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APPLICATION NOTE 72961

Screening and quantitation of pesticide residues in rice using LC-(HESI)-MS/MS

Authors
Ramiz M.R. Azad and Dasharath Oulkar

Customer Solution Center, Ghaziabad,
Thermo Fisher Scientific, India

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TraceFinder, pesticide residues, rice, QuEChERS, LC-MS/MS, TSQ Quantis

Goal

The objective of this work was to develop a screening solution followed by quantitation of 160 pesticide residues in rice matrix using liquid chromatography-triple quadrupole mass spectrometry. The optimized method was validated in accordance with the SANTE guidelines and in compliance with the requirements of FSSAI and the European Commission (EC) MRLs.

Introduction

The Central Insecticide Board and Registration Committee (CIBRC) has few chemicals registered for rice.[1] The European Commission (EC) and FSSAI have set the maximum residue levels (MRLs) for many pesticides in rice at 0.01 mg/kg, but the MRLs for fipronil and fipronil sulfone are set at 0.005 mg/kg.[2,3] The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has been adopted for pesticide residue extraction in most food samples.[4] Additionally, the instrument method plays an important role in delivering accurate and precise results to meet the regulatory requirements.

The aim of this work was the optimization and method validation of a multi-residue method for pesticides in rice by using LC-MS/MS with a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer. Sample

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extraction was carried out using the acetic acid buffered version of the QuEChERS.[4] The data acquisition and processing was carried out by using Thermo Scientific™ TraceFinder™ software. The optimized method was validated according to the SANTE/11813/ 2017 guidelines.[5] This method was applied to real samples to demonstrate the application of streamlined workflow in compliance with the EU and FSSAI MRL requirements.

Experimental

Chemicals and apparatus

- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical
- Methanol, Optima™ LC/MS Grade, Fisher Chemical
- Water, Optima™ LC/MS Grade, Fisher Chemical
- Formic acid, Fisher Chemical
- Acetic acid, Fisher Chemical
- Ammonium formate, LC-MS Grade, Fisher Chemical
- Anhydrous magnesium sulfate, Thermo Fisher Scientific
- Sodium acetate, LR Grade, Fisher Chemical
- Certified reference materials
- Analytical balance (Aczet, CY2202, San Diego, CA) and precision balance (Aczet, CY205C, San Diego, CA)
- Vortex mixer (Thermo Scientific, P/N 88880017TS, also known as 88880017)
- Refrigerated centrifuge (Thermo Scientific™ Sorvall™ ST8 ventilated benchtop centrifuge)
- Variable volume micropipettes (Thermo Scientific)
- QuEChERS Salts (2007.01) Mylar Pouch 6 g magnesium sulfate (anhydrous),1.5 g sodium acetate 50 pk Thermo Scientific™ (P/N 60105-341)

LC-MS/MS analysis

The Thermo Scientific™ Vanquish™ Flex Binary UHPLC system was coupled with the TSQ Quantis triple quadrupole mass spectrometer, which included the Heated Electrospray Ionization (HESI) source. The instrument was operated using an electrospray source in both positive/negative mode. The detailed conditions are given in Table 1. Multiple reaction monitoring (MRM) conditions were optimized for each pesticide during infusion (Appendix, Table 2).

Table 1. LC-MS/MS instrument conditions

Liquid chromatography method				
Instrumentation:	Vanquish Flex Binary UHPLC			
Column:	Thermo Scientific™ Hypersil GOLD™ (100 mm × 2.1 mm × 1.9 µm) (P/N 25002-102130)			
Sample compartment temp.:	10 °C			
Column oven temp.:	25 °C			
Mobile phase A:	2 mM ammonium formate + 0.1% formic acid in water: acetonitrile (90:10, v/v)			
Mobile phase B:	2 mM ammonium formate + 0.1% formic acid in water: acetonitrile (10:90, v/v)			
Total run time:	18.0 min			
Gradient program:	*Time (min)*	*Flow Rate*	*%B*	*Curve*
	0.0	0.400	1	5
	1.5	0.400	1	5
	5.0	0.400	5	5
	8.5	0.400	95	5
	13.5	0.400	95	5
	14.0	0.400	1	5
	18.0	0.400	1	5
Mass spectrometry method				
Instrumentation:	TSQ Quantis triple quadrupole tandem mass spectrometer			
Method type:	Time-based Selective-Reaction Monitoring (T-SRM)			
Ion source type:	H-ESI			
Polarity:	Positive/Negative switching			
Spray voltage:	Static Positive: 3500 V Negative: 2500 V			
Sheath gas:	50 Arb			
Aux gas:	10 Arb			
Sweep gas:	1 Arb			
Ion transfer tube temp.:	325 °C			
Vaporizer temp.:	350 °C			

Sample preparation

The rice grain matrix purchased from a local market was homogenized using a heavy-duty homogenizer to reduce the particle size to approximately 200 to 500 µm. The QuEChERS method was used for extraction as described below without cleanup.

Sample extraction

- Weigh 5 g homogenized sample into a 50 mL extraction tube (Note sample spiking at this step).
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample to soak for 10 min.
- Add 15 mL acetonitrile.
- Shake vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at ambient conditions.
- Filter the acetonitrile supernatant through a 0.2 µm PTFE membrane filter and dilute with HPLC grade water (1:1) before analyzing by LC-MS/MS.

Data acquisition and processing

The data acquisition and processing were carried out using TraceFinder software version 4.1. The data was acquired in Timed-SRM mode, which includes two or more transitions per analyte from the compound database (CDB). For data processing, the ion ratio (±30%), retention time (±0.1 min), linearity (>0.99 with residuals ±20), recovery (70–120%) and precision (±20%) were set as user-defined filters as per SANTE guideline criteria.[5]

Results and discussion

Sample preparation

Rice is a dry powder and complex matrix that contains carbohydrates (9%) and proteins (5%). Rice has close to neutral pH (6.2–6.7), therefore acidification was maintained by using 1% acetic acid in water. It has very low moisture content, so water (1% acetic acid) was used, which is required for liquid-liquid partitioning with acetonitrile. Acidification improved the stability of base-sensitive compounds during extraction. No cleanup was applied, and the extract was diluted 6-fold as per the defined protocol to minimize the matrix effect without losses of target analytes. The final diluted extract, e.g. 0.01 mg/kg, corresponds to 0.0016 mg/kg.

LC-MS/MS analysis

Total LC-MS/MS method conditions were optimized for pesticide residues analysis, showing excellent sensitivity for 160 compounds. The total ion chromatogram (TIC) is shown in Figure 1. The optimized liquid chromatographic method offered excellent separation for the target analytes (spinetoram and spinosad D presented in Figure 2) and the absence of an isobaric interference from the matrix. In this method, the dwell time was automatically optimized in the range of 2–10 ms per transitions, which offered ≥12 points per peak. For early eluting compounds like 3-OH-carbofuran, this offered more than 12 points per peak as shown in Figure 3. This optimized instrument conditions provided excellent selectivity, repeatability, and reproducibility.

