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A simple and robust method for trace level quantitation of pesticide residues in wheat grain using LC-MS/MS

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Keywords

TraceFinder, pesticide residues, wheat grain, QuEChERS, LC-MS/MS, TSQ Quantis

Goal

The objective is to deliver a total solution for trace level quantitation of 145 pesticide residues in wheat grain by using liquid chromatography-tandem mass spectrometry. The optimized method was validated as per SANTE guidelines and evaluated for the fulfillment of the Food Safety and Standards Authority of India (FSSAI) as well as the European Commission (EC) MRLs compliance in wheat grain.

Introduction

Cerials have high nutritional and economic importance, occupying more than 60% of total worldwide crops. Meanwhile, wheat covers more of the lands destined for agriculture than any other crops. It is a valuable source of nutrients, vitamins, minerals, and complex carbohydrates. However, cereals may be a significant source of daily pesticide exposure. Pesticides are widely used in the control or prevention of weeds and crop diseases. In particular, insecticides, fungicides, herbicides, and plant growth regulators are spread on wheat plantations.[1] However, residues that remain may be harmful to human health and the environment. The European Commission (EC) and

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FSSAI have established maximum residue levels (MRLs) for pesticides in wheat grain, and the minimum values are 0.01 mg/kg except for fipronil and fipronil sulfone (0.005 mg/kg).[2,3] The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method has been adopted for pesticides residue extraction in most of the food samples.[4] In addition, the instrument method plays an important role in delivering accurate, precise, and rugged results to meet the regulatory requirements.

The objective of this work was to set up an extraction and analysis method followed by method validation of a multi-residue method for pesticides in wheat grain by using the Thermo Scientific™ TSQ Quantis™ LC-MS/MS system. The data acquisition and processing were 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 Scientific™
- Methanol, Optima™ LC/MS Grade, Fisher Scientific™
- Water, Optima™ LC/MS Grade, Fisher Scientific™
- Formic acid (85%), Fisher Scientific™
- Acetic acid (100%), Fisher Scientific™
- Ammonium formate, LC/MS Grade, Fisher Scientific™
- Anhydrous magnesium sulfate, Fisher Scientific™
- Sodium acetate, LR Grade, Fisher Scientific™
- Certified reference materials (CRMs), procured from Restek™
- 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™ UHPLC system was coupled with the TSQ Quantis triple quadrupole mass spectrometer, which included the Heated Electrospray Ionization (HESI) source. The specific optimized compound-independent LC-MS/MS conditions are given in Table 1. The compound-dependent parameters, like product ions (transitions), collision energy (CE), and retention time (RT, min), are given in Table 2 (Appendix).

Table 1A. LC-MS/MS instrument conditions

Liquid chromatography method				
Instrumentation:	Vanquish 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) 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*	*Flow Rate*	*%B*	*Curve*
	0.000	0.400	1	5
	1.500	0.400	1	5
	5.000	0.400	50	5
	8.500	0.400	95	5
	13.500	0.400	95	5
	14.000	0.400	1	5
	18.000	0.400	1	5

Table 1B. LC-MS/MS instrument conditions

Mass spectrometry method	
Instrumentation:	TSQ Quantis triple quadrupole tandem mass spectrometer
Method type:	Time-based selective-reaction monitoring (t-SRM)
Ion source type:	HESI
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 wheat grains were purchased from the local market and homogenized by using a heavy duty grinder to reduce the particle size to approximately 200 to 500 µm. The QuEChERS method[4] was used for extraction as below without cleanup.

Sample extraction:

- Weigh 5 g sample into a 50 mL extraction tube.
- Spike recovery samples before addition of extraction solvent.
- Add 15 mL of HPLC grade water (containing 1% acetic acid) and leave the sample for 10 min soaking.
- Add 15 mL acetonitrile to the tube.
- Shake vigorously for 1 min 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 min on a vortex mixer at 2500 rpm.
- Centrifuge with 5000 rpm for 5 min at ambient conditions.
- Filter the extract through a syringe filter and dilute with water (50:50).
- Inject into the LC-MS/MS.

