runtime. The first compounds eluted at 1.5 minutes. There were 68 compounds eluted within the first 3 minutes, another 65 compounds between 3 and 5 minutes, and a further 62 compounds between 5 and 10 minutes. In total, 195 compounds eluted within 10 minutes with a gradient from 2 to 10 % ethanol. The elution was broadly distributed in the first 10 minutes.

For the complete set of 223 pesticides measured, a distribution of their LOQs is shown in Figure 4. A total of 102 pesticides had an LOQ of 0.5 ppb with a signal-to-noise (S/N) ratio greater than 10, and 167 had an LOQ of 1 ppb or lower. Only seven pesticides out of the 223 compounds had an LOQ below 10 ppb. Nevertheless, all had LODs below 10 ppb, and thereby met the requirement of the regulations[1]. The calibration curves for all compounds were created from their LOQ up to 100 ppb. All compounds showed a linearity of $R^2 = 0.999$ or better. Figure 5 shows the distribution of retention time precision. The majority of the 165 compounds had a retention time precision better than 1 % RSD. Figure 6 shows the distribution of the area precision. In total, 162 compounds had area RSDs below 5 %, and the majority of the compounds had RSDs between 2 and 5 %.

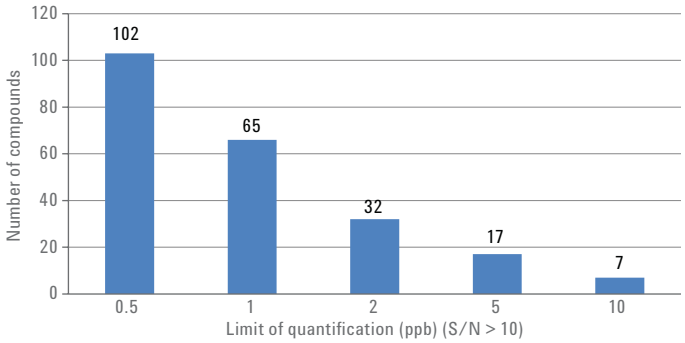

Figure 4. Distribution of LOQ for tested pesticides. There were 102 pesticides with a LOQ of 0.5 ppb, with an S/N > 10 and 167 had an LOQ of 1 ppb or lower. Only seven pesticides out of the 223 compounds had an LOQ of 10 ppb.

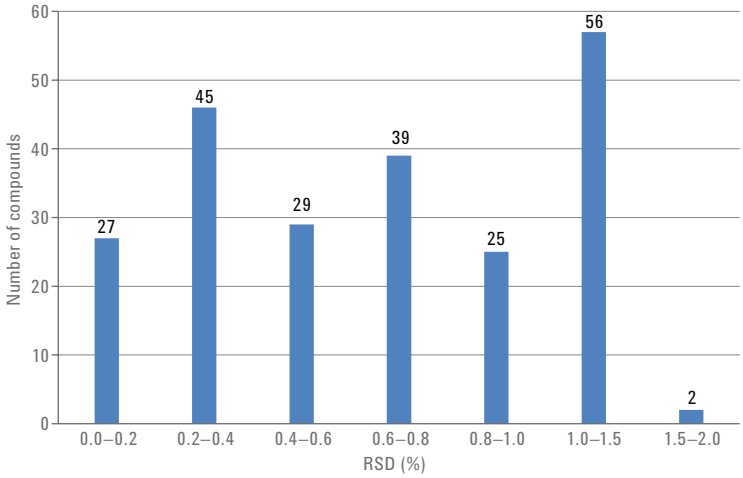

Figure 5. Distribution of retention time precision. There were 165 compounds with a retention time precision below 1 % RSD.

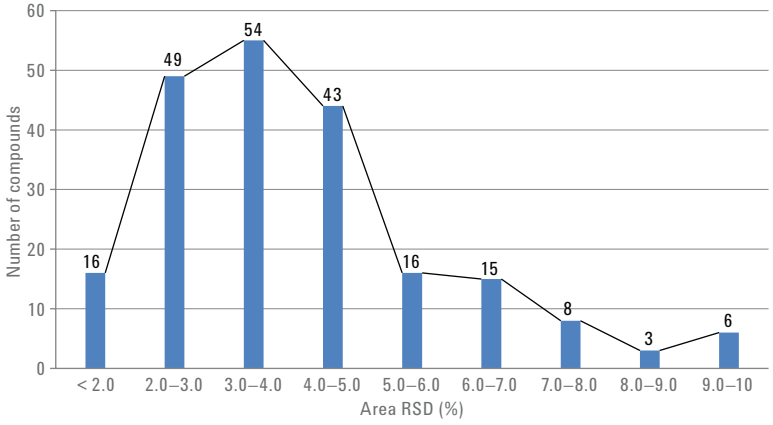

Figure 6. Distribution of area precision. There were 162 compounds with RSDs below 5 %, and the majority of the compounds had RSDs between 2 and 5 %.