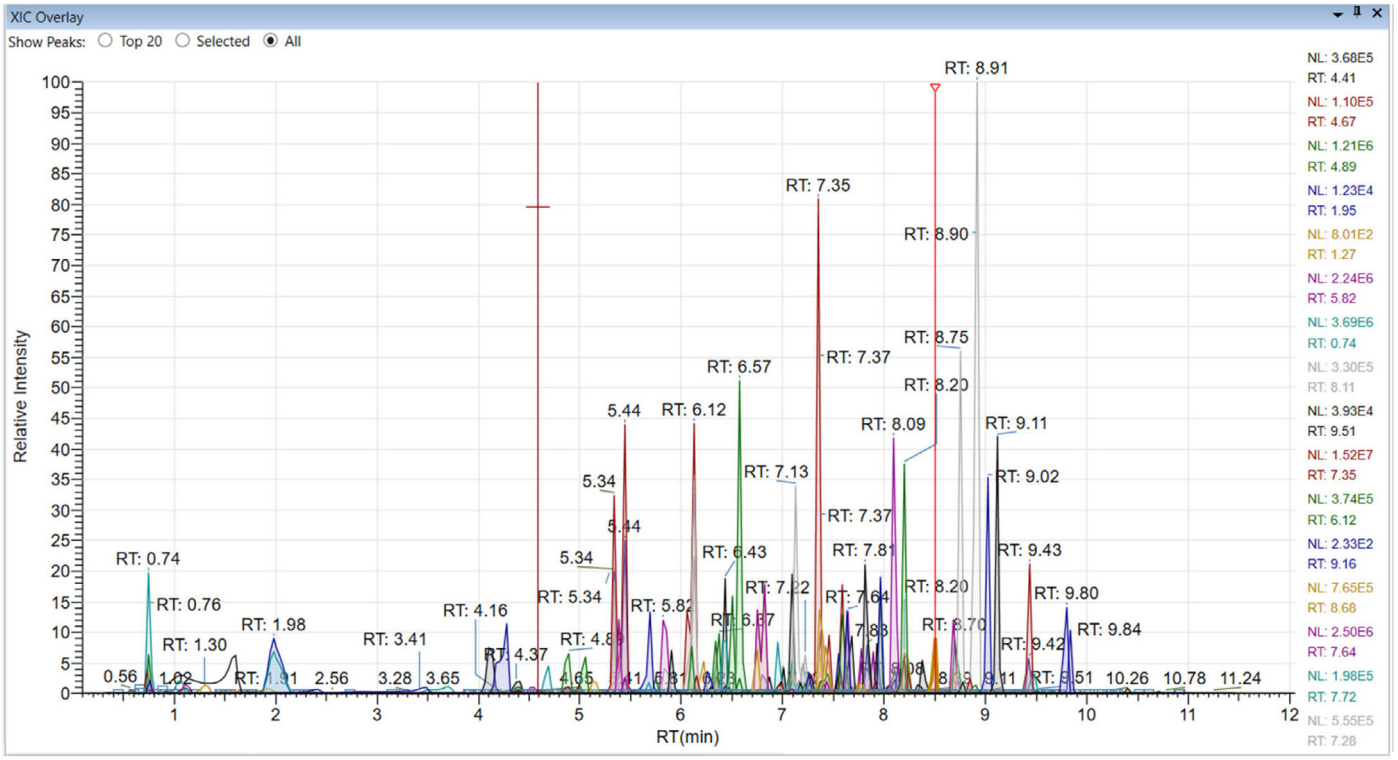

Figure 1. Extracted ion chromatogram for 160 compounds overlaid in a single window

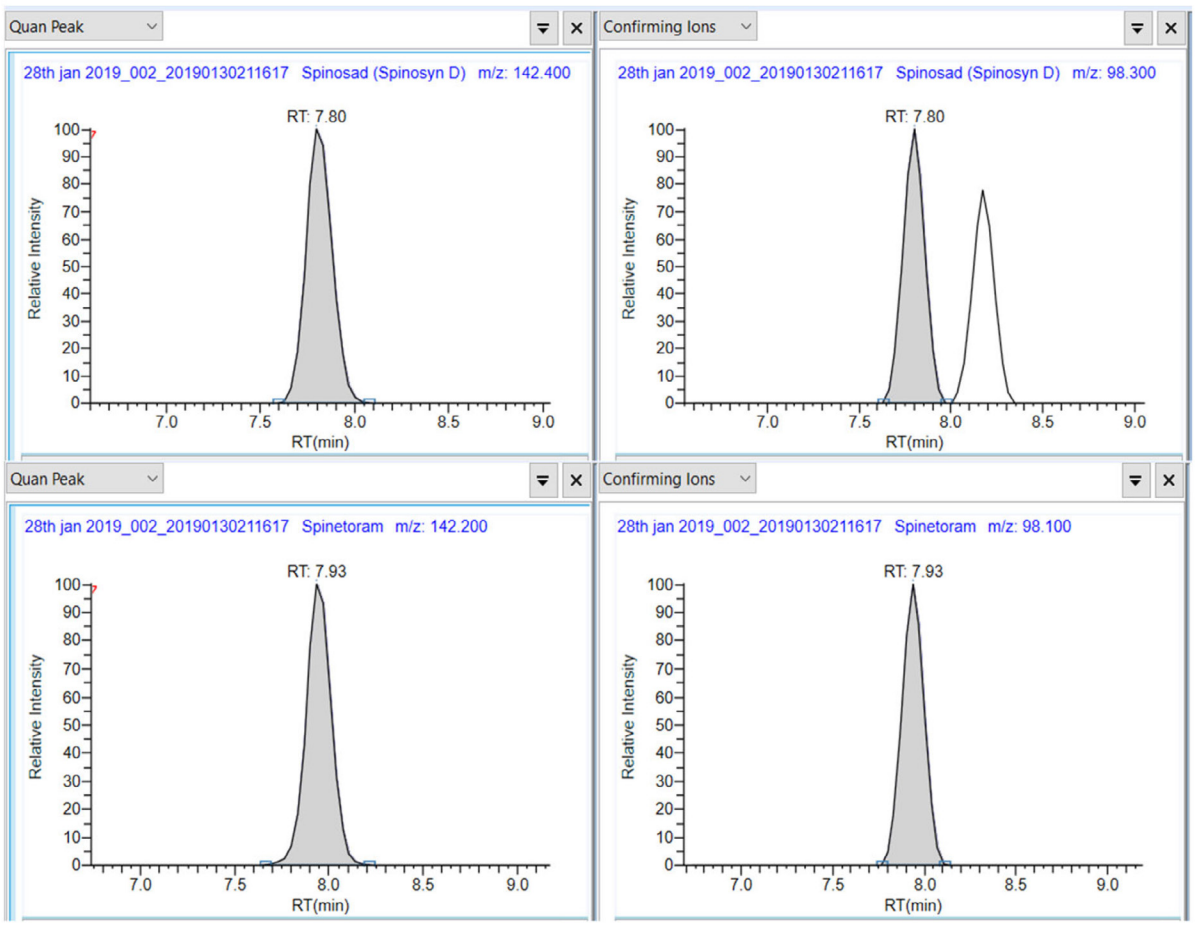

Figure 2. Effect of optimized gradient program on separation of isobaric compounds

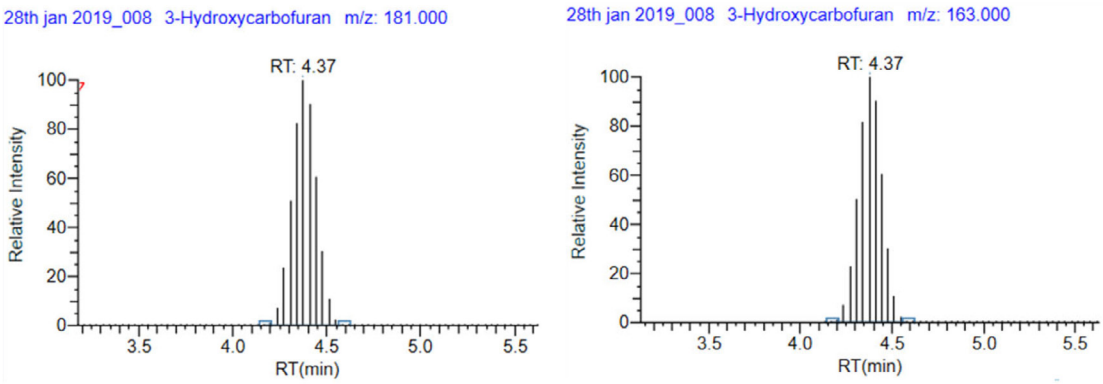

Figure 3. Impact of optimized dwell time on the data points per peak

Identification and quantitation

Parameters set in the TraceFinder Master Method for data processing included two transitions per analyte, retention time, correlation coefficient, and calibration graph residuals. Based on these parameters, the data was processed automatically with flagging. These color-coded flags indicated whether results passed or failed the acceptance criteria set in the processing method. The results passed SANTE guideline criteria as shown by the green colored flags (Figure 4).