Data acquisition and processing

The data acquisition and processing were carried out using Thermo Scientific™ TraceFinder™ software version 4.1. The data was acquired in time-based selective reaction monitoring mode (t-SRM mode), which includes two or more transitions per analyte taken from Thermo Scientific compound database. 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 criteria as per SANTE guidelines.[7]

Results and discussion

Sample preparation

Wheat grain is a dry powder and complex matrix, which has a high content of carbohydrate (72.57%) and proteins (13.70%). Wheat grain has close to neutral pH (6.0–6.8), so acidic pH was maintained by using 1% acetic acid in water. As a low water content matrix and for liquid-liquid partitioning, the aqueous phase should be a nearly equal portion, so 15 mL water was added to maintain moisture content. Acidification improved the stability of base-sensitive and organophosphorus compounds during extraction. The cleanup step has been avoided here, and the extract was diluted with water. This dilution approach offered a reduction in matrix effect without losing target analytes. The final extract was diluted (6x) as per the defined protocol. The final diluted extract (e.g. 0.01 mg/kg corresponds to 0.0016 mg/kg) was easily recognized by LC-MS/MS method. By taking advantage of advances LC-MS/MS system with high sensitivity, the dilution approach offered excellent sensitivity with good recoveries (70–120%) and precision (<20%) at 0.01 mg/kg which is default EU MRLs as well as reporting limit.

LC-MS/MS analysis

Total LC-MS/MS method conditions optimized for the pesticide residues analysis showed excellent sensitivity for 160 compounds. The total ion chromatogram (TIC) is shown in Figure 1 for 145 compounds. The optimized liquid chromatographic method offered excellent separation for the target analytes (mevinphos isomers are shown in Figure 2) and the absence of an isobaric interference from the matrix.

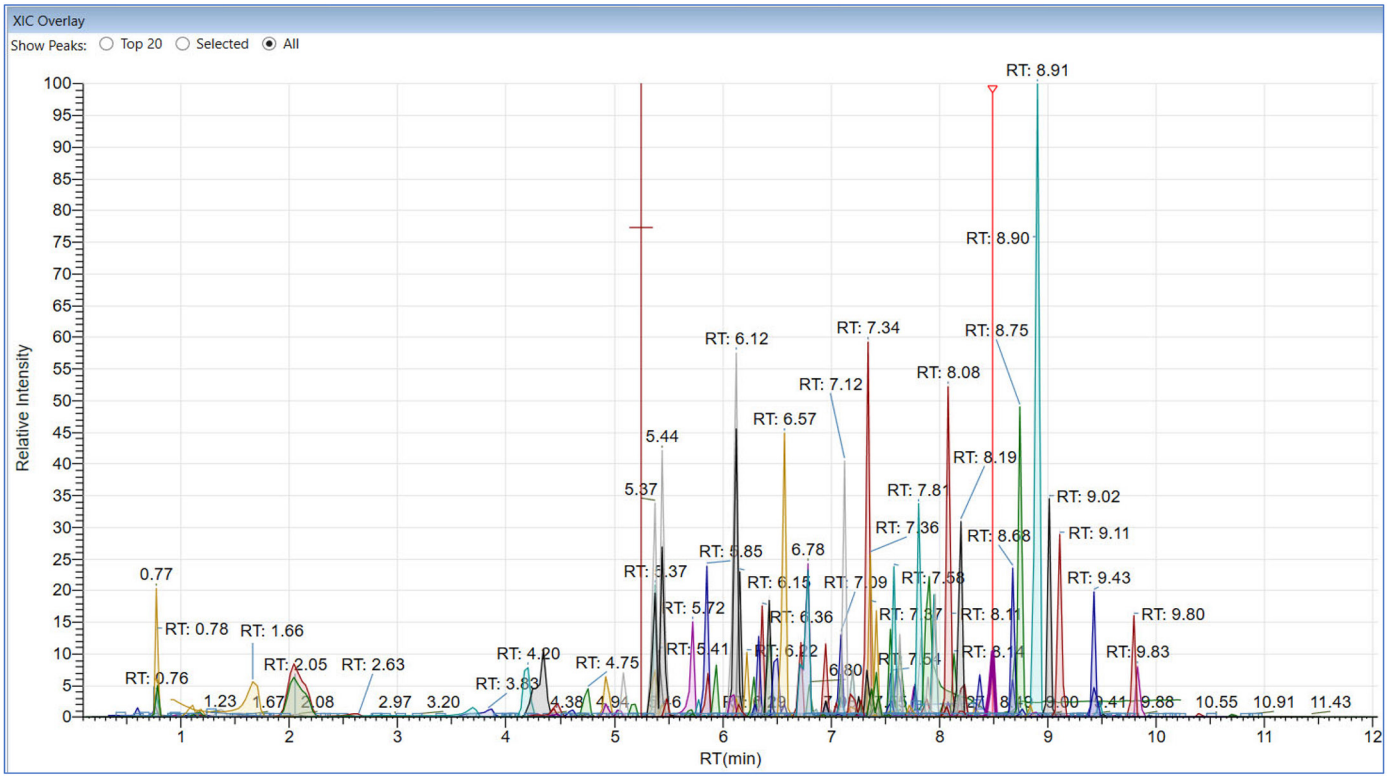

Figure 1. Total ion chromatograms with overlay extraction ions (145 compounds) in a single window