As examples, the compounds displayed in Figure 7 are discussed in more detail. The first example is oxasulfuron, which belongs to the group of sulfonylurea herbicides, and displays good chromatographic behavior when using SFC. The lowest level of the calibration was 10 ppb, the calculated LOQ was 0.14 ppb, and the LOD was 0.04 ppb with a linearity of R^2 = 0.99993 (Figure 7A). The second example is methamidosphos, which is widely used for the protection of rice plants. It is a highly polar compound, and often peak broadening is observed when injecting pure QuEChERS extracts in reversed phase HPLC separations due to early elution. QuEChERS sample preparation results in a final extract of pure acetonitrile. In contrast to HPLC, this solution can be used in SFC directly, without compromising peak shape. Under the SFC conditions, it eluted at 7.055 minutes. The 10 ppb calibration level and the calibration curve are shown in Figure 7B. The calculated LOQ was 0.38 ppb, and the LOD was 0.13 ppb, with a linearity of R^2 = 0.99991.

As an example of real-life samples, strawberries, apples, and tomatoes were extracted according to the described QuEChERS procedure[4], and the obtained acetonitrile extract was injected directly. In this part of the experiment, all 223 pesticides were calibrated from 10 to 100 ppb, whereby the 10-ppb value is the highest legally accepted pesticide residue. From the measured 223 pesticides, only five were detected in minor amounts near the LOD: tebuconazole, triadimenol, chlorantraniliprol, trifloxystrobin, and boscalid.

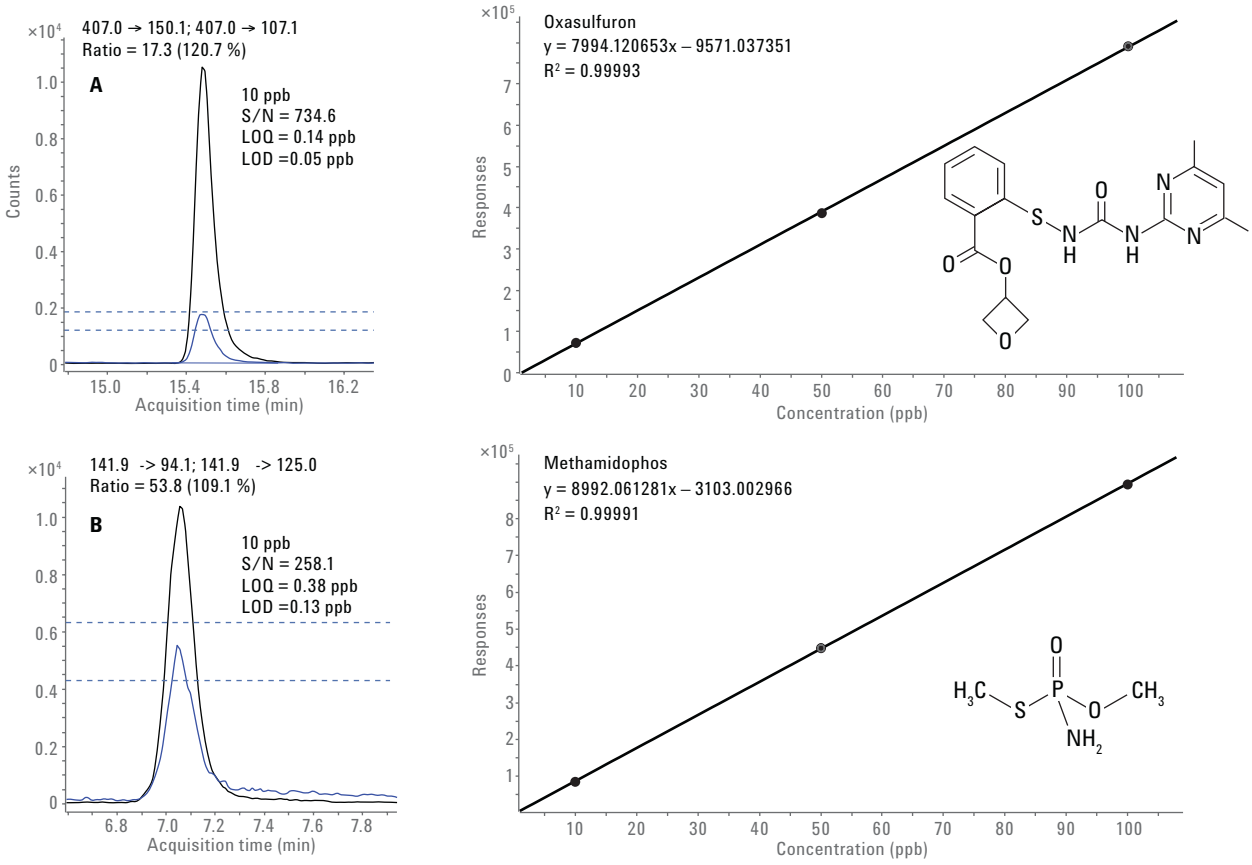

Figure 7. (A) Oxasulfuron, lowest level of the calibration at 10 ppb with an S/N = 734.6, LOQ = 0.14 ppb, LOD = 0.05 ppb, and linearity 0.99993. (B) Methamidophos, lowest level of the calibration at 10 ppb with an S/N = 258.1, LOQ = 0.38 ppb, LOD = 0.13 ppb, and linearity 0.99991.