In Figure 3, an identification of 3-hydroxycarbofuran in rice was demonstrated with two transitions 238.1→181.0 (quantitative) and 238.1→163.0 (confirmatory) at the same retention time (4.37 min, ±0.1), with observed ion ratio of 66.0% (45.21%–83.96%) in rice in comparison with the neat standard.

The linearity for 3-hydroxycarbofuran provided a correlation coefficient >0.999 with <15% residuals in compliance with the SANTE guidelines.

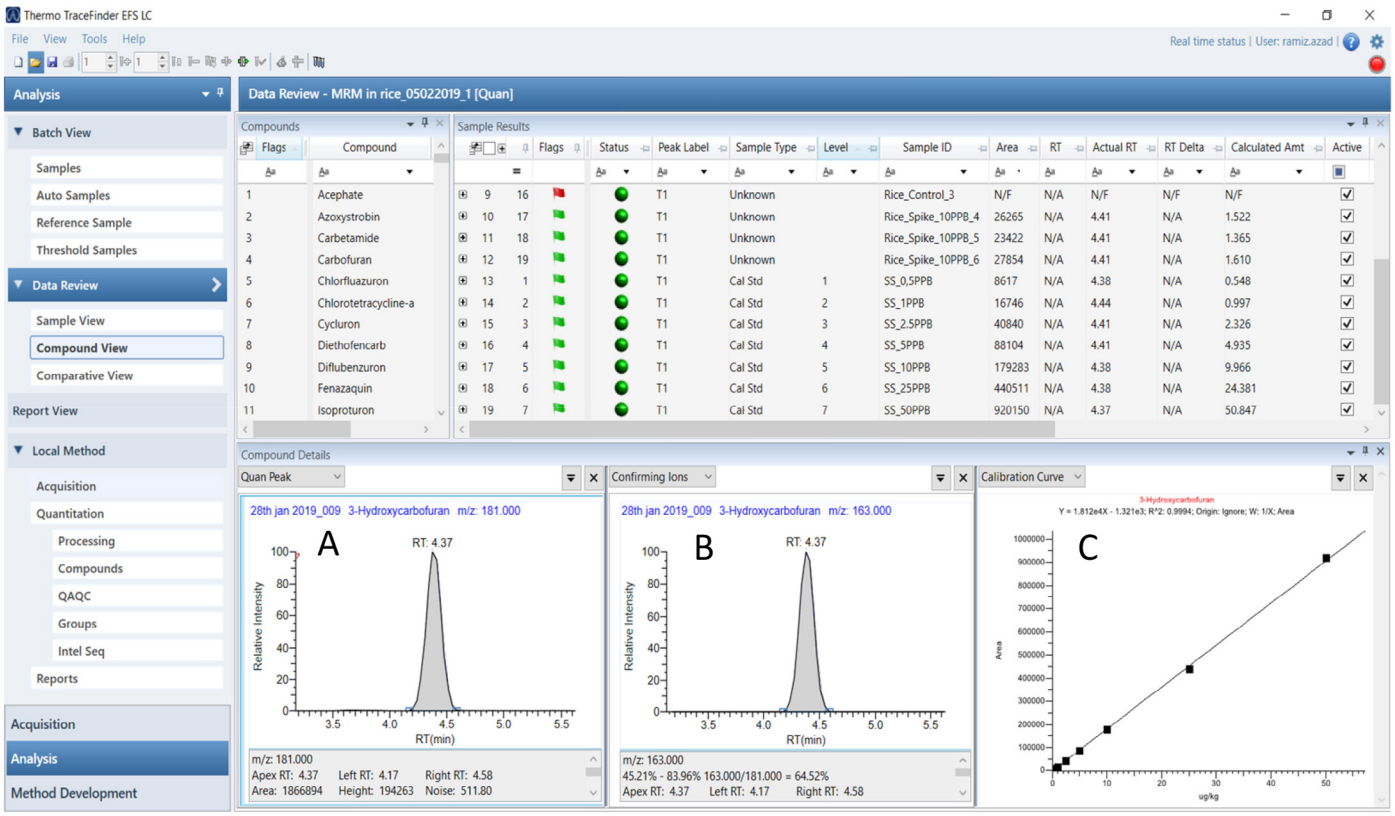

Figure 4. (A) Extracted ion chromatogram for quantifier ion of 3-hydroxycarbofuran, (B) identification based on the selectivity of confirmatory ions and confirmed with ion ratio, and (C) calibration curve

Method performance

The instrument detection limit was 0.05 ng/mL for 95% of the 160 target pesticides (Figure 5). Calibration standards were linear over the range of 0.0005 to 0.1 mg/kg with the correlation coefficient (>0.99) and residuals <20% for all the target analytes in both solvent and rice matrix. Because of the dilution of rice extracts, the LOQs in rice matrix were 0.01 mg/kg with acceptable recoveries (70%–120%) and precision (<20%). The validation experiment was carried out by analysis of six replicates spiked at 0.01 (LOQ) and 0.05 mg/kg. Average recoveries were in the range of 76% to 116% with <15% RSD (Appendix, Table 3), which are in compliance with the SANTE criteria (recovery 70%–120% and precision <20%).5 The repeatability of results obtained by analysis of an extended sequence (n=50 injection) was <10% for the area and <±0.05 min retention time for acetamiprid and thiabendazole in Figures 6 and 7 (Figure 7 next page). This reveals that the optimized method offered excellent repeatability in results.

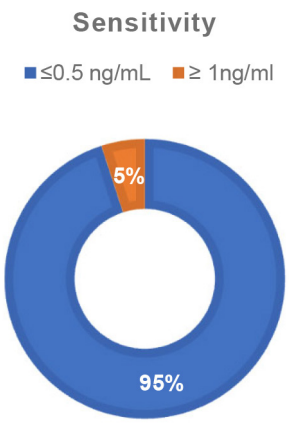

Figure 5. Sensitivity for the target list of analytes in the solvent standard

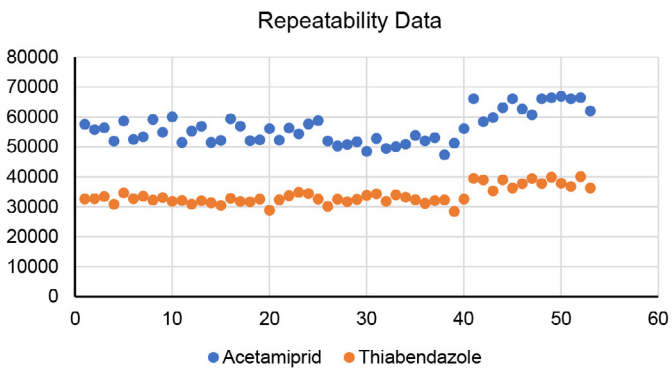

Figure 6. Area repeatability for acetamiprid and thiabendazole (n=54)

Conclusion

The developed method provides a solution for the simultaneous screening and quantitation of pesticide residues (160 pesticides) in rice by using a Thermo Scientific LC-HESI-MS/MS system. Use of the QuEChERS method for extraction followed by LC-MS/MS analysis could increase the overall high throughput of the commercial food testing laboratory. By following this approach at least 50 injections (standards, samples, blank) could be completed in a 24 h cycle. The optimized method results showed that LC separations in combination with SRM windows allowed maintaining the number of transitions monitored in single injection by auto-optimized dwell time without compromising data quality. This validated method data meets the SANTE guidelines.[5] Also, this method complies with the EU and FSSAI MRL requirements by achieving an excellent lower limit of quantitation (LOQ).