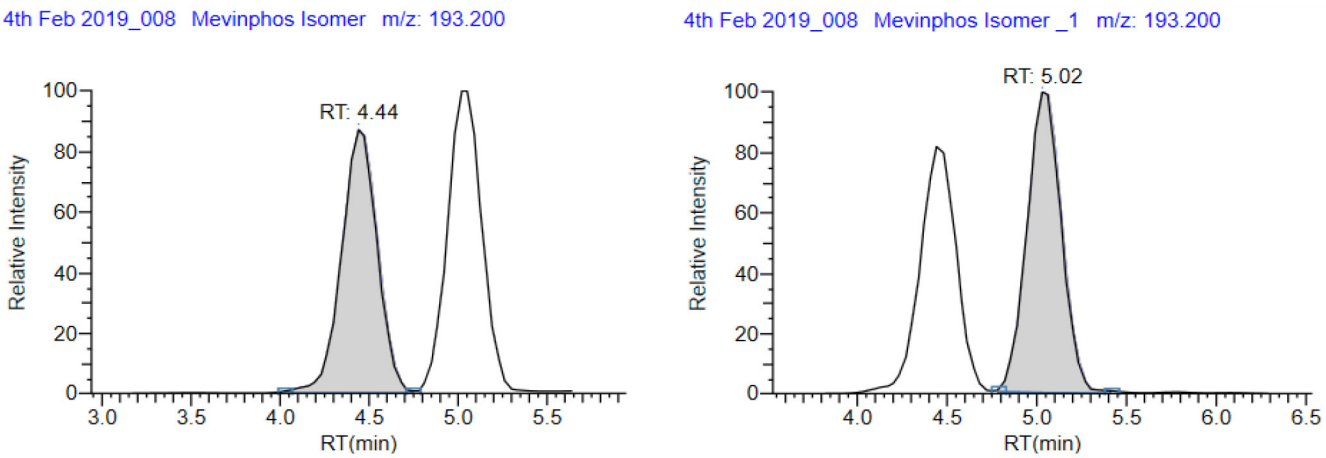

Figure 2. Chromatographic separation of mevinphos isomers

Auto-optimized dwell time (1-10 ms per transition) by software offered at least 12 points per peak. Early eluting compounds like omethoate provided more than 12 points per peak shown in Figure 3. These optimized instrument conditions provided excellent repeatability and reproducibility. The instrumental conditions used in this analysis offered excellent selectivity.

Identification and quantitation

Based on user-defined criteria, the data was processed in TraceFinder software with the flagging option. These color-coded flags indicated whether results passed or failed the acceptance criteria set in the processing method. The results passed user-defined criteria (SANTE guidelines) as shown by the green colored flags (Figure 3). In Figure 4, an identification of ametryn in wheat grain was demonstrated with two transitions 228.1→186.1 (quantitative) and 228.1→96.0 (confirmatory) at the same retention (5.85 min, ±0.1) with ion ratio of 17.23% (11.71–21.76%) observed in wheat grain in comparison with a neat standard. For the quantitative approach, the linearity provided correlation coefficient >0.999 with <15% residuals. This approach meets the requirement of SANTE guidelines for identification and quantitation.