Triadimenol is a systemic fungicide used predominantly against rust and powdery mildew, for example, on fruits, grapes, and tomatoes. Triadimenol is a metabolite of triadimefon, but is also used as an active ingredient itself. Often, it is used in combination with other fungicides such as tebuconazole. In the tomato sample, triademenol was detected at a low level (Figure 8). The lowest level of the calibration was 10 ppb with S/N = 971.2, LOQ = 0.1 ppb, and LOD = 0.03 ppb (Figure 8A). The triadimenol residue detected in tomatoes corresponded to a level of 1.36 ppb (Figure 8B). The calibration curve for triadimenol at levels of 10, 50, and 100 ppb showed a linearity of R^2 = 0.99929. Another example of a low level residue found in the strawberry sample is boscalid. It was detected at a concentration of 0.75 ppb and, thus, very close to the estimated LOD. Boscalid is widely used as a fungicide for the protection of fruits, vegetables, and wine grapes. According to the United States Environmental Protection Agency (EPA), boscalid has some carcinogenicity, but with minor potential on humans[5]. The maximum accepted daily dose is 0.04 mg/kg. However, the minimum reporting level (MRL) for triadimenol in tomatoes and boscalid in strawberries is significantly higher (1,000 and 500 ppb, respectively). These examples show the performance of the presented method for the analysis of trace level residues in complex food matrixes.

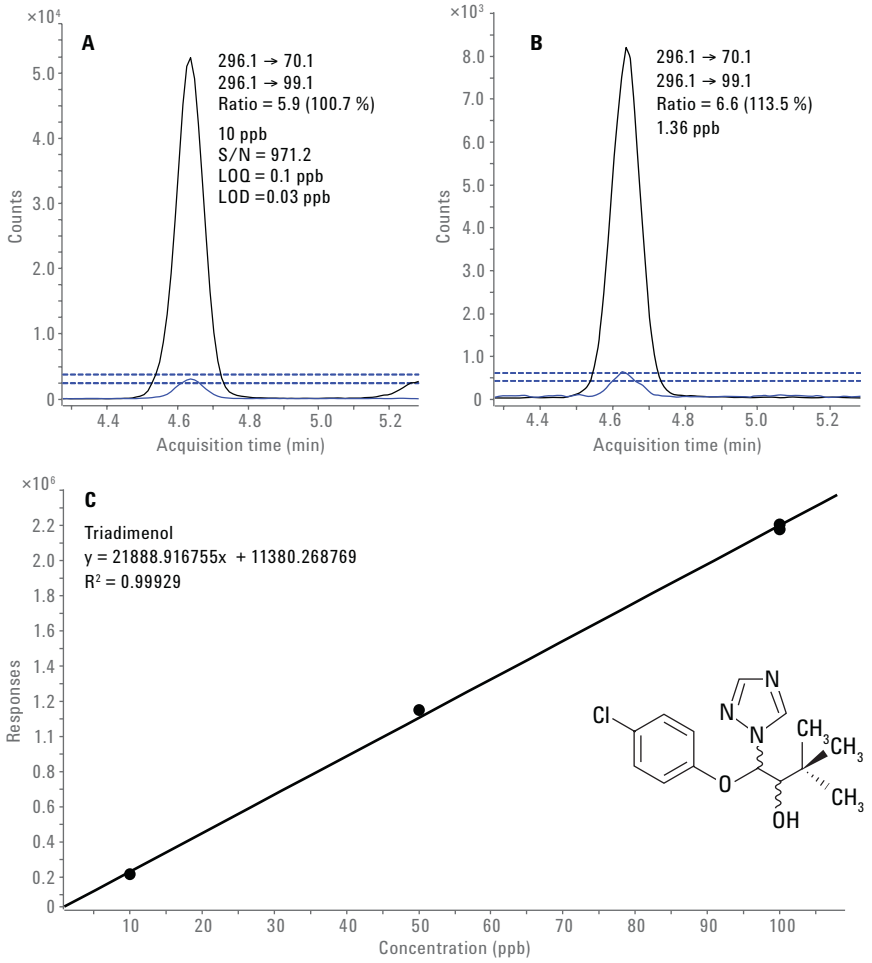

Figure 8. Triadimenol residue in tomatoes. A) Lowest level of the calibration at 10 ppb with an S/N = 971.2, LOQ = 0.1 ppb, and LOD = 0.03 ppb. B) Triadimenol residue detected in tomatoes at 1.36 ppb. C) Calibration curve for triadimenol at levels of 10, 50, and 100 ppb.