References

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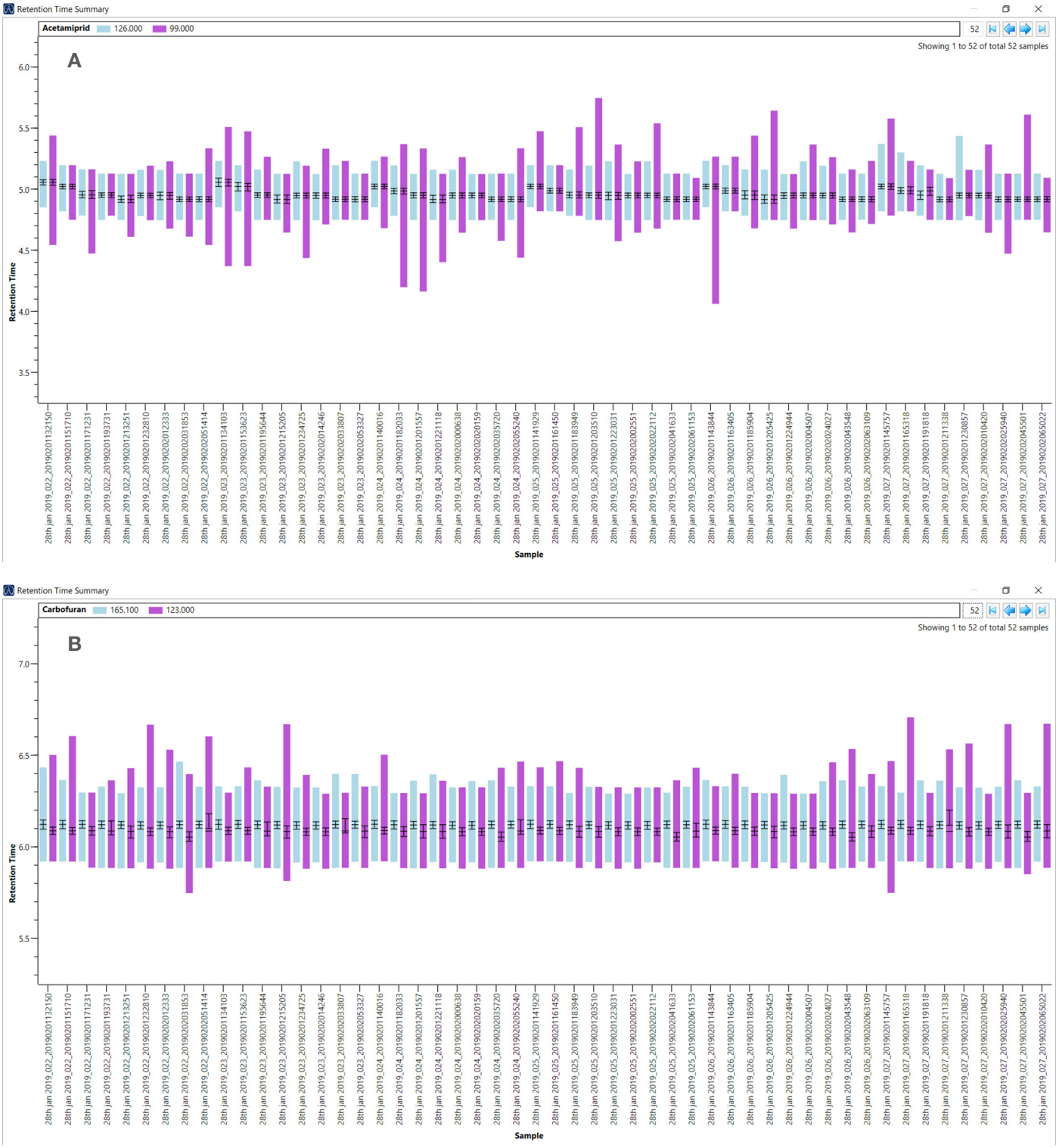

Figure 7. Retention time repeatability for acetamiprid (A) and carbofuran (B) (n=52)

Appendix

Table 2A. List of pesticides with MRM transitions used

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
3-Hydroxycarbofuran	3.89	Positive	238.1	163	19
3-Hydroxycarbofuran	3.89	Positive	238.1	181	15
Acephate	4.17	Positive	184.1	49	35
Acephate	4.17	Positive	184.1	143	11
Acetamiprid	4.51	Positive	223	99	53
Acetamiprid	4.51	Positive	223	126	29
Aldicarb sulfone	1.46	Positive	240.1	86.2	28
Aldicarb sulfone	1.46	Positive	240.1	148.2	19
Aldicarb sulfoxide	0.98	Positive	207.1	89.1	19
Aldicarb sulfoxide	0.98	Positive	207.1	132.1	9
Ametryn	5.75	Positive	228.1	96	35
Ametryn	5.75	Positive	228.1	186.1	25
Aminocarb	0.94	Positive	209.1	137.1	33
Aminocarb	0.94	Positive	209.1	152	19
Amitraz	7.71	Positive	294.2	91.2	57
Amitraz	7.71	Positive	294.2	148.3	22
Avermectin Ba	8.95	Positive	890.5	305	35
Avermectin Ba	8.95	Positive	890.5	567.5	17
Azoxystrobin	6.96	Positive	404.1	344.1	33
Azoxystrobin	6.96	Positive	404.1	372.1	19
Benalaxyl	7.72	Positive	326.2	148.1	29
Benalaxyl	7.72	Positive	326.2	294.1	15
Bendiocarb	5.98	Positive	224.1	109	33
Bendiocarb	5.98	Positive	224.1	167.1	15
Benzoximate	8.1	Positive	364	105	31
Benzoximate	8.1	Positive	364	199	11
Bifenazate	7.64	Positive	301.1	170.1	27
Bifenazate	7.64	Positive	301.1	198.1	13
Bitertanol	7.35	Positive	338.2	70	29
Bitertanol	7.35	Positive	338.2	269.2	13
Boscalid	7.07	Positive	343	140	25
Boscalid	7.07	Positive	343	307	27
Bromucanozole	7.04	Positive	378	70	47
Bromucanozole	7.04	Positive	378	159	37
Bupirimate	7.13	Positive	317	108	35
Bupirimate	7.13	Positive	317	159.1	33
Buprofezin	8.28	Positive	306.2	116.2	23
Buprofezin	8.28	Positive	306.2	201.1	17
Butafenacil	7.6	Positive	492.1	331	27
Butafenacil	7.6	Positive	492.1	349	19