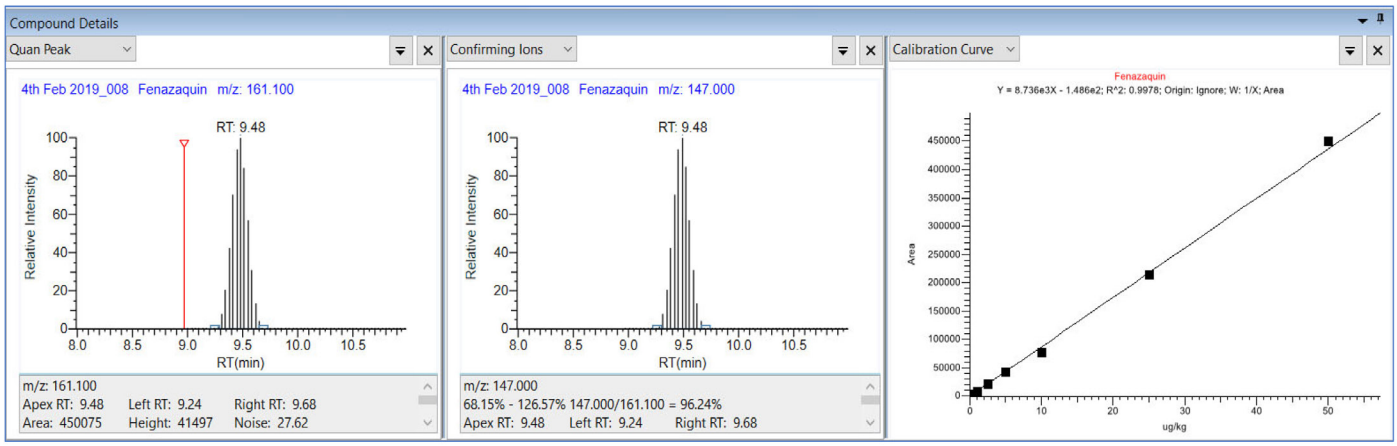

Figure 3. Impact of optimized dwell time on the data points per peak

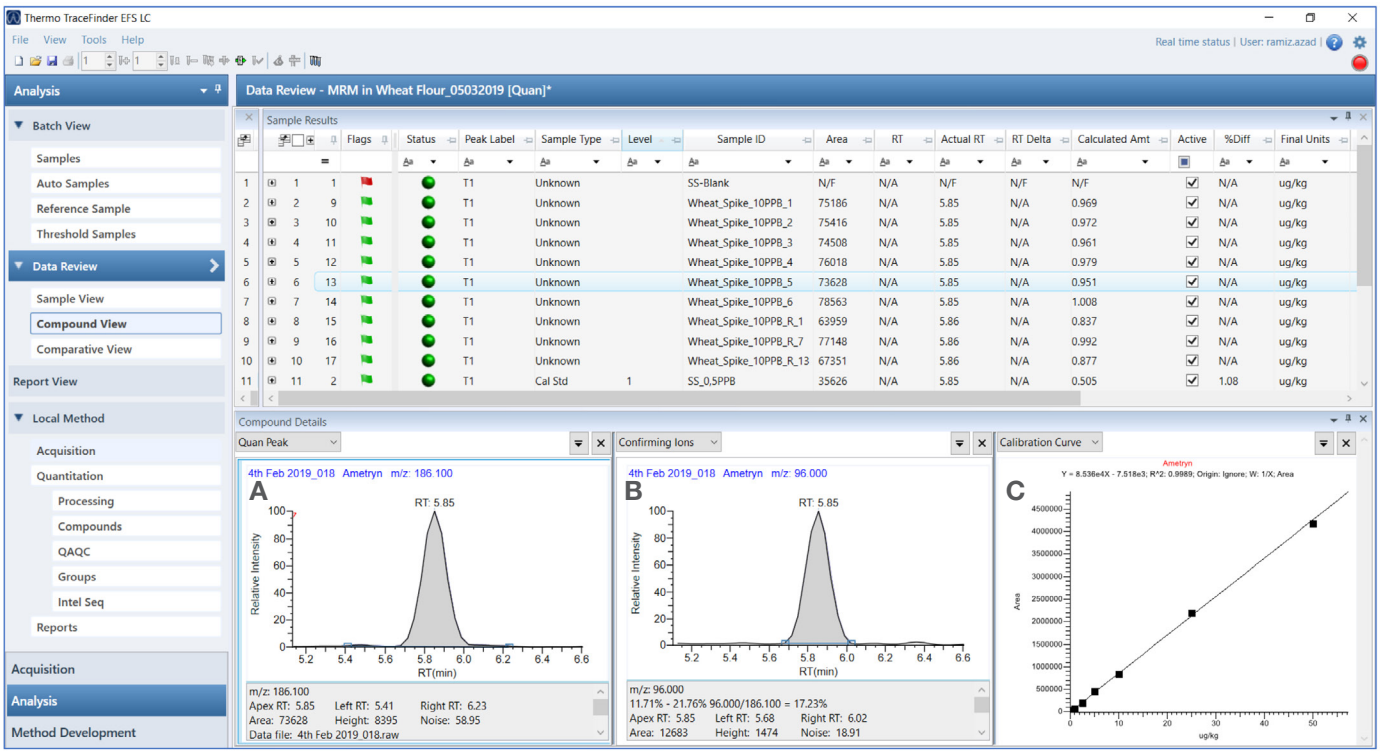

Figure 4. (A) Extracted ion chromatogram for quantifier ion of ametryn, (B) identification based on the selectivity of a confirmatory ion with ion ratio, and (C) calibration curve