The influence of the respective matrix was examined by comparing spiked matrix samples and standards. The recovery for most compounds was in the range of 70 to 120 %, which is accepted by SANCO guidelines for method validation[6]. This was also shown in an earlier work[4]. For instance, for the strawberry matrix, at the 10-ppb level, 193 compounds out of the measured 223 fall in the recovery range of 70 to 120 % (Figure 9). Accounting for the matrix effect, a matrix calibration with compound addition could be done to further improve these results. In addition, standard addition can be used as a means to compensate for matrix effects.

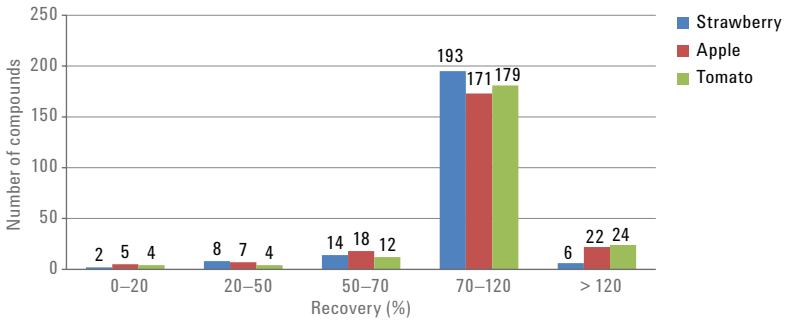

Figure 9. Distribution of pesticide recoveries. Most of the compounds have recoveries in the required range of –30 to +20 %.

Figure 10 shows the standard addition for trifloxystrobin in apple, calculated using the built-in function of the Agilent MassHunter Quantitative Software. The quadratic symbol in the calibration line corresponds to the sample, and the round symbols show the various spiking levels. While the external calibration resulted in a final concentration of 8.1 ppb trifloxystrobin, the standard addition resulted in 11.3 ppb. This shows how a matrix suppression of nearly 30 % can give a result that lies below the actual value. For trifloxystrobin in apples, the MRL is significantly higher (700 ppb) than the default MRL of 10 ppb and, therefore, no MRL exceeding has to be reported.

Conclusion

This Application Note describes the development of a multipesticide method for SFC coupled to triple quadrupole MS for the determination of 223 pesticide compounds. In this method, the majority of 195 pesticide compounds eluted within 10 minutes using a gradient from 2 to 10 % organic modifier. By focusing on these pesticides, this could shorten the method dramatically compared to typical HPLC methods for the measurement of the same number of compounds. The targeted pesticides were determined with typical LOQs at or below 1 ppb, and calibration linearity better than $R^2 = 0.999$. Polar pesticide compounds that are difficult to determine by standard reversed phase HPLC/MS are easily separated and determined by SFC/MS directly from the organic sample extract. Matrix effects are in the same range as reported before, and matrix calibration or the use of internal standards is recommended to compensate for strong matrix effects for specific compounds.