Continued on next page

Table 2B. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Butoxycarboxim	1.87	Positive	223.1	106	13
Butoxycarboxim	1.87	Positive	223.1	159	11
Carbendazim	1.64	Positive	192.2	132.1	41
Carbendazim	1.64	Positive	192.2	160.2	25
Carbetamide	5.02	Positive	237.1	118.1	17
Carbetamide	5.02	Positive	237.1	192	13
Carbofuran	5.98	Positive	222.1	123	29
Carbofuran	5.98	Positive	222.1	165.1	17
Carboxin	5.98	Positive	236.1	87	33
Carboxin	5.98	Positive	236.1	143	21
Chlorantraniliprole	6.47	Positive	484	285.9	17
Chlorantraniliprole	6.47	Positive	484	452.9	21
Chlorotoluron	5.97	Positive	213.1	46.2	35
Chlorotoluron	5.97	Positive	213.1	72.2	31
Chloroxuron	6.99	Positive	291.1	72.4	47
Chloroxuron	6.99	Positive	291.1	218.1	33
Clethodim	8.27	Positive	360.101	164	29
Clethodim	8.27	Positive	360.101	268.1	17
Clothianidin	3.86	Positive	250	132	21
Clothianidin	3.86	Positive	250	169	19
Cyazofamid	7.72	Positive	325.2	108	18
Cyazofamid	7.72	Positive	325.2	261.2	14
Cycluron	6.24	Positive	199.1	89	21
Cycluron	6.24	Positive	199.1	89.1	21
Cyproconazole Isomer	6.79	Positive	292	70	31
Cyproconazole Isomer	6.79	Positive	292	125	29
Cyprodinil	7.29	Positive	226	77	61
Cyprodinil	7.29	Positive	226	93	47
Desmedipham	6.73	Positive	318.1	154	35
Desmedipham	6.73	Positive	318.1	182	19
Diclobutrazol	7.54	Positive	328.2	59.1	48
Diclobutrazol	7.54	Positive	328.2	70.2	48
Dicrotophos	2.78	Positive	238.1	112.1	17
Dicrotophos	2.78	Positive	238.1	193	13
Diethofencarb	6.75	Positive	268.1	124	43
Diethofencarb	6.75	Positive	268.1	226.1	13
Difenoconazole	7.76	Positive	406.1	251.1	41
Difenoconazole	7.76	Positive	408.2	253.1	31
Dimethoate	4.15	Positive	230	125	29
Dimethoate	4.15	Positive	230	199	13
Dimethomorph Isomer	6.38	Positive	388.1	165.1	45
Dimethomorph Isomer	6.38	Positive	388.1	301	29

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Table 2C. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Dimethomorph Isomer _1	6.6	Positive	388.101	165.1	45
Dimethomorph Isomer _1	6.6	Positive	388.101	301	29
Dimoxystrobin	7.41	Positive	327.1	116	29
Dimoxystrobin	7.41	Positive	327.1	205	23
Diniconazole	7.61	Positive	326.2	70.2	50
Diniconazole	7.61	Positive	326.2	159	43
Dinotefuran	1.07	Positive	203.1	129.2	17
Dinotefuran	1.07	Positive	203.1	157.2	11
Dioxacarb	4.11	Positive	224.1	123	21
Dioxacarb	4.11	Positive	224.1	167	11
Diuron	6.21	Positive	233.1	72	37
Diuron	6.21	Positive	235.1	72.1	33
Doramectin	9.31	Positive	916.6	331.4	33
Doramectin	9.31	Positive	916.6	593.5	19
Emamectin-benzoate b1a	8.54	Positive	886.5	82.1	65
Emamectin-benzoate b1a	8.54	Positive	886.5	158.1	49
Epoxiconazole	7.1	Positive	330	101.1	65
Epoxiconazole	7.1	Positive	330	121.1	49
Eprinomectin	8.65	Positive	914.6	154.2	49
Eprinomectin	8.65	Positive	914.6	186.2	25
Etaconazole	7.17	Positive	328.1	159	31
Etaconazole	7.17	Positive	328.1	205	23
Ethirimol	4.2	Positive	210.2	98.1	39
Ethirimol	4.2	Positive	210.2	140.1	31
Etoxazole	8.9	Positive	360.1	57.2	51
Etoxazole	8.9	Positive	360.1	141	59
Fenamidone	6.96	Positive	312.1	92	35
Fenamidone	6.96	Positive	312.1	236.1	21
Fenarimol	6.9	Positive	331	81	55
Fenarimol	6.9	Positive	331	268	35
Fenazaquin	9.69	Positive	307.1	147	25
Fenazaquin	9.69	Positive	307.1	161.1	27
Fenbuconazole	7.31	Positive	337	70	39
Fenbuconazole	7.31	Positive	337	124.9	55
Fenpropimorph	7.23	Positive	304	117	65
Fenpropimorph	7.23	Positive	304	147	39
Fenpyroximate	8.92	Positive	422	135.1	53
Fenpyroximate	8.92	Positive	422	366.1	23
Fenuron	3.8	Positive	165.1	46	29
Fenuron	3.8	Positive	165.1	72.1	45
Fipronil	7.7	Negative	435	250	35
Fipronil	7.7	Negative	435	330	20

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Table 2D. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Fluazinam	8.63	Positive	465	91	43
Fluazinam	8.63	Positive	465	148.9	43
Flubendiamide	7.63	Positive	683.1	274.1	41
Flubendiamide	7.63	Positive	683.1	408	9
Flufenacet	7.42	Positive	364.1	152.1	27
Flufenacet	7.42	Positive	364.1	194.2	17
Fluometuron	5.95	Positive	233.1	46	37
Fluometuron	5.95	Positive	233.1	72.1	35
Fluoxastrobin	7.46	Positive	459.2	188	47
Fluoxastrobin	7.46	Positive	459.2	427.2	23
Flusilazole	7.34	Positive	316.1	165.1	37
Flusilazole	7.34	Positive	316.1	247.1	21
Flutolanil	7.38	Positive	324.1	242.1	35
Flutolanil	7.38	Positive	324.1	262.1	31
Forchlorfenuron	6.05	Positive	248	93.1	49
Forchlorfenuron	6.05	Positive	248	129.1	25
Formetanate HCl	1.05	Positive	222.1	120	37
Formetanate HCl	1.05	Positive	222.1	165	23
Fuberidazole	2.5	Positive	185	65	57
Fuberidazole	2.5	Positive	185	157	37
Furalaxyl	6.76	Positive	302.1	95	39
Furalaxyl	6.76	Positive	302.1	242.1	21
Furathiocarb	8.43	Positive	383.1	195.1	25
Furathiocarb	8.43	Positive	383.1	252.1	17
Hexaconazole	7.44	Positive	314.1	70	55
Hexaconazole	7.44	Positive	314.1	159	33
Hexafluomuron	7.49	Positive	461.1	141.1	57
Hexafluomuron	7.49	Positive	461.1	158.2	23
Hexythiazox	8.67	Positive	353.1	168	37
Hexythiazox	8.67	Positive	353.1	228	19
Imidacloprid	4.09	Positive	256	175.1	25
Imidacloprid	4.09	Positive	256	209.1	21
Indoxacarb	8.15	Positive	528	203	47
Indoxacarb	8.15	Positive	528	218	35
Ipconazole	7.68	Positive	334.2	70	37
Ipconazole	7.68	Positive	334.2	125	47
Iprovalicarb	6.83	Positive	321.2	119	47
Iprovalicarb	6.83	Positive	321.2	203.1	13
Isoprocarb	6.2	Positive	194.1	95	21
Isoprocarb	6.2	Positive	194.1	137	13
Isoproturon	6.14	Positive	207.2	46.1	35
Isoproturon	6.14	Positive	207.2	72.1	29