Method performance

In this method, the linearity was plotted in the range of 0.0005 to 0.1 mg/kg. This range offered an excellent correlation coefficient (>0.99) with <20% residuals for all the target analytes in both solvents as well as in wheat grain matrix. Based on the lower calibration level (0.0005 mg/kg) showed good sensitivity with ≥15 signal-to-noise ratio. But as per the extraction protocol, the sample gets diluted (six times). Hence the limit of quantitation (LOQ) value observed in wheat grain matrix was 0.01 mg/kg with the acceptable recoveries (70–120%) and precision (<20%). The recovery experiment was carried out at by spiking wheat grain at 0.01 (LOQ) and 0.05 (LOQ × 5) mg/kg before the addition of extraction solvents to demonstrate the performance of the method in terms of accuracy and precision. An average recoveries were observed in the range of 76% to 116% with <15% RSD (Table 2, Appendix) for both the spiking levels, which were within acceptance criteria of SANTE guidelines.[5] Also, the optimized method was tested for repeatability of results obtained in a large batch (n=50 injection) by considering the commercial food testing lab schedule. The repeatability was <15% for the area and <±0.05 min retention time. This reveals that the optimized method offered excellent reproducibility in results. The repeatability in terms of area, retention time, and ion ratio are shown for a few compounds in Figures 5, 6, and 7, respectively.

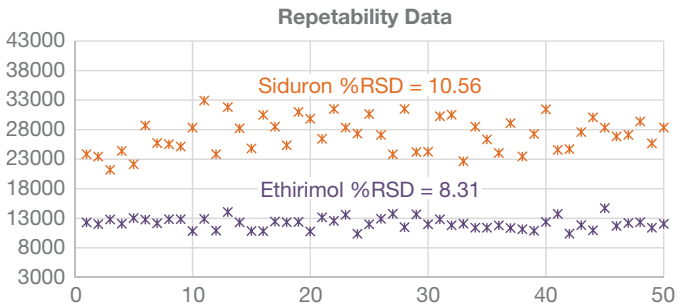

Figure 5. Area repeatability for ethirimol and siduron (n=50)