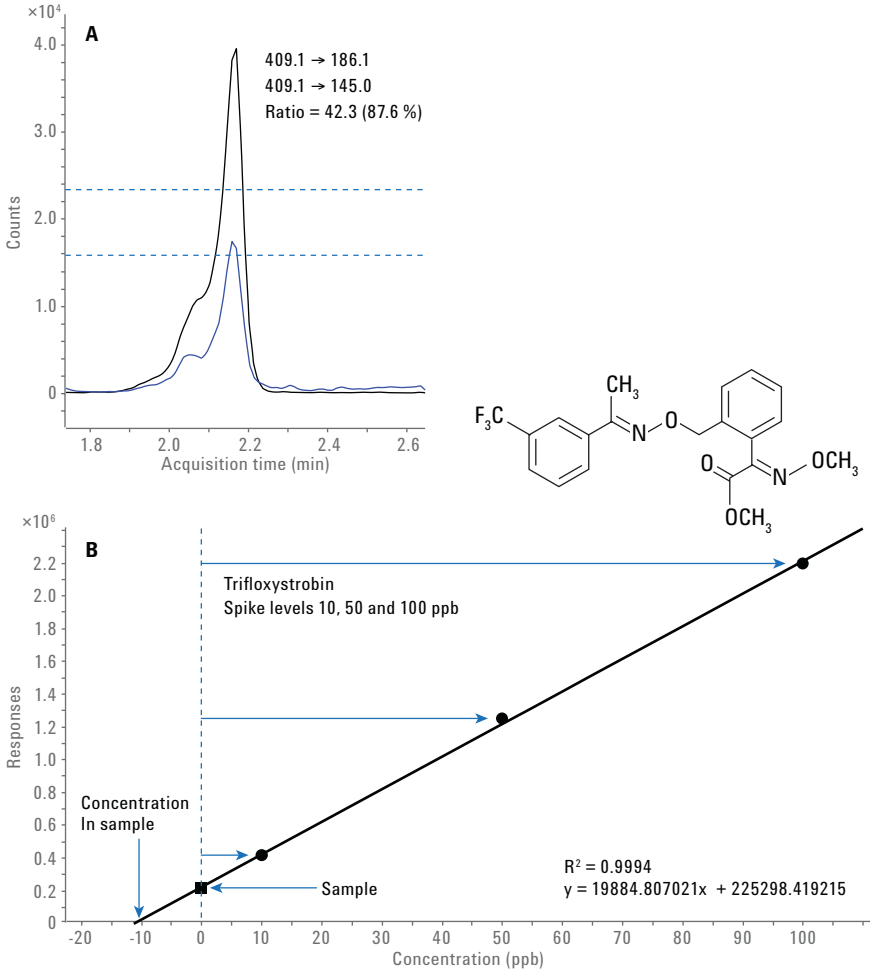

Figure 10. Trifloxystrobin residue in apples. A) Trifloxystrobin residue detected in tomatoes at 8.1 ppb by external calibration. B) Calibration curve for trifloxystobin including standard addition at levels of 10, 50, and 100 ppb. This approach resulted in 11.3 ppb.

References

1. 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

2. Naegele, E., Analysis of Pesticides by Supercritical Fluid Chromatography/ Mass Spectrometry – Optimizing the Agilent 1260 Infinity Analytical SFC System in Combination with the Agilent 6460 Triple Quadrupole LC/MS, *Agilent Application Note*, publication number 5991-5256EN, **2014**

3. Naegele, E., Glauner, T., Analysis of pesticides in vegetable samples with the Agilent 1260 Infinity Analytical SFC System with triple quadrupole MS detection, *Agilent Application Note*, publication number 5991-5443EN, **2015**

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5. United States Environmental Protection Agency: Pesticide Fact Sheet Boscalid, July 2006, last updated December 11, 2011

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Appendix

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages.

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell accel. (V)
1	Methacrifos	1.56	241	55	209.1	0	125.1	28	3
2	Carfentrazone-ethyl	1.61	412	150	366	15	346.1	20	3
3	Pendimethalin	1.61	282.1	85	212.1	5	194.1	15	3
4	Dichlorvos	1.62	220.9	100	109	12	79	24	4
5	Molinate	1.62	188.1	90	126	10	83.2	15	3
6	Diazinon	1.63	305.1	105	169	20	153.1	20	4
7	Malathion	1.65	331	80	126.9	5	99	10	3
8	Oxadiazon	1.65	345	90	303	10	220	15	3
9	Prosulfocarb	1.66	252.1	90	128.1	5	91.1	20	3
10	Pirimiphos-methyl	1.67	306	130	164.2	20	108.1	30	3
11	Phoxim	1.72	299.1	70	129.1	4	77.1	24	3
12	Tolclofos-methyl	1.76	300.9	115	269	10	125	15	3
13	Bifenthrin	1.78	440.2	100	181	5	–	–	4
13	Bifenthrin	1.78	442.2	100	–	–	181	5	4
14	Ethion	1.81	385	95	199	4	143	20	4
15	Mecarbam	1.85	330	70	227	0	97.1	45	3
16	Mevinphos	1.85	225	65	193.1	0	127	10	3
17	Ethoprophos	1.89	243	90	131	15	97	30	4
18	Quinalphos	1.89	299	90	163	20	147	20	7
19	Chlorpyriphos-methyl	1.90	322	110	290	10	125	25	4
20	Phenthoate	1.90	321	75	247	4	79.1	48	3
21	Propargit	1.93	368.1	80	231.2	5	175.1	10	3
22	Ethofumesat	1.97	287	80	259.1	0	121.1	10	3
23	Clomazone	1.98	240	70	125.1	15	89.1	45	3
24	Ethoxyquin	1.98	218	120	174	30	160	35	3

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages. (continued)