Continued on next page

Table 2E. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Ivermectin	9.78	Positive	892.6	307.3	31
Ivermectin	9.78	Positive	892.6	569.5	19
Linuron	6.85	Positive	249.1	160	25
Linuron	6.85	Positive	249.1	182.1	21
(Monceren) Pencycuron	7.95	Positive	329.1	125	31
(Monceren) Pencycuron	7.95	Positive	329.1	218.1	23
Mandipropamid	7.08	Positive	412.1	328.1	19
Mandipropamid	7.08	Positive	412.1	356.1	15
Mefenacet	7.06	Positive	299	120.1	35
Mefenacet	7.06	Positive	299	148.1	21
Mepanipyrim	7.36	Positive	224	77	55
Mepanipyrim	7.36	Positive	224	106	35
Mepronil	7.26	Positive	270.1	119.1	31
Mepronil	7.26	Positive	270.1	228	21
Metalaxyl	6.02	Positive	280.1	192.2	25
Metalaxyl	6.02	Positive	280.1	220.2	19
Metconazole	7.51	Positive	320.1	70	43
Metconazole	7.51	Positive	320.1	125	53
Methabenzthiazuron	5.98	Positive	222.1	150.3	45
Methabenzthiazuron	5.98	Positive	222.1	165.2	21
Methamidophos	0.63	Positive	142	94	19
Methamidophos	0.63	Positive	142	125	19
Methiocarb	6.71	Positive	226.1	121.1	27
Methiocarb	6.71	Positive	226.1	169.1	13
Methoprotryne	5.72	Positive	272.2	198	31
Methoprotryne	5.72	Positive	272.2	240.2	27
Methoxyfenozide	7.25	Positive	369.1	149.1	21
Methoxyfenozide	7.25	Positive	369.1	313.2	11
Metobromuron	6.28	Positive	259	148.2	21
Metobromuron	6.28	Positive	259	170.2	25
Metribuzin	5.28	Positive	215.1	84.1	31
Metribuzin	5.28	Positive	215.1	187.1	25
Mevinphos Isomer	3.91	Positive	225.101	127.1	21
Mevinphos Isomer	3.91	Positive	225.101	193.2	11
Mevinphos Isomer_1	4.55	Positive	225.102	127.1	21
Mevinphos Isomer_1	4.55	Positive	225.102	193.2	11
Mexacarbate	1.88	Positive	223.2	151	31
Mexacarbate	1.88	Positive	223.2	159.1	21
Monocrotophos	1.94	Positive	224.1	98	17
Monocrotophos	1.94	Positive	224.1	127.1	21
Monolinuron	6.07	Positive	215.1	99	47

Continued on next page

Table 2F. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Monolinuron	6.07	Positive	215.1	126.1	23
Moxidectin	9.74	Positive	640.4	498.5	17
Moxidectin	9.74	Positive	640.4	528.5	13
Myclobutanil	6.99	Positive	289	70	41
Myclobutanil	6.99	Positive	289	125	39
Nitenpyram	2.22	Positive	271	126	35
Nitenpyram	2.22	Positive	271	225.2	17
Nuarimol	6.72	Positive	315	81	49
Nuarimol	6.72	Positive	315	251.1	37
Omethoate	0.85	Positive	214	124.9	31
Omethoate	0.85	Positive	214	182.8	17
Oxadixyl	5.26	Positive	279.1	132.1	43
Oxadixyl	5.26	Positive	279.1	219.1	15
Paclobutrazol	6.65	Positive	294	70	49
Paclobutrazol	6.65	Positive	294	125	41
Penconazole	7.57	Positive	284.1	70	37
Penconazole	7.57	Positive	284.1	159	35
Phenmedipham	6.72	Positive	301.2	107.9	44
Phenmedipham	6.72	Positive	301.2	168	12
Picoxystrobin	7.62	Positive	368	145	29
Picoxystrobin	7.62	Positive	368	205	13
Piperonyl butoxide	8.37	Positive	356.2	119.1	47
Piperonyl butoxide	8.37	Positive	356.2	177.2	13
Pirimicarb	3.89	Positive	239.2	72.1	33
Pirimicarb	3.89	Positive	239.2	182.1	21
Prochloraz	7.56	Positive	376	70	43
Prochloraz	7.56	Positive	376	308	15
Promecarb	6.85	Positive	208.1	109	21
Promecarb	6.85	Positive	208.1	151	13
Prometon	5.32	Positive	226.1	86	39
Prometon	5.32	Positive	226.1	142	33
Prometryne	6.41	Positive	242.2	158.1	33
Prometryne	6.41	Positive	242.2	200.1	25
Propamocarb	1.13	Positive	189.2	102	25
Propamocarb	1.13	Positive	189.2	144	19
Propargite	8.91	Positive	368.2	175.1	23
Propargite	8.91	Positive	368.2	231.1	15
Propham	6.08	Positive	180.1	120	23
Propham	6.08	Positive	180.1	138	11
Propiconazole	7.65	Positive	342.1	69	39
Propiconazole	7.65	Positive	342.1	159	31
Propoxur	5.54	Positive	210.1	111	19

Continued on next page

Table 2G. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Propoxur	5.54	Positive	210.1	168.1	11
Pyracarbolid	5.78	Positive	218.1	97	37
Pyracarbolid	5.78	Positive	218.1	125	25
Pyraclostrobin	7.83	Positive	388	163	31
Pyraclostrobin	7.83	Positive	388	194	17
Pyridaben	9.23	Positive	365	147	33
Pyridaben	9.23	Positive	365	309	19
Pyrimethanil	6.24	Positive	200	82	37
Pyrimethanil	6.24	Positive	200	107	33
Pyriproxyfen	8.57	Positive	322	96	21
Pyriproxyfen	8.57	Positive	322	185	31
Quinoxyfen	8.91	Positive	308.1	162.1	63
Quinoxyfen	8.91	Positive	308.1	197.1	45
Rotenone	7.42	Positive	395.1	192.1	33
Rotenone	7.42	Positive	395.1	213.1	31
Secbumeton	5.38	Positive	226.2	100	37
Secbumeton	5.38	Positive	226.2	170.1	25
Siduron	6.61	Positive	233.3	94	31
Siduron	6.61	Positive	233.3	137.2	23
Simetryn	5.04	Positive	214	124	29
Simetryn	5.04	Positive	214	144	29
Spinetoram	8.44	Positive	748.5	98.1	65
Spinetoram	8.44	Positive	748.5	142.2	43
Spinosad (Spinosyn A)	7.85	Positive	732.5	98.1	65
Spinosad (Spinosyn A)	7.85	Positive	732.5	142.2	39
Spinosad (Spinosyn D)	8.24	Positive	746.8	98.3	65
Spinosad (Spinosyn D)	8.24	Positive	746.8	142.4	41
Spirodiclofen	9.91	Positive	411.3	71.3	31
Spirodiclofen	9.91	Positive	411.3	313.3	17
Spiromesifen	9.21	Positive	371.2	255.2	31
Spiromesifen	9.21	Positive	371.2	273.2	11
Spirotetramat	6.8	Positive	374.2	302.2	27
Spirotetramat	6.8	Positive	374.2	330.2	23
Spiroxamine	7.3	Positive	298.2	100.1	43
Spiroxamine	7.3	Positive	298.2	144.2	29
Sulfentrazone	5.93	Positive	387	146	59
Sulfentrazone	5.93	Positive	387	307.1	29
Tebufenozide	7.59	Positive	353.2	133	23
Tebufenozide	7.59	Positive	353.2	297.2	11
Tebufenpyrad	8.28	Positive	334	117	47
Tebufenpyrad	8.28	Positive	334	145	37
Tebuthiuron	5.05	Positive	229.1	116.1	37

Continued on next page

Table 2H. List of pesticides with MRM transitions used (continued from previous page)