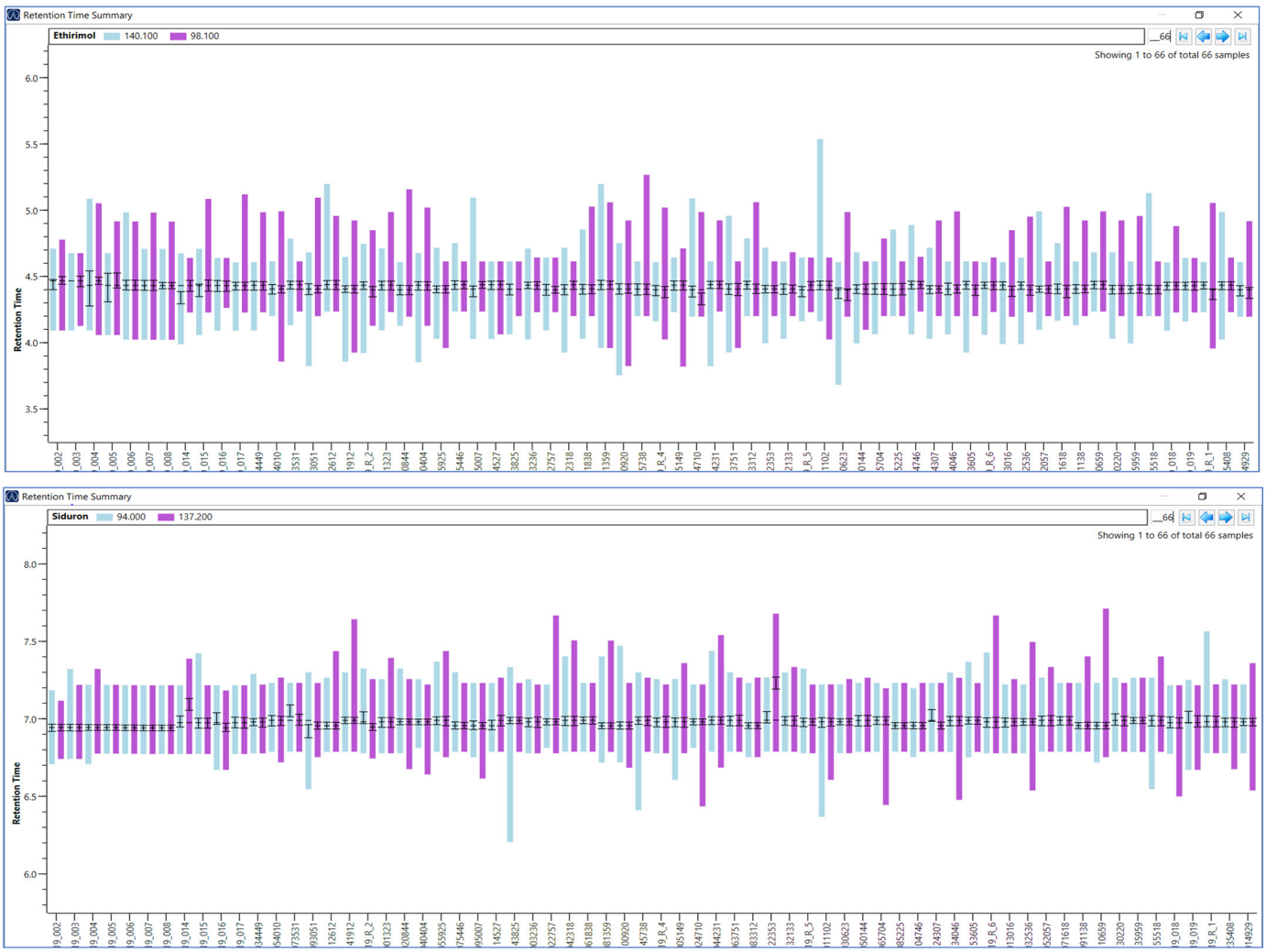

Figure 6. Retention time repeatability for ethirimol and siduron (n=66)

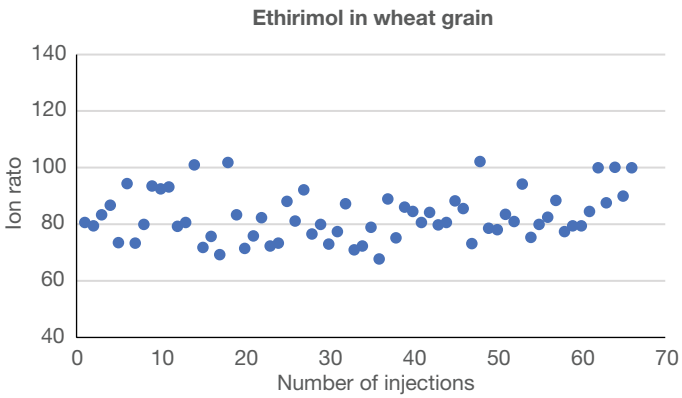

Figure 7. Ion ratio observed in replicate injections (n=66) in spiked wheat grain matrix

Conclusion

This application note offered a solution for a trace-level accurate quantitation of pesticide residues in wheat grain by using Thermo Scientific LC-HESI-MS/MS analysis. 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 day (24-hour 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 requirement of SANTE guidelines. Also, this method complies the EU and FSSAI MRL requirements by achieving an excellent LOQ.

References

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5. SANTE guidelines. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf

Appendix

Table 2 (part 1). Target list pesticides with their SRM transitions and validation data at 0.01 and 0.05 mg/kg in wheat