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell Accel. (V)
25	Flufenacet	2.00	364	90	194.2	5	152.1	15	3
26	Proquinazid	2.03	372.9	85	331	12	289	24	3
27	Isoxaflutole	2.04	359.8	95	250.9	20	220	35	3
28	Propetamophos	2.05	282.1	125	156	10	138	15	3
29	Triadimefon	2.06	294.1	90	197.1	10	69.1	20	3
30	Metolachlor	2.13	284.1	100	252.2	10	176.1	20	3
31	Kresoxim-methyl	2.14	314.1	85	267.1	0	222.2	10	3
32	Profenofos	2.15	374.9	120	347	5	304.9	15	3
33	Trifloxystrobin	2.19	409.1	110	186.1	10	145	45	3
34	Malaoxon	2.20	315.1	85	127	4	99	20	3
35	Diflufenican	2.21	395	150	266	25	246	40	3
36	Methidathion	2.25	302.9	55	145	0	85.1	15	3
37	Dimethachlor	2.26	256	120	224	10	148	25	3
38	Etofenprox	2.27	394.2	100	177.2	10	107.1	45	3
39	Pyripoxyfen	2.28	322.1	110	185.1	20	96.1	10	3
40	Carbosulfan	2.30	381.1	105	160.1	8	118.1	16	3
41	Furathiocarb	2.30	383.1	110	252.1	5	195.1	15	3
42	Propham	2.33	180.1	60	138.1	4	120	12	3
43	Quinoxyfen	2.33	308	115	197	35	162	45	7
44	Tolylfluanide	2.37	346.9	70	238.1	0	137	25	3
45	Tebufenpyrad	2.39	334.1	145	145.1	25	117.1	40	3
46	Chlorfenvinphos	2.39	358.9	105	170	40	155.1	8	4
47	Metazachlor	2.41	278	70	210.1	0	134.1	15	3
48	Spirodiclofen	2.42	411.1	110	313	5	71.2	15	3
49	Picoxystrobin	2.44	368.1	70	205.1	0	145.1	20	3
50	Pirimicarb	2.45	239.1	100	182.2	10	72.1	20	3
51	Spiromesifen	2.47	388.2	110	273	10	255	25	3
52	Phosalone	2.49	368	70	182	10	111.1	45	3
53	Fenazaquin	2.50	307.2	105	161.1	10	57.1	25	3
54	Hexythiazox	2.50	353	90	228.1	10	168.1	25	3
55	Benfuracarb	2.51	411.1	95	252.1	10	195.1	20	3
56	Spiroxamine	2.55	298.2	125	144.2	15	100.2	35	3
57	Picolinafen	2.56	377.1	120	359	24	238	32	3
58	Fenpyroximat	2.59	422.1	135	366.1	15	135.1	30	3
59	Propaquizafop	2.60	444	125	371	10	100.2	15	3
60	Benalaxyl	2.62	326.1	90	294.2	5	148.1	15	4
61	Propiconazole	2.70	342	115	158.9	30	69.1	15	4
62	DEET	2.71	192.14	110	119	16	91.1	32	3
63	Metalaxyl	2.76	280.1	95	220.1	10	160.1	20	3
64	Indoxacarb	2.82	528	110	203	45	149.9	20	3
65	Cymoxanil	2.83	199	50	128	0	111.1	15	3
66	Buprofezin	2.85	306.1	105	201.2	5	116.1	10	3
67	Trietazin	2.95	230.1	105	202.1	15	99	25	3
68	Bupirimate	2.97	317.1	125	166.1	20	108.1	25	4
69	Phosmet	3.03	317.9	70	160	10	133	40	3
70	Silthiopham	3.03	268	135	252.1	5	139	15	3

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages. (continued)

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell Accel. (V)
71	Pyrimethanil	3.05	200.1	120	107.1	20	82.1	25	3
72	Benzoximate	3.08	364.1	80	198.1	4	104.9	20	3
73	Aldicarb-fragment	3.16	116	70	89.1	4	70.1	4	3
74	Clofentezin	3.16	303	110	138	10	102.1	40	3
75	Flumioxazin	3.23	355.1	100	327.1	20	299	28	3
76	Diethofencarb	3.25	268.1	70	226	0	124	30	3
77	Azinphos-ethyl	3.28	346.05	70	132	8	97	32	3
78	Fluquinconazole	3.28	376	120	349.1	16	307	24	4
79	Fenoxycarb	3.29	302.1	90	116.1	5	88.1	15	3
80	Epoxyconazol	3.34	330	100	121.1	20	101.1	45	4
81	Tetraconazole	3.34	372	130	159	30	70.1	20	4
82	Butocarboxim	3.35	213	70	156.1	5	75	10	3
83	Beflubutamid	3.37	356	145	162.1	25	91	30	3
84	Metobromuron	3.37	259	120	170	15	148	10	3
85	Penconazole	3.4	284	70	159	30	70.1	15	3
86	Flusilazole	3.42	316	120	247.2	15	165.1	25	4
87	Promecarb	3.42	208.1	80	151	0	109.1	10	3
88	Cyprodinil	3.43	226.1	140	93.1	40	77.1	45	3
89	Azamethiphos	3.44	325	120	182.9	12	111.9	40	4
90	Phosphamidon	3.44	300.1	110	174.1	8	127	16	3
91	Azinphos-methyl	3.46	318.02	60	261	0	132	8	3
92	Coumaphos	3.46	363	120	307	16	226.9	28	4
93	Temephos	3.47	467	155	419	20	124.9	44	3
94	Triflumizol	3.48	346	85	278.1	5	73.1	10	3
95	Pyridaben	3.49	365.1	80	309.1	10	147.1	25	3
96	Isocarbophos	3.54	231	100	121	20	65	40	3
97	Fosthiazate	3.55	284	90	228.1	5	104.1	20	3
98	Propyzamid	3.59	256	105	190	10	173	20	3
99	Metrafenon	3.6	409	110	226.9	25	209.1	10	3
100	Cymiazol	3.61	219	95	171	25	144	35	3
101	Prometon	3.62	226.2	100	184	16	142.1	24	3
102	Isoprothiolane	3.63	291.1	80	231	8	188.8	20	3
103	Fenobucarb	3.70	208.1	65	152.1	5	95.1	10	3
104	Triazophos	3.70	314	110	162.1	15	119.1	35	3
105	Tralkoxydim	3.71	330.1	170	284.2	5	138.1	15	3
106	Furalaxyl	3.72	302.1	110	242.1	10	95	27	3
107	Iprovalicarb	3.74	321.1	80	203.1	0	119.1	20	3
108	Trimethacarb	3.77	194.1	80	137	4	122.1	28	3
109	Mexacarbate	3.82	223.1	110	166.1	12	151	24	3
110	Azaconazole	3.83	300	130	230.8	16	158.9	32	3
111	Propoxur	3.83	210.1	55	168.1	0	111.1	10	3
112	Mepanipyrim	3.88	224	140	209.1	16	106.1	25	3
113	Cyazofamid	3.89	325	90	261.1	5	108.1	10	3
114	Bromuconazole	3.98	377.9	115	159	35	70.1	20	4
115	Methoprotryne	4.10	272.2	140	198	24	169.9	28	3
116	Carbofuran	4.11	222.1	80	165.1	5	123.1	20	3