Compound	RT (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	CE (V)
Tebuthiuron	5.05	Positive	229.1	172.4	25
Terbumeton	5.38	Positive	226.1	100	41
Terbumeton	5.38	Positive	226.1	170.1	23
Terbutryn	6.51	Positive	242.1	68.1	61
Terbutryn	6.51	Positive	242.1	186.1	25
Tetraconazole	7.18	Positive	372.1	70	47
Tetraconazole	7.18	Positive	372.1	159	35
Thiabendazole	2.41	Positive	202.1	131.2	45
Thiabendazole	2.41	Positive	202.1	175.1	35
Thiacloprid	5.03	Positive	253	99	59
Thiacloprid	5.03	Positive	253	126	29
Thiamethoxam	2.86	Positive	292	181	31
Thiamethoxam	2.86	Positive	292	211	17
Thidiazuron	5.28	Positive	221.1	102.1	23
Thidiazuron	5.28	Positive	221.1	127.9	23
Thiophanate-methyl	5.5	Positive	343	151.1	31
Thiophanate-methyl	5.5	Positive	343	311	17
Triadimefon	7.06	Positive	294	197.1	21
Triadimefon	7.06	Positive	294	225	19
Triadimenol	6.65	Positive	296.1	70	33
Triadimenol	6.65	Positive	296.1	227.1	17
Trichlorfon	5.03	Positive	256.9	109.1	25
Trichlorfon	5.03	Positive	256.9	127	23
Tricyclazole	5	Positive	190	136	39
Tricyclazole	5	Positive	190	163	33
Trifloxystrobin	8.2	Positive	409	186	21
Trifloxystrobin	8.2	Positive	409	206	19
Triflumizole	7.85	Positive	346.1	73	27
Triflumizole	7.85	Positive	346.1	278.1	17
Vamidothion	3.86	Positive	288	118	37
Vamidothion	3.86	Positive	288	146	17

Table 3A. Method validation data (Linearity, LOQ, % recovery, and precision)

Sr. No.	Compound	R^2	LOQ (mg/kg)	0.010 mg/kg		0.050 mg/kg	
				% Rec	%RSD	% Rec	%RSD
1	3-Hydroxycarbofuran	0.9992	0.01	88	4.79	89	3.32
2	Acephate	0.9994	0.01	101	11.66	93	7.89
3	Acetamiprid	0.9993	0.01	95	1.55	90	2.15
4	Aldicarb sulfone	0.9991	0.01	100	8.59	93	6.41
5	Aldicarb sulfoxide	0.9933	0.05	ND	ND	101	3.76
6	Ametryn	0.9984	0.01	103	5.59	101	7.63
7	Aminocarb	0.9993	0.01	119	3.28	118	3.4
8	Amitraz	0.9994	0.01	108	10.71	102	15.01
9	Avermectin B1a	0.9947	0.01	89	17.09	112	11.89
10	Azoxystrobin	0.9991	0.01	71	19.1	76	7.71
11	Benalaxyl	0.9992	0.01	92	19.04	99	7.54
12	Bendiocarb	0.9991	0.01	96	14.82	101	5.34
13	Benzoximate	0.9978	0.01	77	13.86	110	19.14
14	Bifenazate	0.9986	0.01	91	17.7	107	5.12
15	Bitertanol	0.9991	0.01	74	16.95	84	3.71
16	Boscalid	0.9925	0.05	ND	ND	100	8.34
17	Bromucanozole Isomer 1	0.9973	0.01	74	11.04	90	11.32
18	Bromucanozole Isomer 2	0.9946	0.01	87	9.06	86	18.16
19	Bupirimate	0.9996	0.01	82	8.52	95	3.47
20	Buprofezin	0.9933	0.01	73	17.16	119	11.56
21	Butafenacil	0.9941	0.01	89	15.07	105	15.96
22	Butoxycarboxim	0.9997	0.01	92	1.16	94	1.59
23	Carbendazim	0.9968	0.01	164	2.77	244	1.4
24	Carbetamide	0.9992	0.01	87	8.51	109	6.07
25	Carbofuran	0.9989	0.01	83	4.49	83	6.53
26	Carboxin	0.9962	0.01	94	8.94	85	3.51
27	Chlorantraniliprole	0.9967	0.01	118	14.16	90	9.13
28	Chlorotoluron	0.9918	0.01	93	8.24	85	7.27
29	Chloroxuron	0.9939	0.01	91	15.79	97	9.22
30	Clethodim	0.9942	0.01	84	7.72	81	12.66
31	Clothianidin	0.9989	0.01	78	17.93	92	5.82
32	Cyazofamid	0.9953	0.01	82	16.75	92	14.09
33	Cycluron	0.9986	0.01	80	5.29	85	3.49
34	Cyproconazole Isomer 1	0.999	0.01	90	13.56	95	5.41
35	Cyproconazole Isomer 2	0.999	0.01	88	6.94	95	5.29
36	Cyprodinil	0.9977	0.01	84	12.94	81	9.31
37	Desmedipham	0.9943	0.01	78	6.67	79	17.01
38	Diclobutrazol	0.9942	0.01	85	11.09	79	13.62
39	Dicrotophos	0.9971	0.01	100	18.15	95	16.84
40	Diethofencarb	0.9997	0.05	ND	ND	95	19.61

Continued on next page. Those analytes without MRL values were considered as 0.01 mg/kg (default MRL set at LOQ

Table 3B. Method validation data (Linearity, LOQ, % recovery, and precision; continued from previous page)

Sr. No.	Compound	R^2	LOQ (mg/kg)	0.010 mg/kg		0.050 mg/kg	
				% Rec	%RSD	% Rec	%RSD
41	Difenoconazole	0.9981	0.01	117	10.97	101	7.3
42	Dimethoate	0.9991	0.01	87	3.12	84	1.34
43	Dimethomorph Isomer 1	0.9975	0.01	77	11.99	82	15.07
44	Dimethomorph Isomer 2	0.9986	0.01	80	12.26	84	12.55
45	Dimoxystrobin	0.9958	0.01	103	15.13	113	10.33
46	Diniconazole	0.9917	0.01	110	14.11	85	11.01
47	Dinotefuran	0.9981	0.01	99	19.28	91	4.24
48	Dioxacarb	0.9982	0.01	91	5.73	83	5.43
49	Diuron	0.9979	0.01	99	14.7	85	8.01
50	Doramectin	0.9975	0.01	117	8.69	117	7.9
51	Emamectin-benzoate b1a	0.9942	0.01	86	9.43	79	11.45
52	Epoxiconazole	0.9956	0.01	109	17.8	102	8.28
53	Eprinomectin	0.9923	0.01	120	14.45	117	7.75
54	Etaconazole	0.9973	0.01	95	9.16	108	4.98
55	Ethirimol	0.9987	0.01	77	10.19	87	4.37
56	Etoxazole	0.9977	0.01	94	10.05	97	10.01
57	Fenamidone	0.9936	0.01	97	13.4	94	19.98
58	Fenarimol	0.9948	0.01	70	11.45	106	17.26
59	Fenazaquin	0.9967	0.01	75	7.99	85	12
60	Fenbuconazole	0.9941	0.01	101	15.97	100	14.81
61	Fenpropimorph	0.9913	0.01	95	16.68	71	4.21
62	Fenpyroximate	0.9982	0.01	75	6.53	89	9.19
63	Fenuron	0.9987	0.01	86	6.92	88	2.66
64	Fipronil	0.9949	0.0025	89	17.37	105	17.8
65	Fluazinam†	0.9973	0.01	115	14.08	116	12.36
66	Flubendiamide	0.9959	0.01	112	18.06	120	3.34
67	Flufenacet	0.9999	0.01	115	4.67	119	2.93
68	Fluometuron	0.9942	0.01	94	8.56	79	6.77
69	Fluoxastrobin	0.9999	0.01	96	13.56	99	14.47
70	Flusilazole	0.9975	0.01	114	14.2	102	19.46
71	Flutolanil	0.9944	0.01	77	19.85	114	14.81
72	Forchlorfenuron	0.9975	0.01	90	11.16	71	10.03
73	Formetanate HCl	0.9985	0.01	106	4.94	110	2.49
74	Fuberidazole	0.999	0.01	91	1.62	91	1.64
75	Furalaxyl	0.9992	0.01	97	17.52	119	4.19
76	Furathiocarb	0.9954	0.01	79	6.2	88	10.4
77	Hexafluomuron	0.9938	0.05	ND	ND	108	12.19
78	Hexaconazole	0.9947	0.01	91	12.82	74	12.29
79	Hexythiazox	0.9947	0.01	83	11.82	83.71	19.71
80	Imidacloprid	0.9993	0.01	114	6.07	110	4.92