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages. (continued)

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell Accel. (V)
117	Methabenzthiazuron	4.11	222	90	165	15	150	35	3
118	Linuron	4.14	249	100	182.1	10	160	15	3
119	Pyraclostrobin	4.18	388	95	194.1	5	163.1	20	3
120	Difenoconazole	4.20	406	120	337.1	15	251.1	25	3
121	Secbumeton	4.24	226.2	100	170.1	16	67.9	50	3
122	Aminocarb	4.34	209.1	105	152	12	137.2	24	3
123	Fenamiphos	4.39	304.1	120	217.1	20	202	35	3
124	Prochloraz	4.39	376	70	308	5	266	10	3
125	Methiocarb	4.40	226.1	70	169.1	0	121.1	15	3
126	Fenpropidin	4.43	274	120	147	30	86	25	3
127	Myclobutanil	4.50	289.1	110	125	35	70.1	15	3
128	Clethodim	4.67	360.1	100	268.2	10	164.1	15	3
129	Imazalil	4.69	297	115	201	15	159	20	4
130	Fluopicolide	4.72	382.9	110	172.9	25	144.9	45	3
131	Triadimenol	4.78	296.1	70	99.1	10	70.1	5	3
132	Rotenone	4.79	395	145	213.1	20	192.1	20	3
133	Cycluron	4.84	199.2	120	88.9	12	72.1	28	3
134	Dimethomorph	5.05	388	145	301.1	20	165.1	30	3
135	Dimoxystrobin	5.16	327.1	115	205.1	5	116	20	3
136	Hexaconazole	5.27	314	95	159	30	70.1	15	4
137	Triflumuron	5.34	359	90	156	10	139	35	3
138	Paclobutrazol	5.46	294.1	115	125	40	70.1	20	3
139	Aldicarb	5.49	208	70	116	0	89.1	10	3
140	Quinoclamin	5.49	208	125	88.9	44	76.9	44	3
141	Carboxin	5.51	236	105	143	10	93	40	3
142	Tebuconazole	5.69	308.1	100	125	40	70.1	20	4
143	Azoxystrobin	5.75	404	110	372.2	10	344	25	3
144	Fenbuconazol	5.78	337.1	145	125.1	35	70.1	15	4
145	Dioxacarb	5.81	224	80	167	10	123	10	3
146	Monocrotophos	5.89	224	65	193.1	0	127	10	3
147	Bitertanol	5.91	338.1	70	269.2	0	70.1	0	3
148	Fenarimol	5.99	331	130	268.1	20	81.1	30	4
149	Fenamidon	6.05	312.1	100	236.2	10	92.1	25	3
150	Flutriafol	6.05	302	90	123	30	70.1	15	3
151	Pyracarbolid	6.14	218.1	145	125	16	96.9	28	3
152	Tebuthiuron	6.18	229.1	105	172.1	12	116	24	3
153	Omethoat	6.19	214	80	125	20	109	25	3
154	Spinosyn A	6.19	732.4	155	142.1	30	98.1	45	3
155	Bifenazate	6.23	301.1	95	198.2	5	170.1	15	3
156	Lufenuron	6.23	510.9	138	158	20	141	45	3
157	Metconazole	6.25	320.1	130	125.1	40	70.1	20	4
158	Diniconazole	6.27	326	75	159	28	70.1	28	4
159	Spinosyn D	6.30	746.5	145	142.1	35	98	55	3
160	Novaluron	6.31	493.1	90	158.1	20	141.1	45	3
161	Tepraloxydim	6.33	342.1	130	250.2	10	166.1	20	3
162	Cyproconazole	6.36	292.1	100	125.1	35	70.1	15	3

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages. (continued)

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell Accel. (V)
163	Uniconazole-P	6.36	292.1	135	125	36	70	24	4
164	Ipconazole	6.39	334.1	115	125	45	70	25	4
165	Dimethoate	6.41	230	70	199	0	125	20	3
166	Alanycarb	6.45	400.1	130	238	4	91	50	3
167	Mandipropamid	6.52	411.9	110	356.1	5	328.1	10	3
168	Carbaryl	6.54	202	65	145	0	127.1	25	3
169	Diflubenzuron	6.55	311	80	158	10	141	35	3
170	Flufenoxuron	6.68	489	100	158	15	141	45	3
171	Oxadixyl	6.88	279.1	70	219.1	5	132.1	35	3
172	Triticonazole	6.92	318.1	90	125.1	40	70.1	10	4
173	Fluoxastrobin	6.94	459	130	427.1	15	188.1	40	3
174	Spirotetramat	7.13	374.1	120	330.1	10	302.1	10	3
175	Vamidothion	7.17	288.1	95	146	8	146	8	4
176	Pencycuron	7.21	329.1	120	218.1	10	125	25	3
177	Methamidophos	7.26	141.9	85	125	10	94.1	10	3
178	Diuron	7.27	235	110	72.1	20	–	–	3
178	Diuron	7.27	233	110	–	–	72.1	20	3
179	Famoxadone	7.27	392.1	85	331.2	0	238.2	10	3
180	Fluometuron	7.27	233.1	105	72.1	15	46.2	15	3
181	Zoxamide	7.32	336	120	187	20	159	45	3
182	Carbendazim	7.50	192	105	160.1	15	132.1	30	3
183	Methomyl	7.58	162.9	50	106.1	5	88.1	0	3
184	Bosclid	7.68	343	145	307.1	12	271	28	3
185	Acephate	7.73	183.9	70	143	0	125	15	3
186	Flonicamid	7.98	230	110	203	15	174	15	3
187	Hexaflumuron	8.09	461	120	158	15	141	45	3
188	Tricyclazol	8.28	190	130	163	20	136	30	4
189	Isoxaben	8.30	333.2	100	165	16	150	48	3
190	Sulfentrazone	8.31	404	110	306.9	28	273	36	3
191	Chlorotoluron	8.85	213.1	120	140	20	72	20	3
192	Lenacil	8.93	235.2	85	153.1	15	136	35	3
193	Oxamyl	9.17	237	60	90.1	0	72.1	15	3
194	Metaflumizone	9.45	507	150	287.1	20	178.1	20	3
195	Tebufenozid	9.45	353	95	297.2	0	133.1	15	3
196	Moxidectin	10.16	640.4	148	622.2	12	528.2	4	3
197	Metamitron	10.18	203.1	100	175.1	15	104.1	20	3
198	Fenuron	10.25	165.1	180	76.9	32	72	16	3
199	Chloroxuron	10.27	291	130	164	10	72.1	20	3
200	Thiodicarb	10.28	355	82	108.1	10	88.1	10	3
201	Methoxyfenozide	10.56	369.2	85	313.2	0	149.1	10	3
202	Tribenuron-methyl	11.08	396	110	181.1	15	155.1	5	3
203	Thiabendazol	11.24	202	130	175.1	25	131.1	35	3
204	Desmedipham	11.47	318.1	80	182.2	5	136.1	25	3
205	Phenmedipham	11.47	318.1	90	168.1	4	136	20	3
206	Propamocarb	11.88	189.1	90	144	5	102.1	15	3
207	Ethidimuron	11.98	265.1	120	207.9	12	57	32	3

Table 1. Dynamic MRM method information for the 223 measured pesticides, including retention times, molecular and fragment masses, and fragmentor, collision, and cell acceleration voltages. (continued)

No.	Compound name	Retention time (min)	Precursor ion (*m/z*)	Fragmentor (V)	Product ion 1 (*m/z*)	Collision energy (V)	Product ion 2 (*m/z*)	Collision energy (V)	Cell Accel. (V)
208	Acetamiprid	12.06	223	80	126.1	2	90.1	35	3
209	Chlorantraniliprole	12.31	483.9	105	452.9	15	285.9	10	3
210	Fuberidazol	12.34	185.1	145	157.1	20	156.1	30	3
211	Fenhexamid	12.56	302	130	97.2	20	55.1	40	3
212	Pymetrozin	12.84	218	110	105.1	20	78.1	45	3
213	Ethirimol	12.87	210.1	145	140.1	20	98.1	25	3
214	Hydramethylnon	12.99	495.2	200	323	36	170.9	48	3
215	Imidacloprid	13.48	256	80	209.1	10	175.1	15	3
216	Thiamethoxam	13.69	292	85	211.1	5	181.1	20	3
217	Chloridazon	13.93	222	130	104.1	25	77.1	35	3
218	Thiacloprid	14.15	253	100	126	20	90.1	40	3
219	Nitenpyram	14.60	271.1	95	225.2	3	56.1	30	3
220	Oxasulfuron	15.57	407	120	150.1	15	107.1	45	3
221	Forchlorfenuron	16.02	248.1	110	129	16	92.9	40	3
222	Mesosulfuron-metyl	16.30	504.1	125	182.1	25	139.1	45	3
223	Triasulfuron	17.86	401.9	130	167.1	10	141	10	3

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