Continued on next page. Those analytes without MRL values were considered as 0.01 mg/kg (default MRL set at LOQ

Table 3C. Method validation data (Linearity, LOQ, % recovery, and precision; continued from previous page)

Sr. No.	Compound	R^2	LOQ (mg/kg)	0.010 mg/kg		0.050 mg/kg	
				% Rec	%RSD	% Rec	%RSD
81	Indoxacarb	0.9969	0.01	116	14.52	105	17.95
82	Ipconazole	0.9955	0.01	98	11.5	114	16.95
83	Iprovalicarb	0.995	0.01	111	12.8	119	6.99
84	Isoprocarb	0.9928	0.01	89	18.67	120	12.11
85	Isoproturon	0.9974	0.01	101	5.71	93	9.63
86	Ivermectin	0.9985	0.01	119	8.66	110	5.24
87	Linuron	0.9925	0.01	83	12.62	79	7.65
88	(Monceren) Pencycuron	0.9999	0.01	113	10.71	110	1.37
89	Mandipropamid	0.9975	0.01	103	18.35	120	19.38
90	Mefenacet	0.9927	0.01	117	14.89	118	1.63
91	Mepanipyrim	0.9936	0.01	86	16.23	82	16.03
92	Mepronil	0.9966	0.01	91	13.52	81	11.19
93	Metalaxyl	0.9968	0.01	106	5.86	120	11.61
94	Metconazole	0.996	0.01	93	10.18	99	11.55
95	Methabenzthiazuron	0.9971	0.01	97	4.09	104	4.88
96	Methamidophos	0.9944	0.01	97	5.29	90	3.93
97	Methiocarb	0.9934	0.01	117	7.97	79	5.65
98	Methoprotryne	0.9992	0.01	93	8.69	118	2.67
99	Methoxyfenozide	0.9905	0.01	110	14.66	117	16.96
100	Metobromuron	0.9936	0.01	101	10.46	116	8.2
101	Metribuzin	0.9951	0.01	100	5.99	83	7.54
102	Mevinphos Isomer 1	0.9982	0.01	90	7.47	87	4.37
103	Mevinphos Isomer 2	0.9988	0.01	85	8.44	88	6.16
104	Mexacarbate	0.9997	0.01	91	1.61	93	1.65
105	Monocrotophos	0.9985	0.01	118	9.42	118	2.11
106	Monolinuron	0.9979	0.01	98	9.65	107	4.73
107	Moxidectin	0.9979	0.01	104	6.15	113	5.8
108	Myclobutanil	0.994	0.01	84	19.98	86	11.16
109	Nitenpyram	0.9924	0.01	119	7.91	120	3.44
110	Nuarimol	0.9978	0.05	ND	ND	19.45	94.54
111	Omethoate	0.9951	0.01	114	5.1	114	3.87
112	Oxadixyl	0.9985	0.01	86	3.68	98	5.56
113	Paclobutrazol	0.9954	0.01	96	17.77	76	3.61
114	Penconazole	0.9975	0.01	83	11.76	76	15.87
115	Phenmedipham	0.9918	0.01	98	16.74	106	5.52
116	Picoxystrobin	0.9962	0.01	91	15.02	88	8.86
117	Piperonyl butoxide	0.9951	0.01	99	14.39	107	5.99
118	Pirimicarb	0.9993	0.01	89	1.79	94	1.69
119	Prochloraz	0.9969	0.01	95	7.55	92	5.94
120	Promecarb	0.9938	0.01	87	4.8	88	3.13

Continued on next page. Those analytes without MRL values were considered as 0.01 mg/kg (default MRL set at LOQ

Table 3D. Method validation data (Linearity, LOQ, % recovery, and precision; continued from previous page)

Sr. No.	Compound	R^2	LOQ (mg/kg)	0.010 mg/kg		0.050 mg/kg	
				% Rec	%RSD	% Rec	%RSD
121	Prometon	0.9993	0.01	79	7.66	100	4.61
122	Prometryne	0.9991	0.01	94	3.97	98	1.9
123	Propamocarb	0.9986	0.01	86	4.91	82	3.17
124	Propargite	0.9958	0.05	ND	ND	93	15.98
125	Propiconazole	0.9947	0.01	110	16.5	94	15.46
126	Propoxur	0.992	0.05	ND	ND	96	7.65
127	Pyracarbolid	0.9957	0.01	76	7	81	4.02
128	Pyraclostrobin	0.9972	0.01	107	13.18	113	18.22
129	Pyridaben	0.9978	0.01	79	12.33	93	7.27
130	Pyrimethanil	0.9985	0.01	80	6.44	86	5.28
131	Pyriproxyfen	0.9931	0.01	76	11.98	84	11.86
132	Quinoxyfen	0.9973	0.01	84	15.61	94	6.53
133	Rotenone	0.9978	0.01	104	18.1	88	18.8
134	Secbumeton	0.999	0.01	87	5.41	90	2.16
135	Siduron	0.9938	0.01	101	8.39	94	10.02
136	Simetryn	0.9996	0.01	93	10.76	98	5
137	Spinetoram	0.996	0.01	85	9.39	90	8.9
138	Spinosad (Spinosyn A)	0.9963	0.01	92	11.51	102	15.45
139	Spinosad (Spinosyn D)	0.996	0.01	104	10.32	104	5.26
140	Spirodiclofen	0.9926	0.01	104	10.33	107	7.48
141	Spiromesifen	0.9968	0.01	104	10.33	99	6.15
142	Spirotetramat	0.9961	0.01	91	10.04	102	14.87
143	Spiroxamine	0.9983	0.01	88	4.19	94	1.1
144	Tebufenozide	0.9924	0.01	89	16.12	100	17.95
145	Tebufenpyrad	0.9929	0.01	80	13.74	98	10.26
146	Tebuthiuron	0.9968	0.01	83	8.23	120	3.29
147	Terbumeton	0.9991	0.01	86	4.69	93	1.65
148	Terbutryn	0.9994	0.01	87	2.87	99	2.52
149	Tetraconazole	0.9954	0.01	100	9.28	93	8.94
150	Thiabendazole	0.9996	0.01	91	3.52	88	0.56
151	Thiacloprid	0.9984	0.01	76	5.05	106	9.01
152	Thiamethoxam	0.9994	0.01	94	4.3	95	5.22
153	Thidiazuron	0.9973	0.01	101	10.26	74	12.76
154	Thiophanate-methyl	0.9972	0.01	91	7.15	91	6.27
155	Triadimefon	0.9958	0.01	73	14.39	90	16.51
156	Triadimenol	0.9952	0.01	101	6.91	78	6.77
157	Tricyclazole	0.9993	0.01	101	15.27	113	15.56
158	Trifloxystrobin	0.9947	0.01	77	15.52	87	16.37
159	Triflumizole	0.9942	0.01	86	7.8	82	7.1
160	Vamidothion	0.9994	0.01	87	3.51	86	2.68

Those analytes without MRL values were considered as 0.01 mg/kg (default MRL set at LOQ

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