Compound	RT (min)	Q1 (*m/z*)	Q3* (*m/z*)	CE (V)	Q3** (*m/z*)	CE (V)	0.01 mg/kg		0.05 mg/kg		Ion ratio at 0.05 (mg/kg)	Ion ratio range
							% Rec	% RSD	% Rec	% RSD		
3-Hydroxycarbofuran	3.89	238.1	163	19	181	15	76	3.9	75	2.5	72.89	46.49–86.35
Acephate	4.17	184.1	49	35	143	11	78	11.8	80	8.4	1.53	1.07–2.00
Acetamiprid	4.51	223	99	53	126	29	80	2.9	81	1.8	11.33	6.96–12.92
Aldicarb sulfone	1.46	240.1	86.2	28	148.2	19	98	4.3	94	6.2	91.39	57.97–107.66
Aldicarb sulfoxide	0.98	207.1	89.1	19	132.1	9	87	5.7	96	7.3	124.55	87.19–161.92
Ametryn	5.75	228.1	96	35	186.1	25	72	1.3	84	3.6	14.90	11.71–21.76
Aminocarb	0.94	209.1	137.1	33	152	19	98	2.7	103	1.1	84.97	63.71–118.32
Amitraz	7.71	294.2	91.2	57	148.3	22	93	7.5	74	3.1	3.61	2.21–4.10
Azoxystrobin	6.96	404.1	344.1	33	372.1	19	71	5.4	83	9.3	5.17	3.74–6.95
Benalaxyl	7.72	326.2	148.1	29	294.1	15	71	2.8	70	6.4	24.75	18.91–35.11
Bendiocarb	5.98	224.1	109	33	167.1	15	84	10.3	114	3.4	0.56	0.36–0.68
Benzoximate	8.1	364	105	31	199	11	75	14.2	97	14.1	5.25	3.71–6.89
Bitertanol	7.35	338.2	70	29	269.2	13	80	14.1	85	6.9	93.17	62.37–115.82
Boscalid	7.07	343	140	25	307	27	87	10.5	73	5.3	4.84	4.47–8.30
Bromucanozole	7.04	378	70	47	159	37	95	9.8	114	10	53.68	45.66–84.80
Bupirimate	7.13	317	108	35	159.1	33	72	12.4	119	3.3	31.43	24.42–45.35
Buprofezin	8.28	306.2	116.2	23	201.1	17	101	16.6	118	10.2	24.20	13.33–24.75
Butafenacil	7.6	492.1	331	27	349	19	108	8.1	91	8.5	29.53	24.57–45.62
Butocarboxim	5.26	213.1	75	19	116	17	73	9.8	97	17	8.25	5.92–10.99
Butoxycarboxim	1.87	223.1	106	13	159	11	92	1.4	98	0.8	0.36	0.30–0.59

*Quantitation ion, **Confirmatory ion

Table 2 (part 2). Target list pesticides with their SRM transitions and validation data at 0.01 and 0.05 mg/kg in wheat

Compound	RT (min)	Q1 (*m/z*)	Q3* (*m/z*)	CE (V)	Q3** (*m/z*)	CE (V)	0.01 mg/kg		0.05 mg/kg		Ion ratio at 0.05 (mg/kg)	Ion ratio range
							% Rec	% RSD	% Rec	% RSD		
Carbendazim	1.64	192.2	132.1	41	160.2	25	74	5.5	85	1.8	8.99	6.34–11.78
Carbetamide	5.02	237.1	118.1	17	192	13	81	16.7	94	6	86.85	65.15–120.99
Carbofuran	5.98	222.1	123	29	165.1	17	72	4.8	82	3.2	33.68	18.14–33.70
Carboxin	5.98	236.1	87	33	143	21	118	3.7	88	5.3	8.28	4.86–9.03
Chlorantraniliprole	6.47	484	285.9	17	452.9	21	73	7.1	92	11.3	74.25	59.31–110.14
Chlorotoluron	5.97	213.1	46.2	35	72.2	31	104	2.3	120	3.7	11.54	8.82–16.38
Chloroxuron	6.99	291.1	72.4	47	218.1	33	78	7.4	108	8.3	17.56	12.59–23.39
Clethodim	8.27	360.101	164	29	268.1	17	78	12	74	5.7	21.45	11.83–21.97
Clothianidin	3.86	250	132	21	169	19	89	15	86	9.1	74.68	42.10–78.18
Cyazofamid	7.72	325.2	108	18	261.2	14	76	18.9	76	0.2	28.92	24.85–46.16
Cycluron	6.24	199.1	89	21	89.1	21	79	4.5	79	1.8	96.66	66.45–123.41
Cyproconazole Isomer	6.79	292	70	31	125	29	91	10.9	110	6.1	86.19	66.45–123.41
Cyprodinil	7.29	226	77	61	93	47	102	6.7	109	3.1	51.93	28.60–53.11
Cyromazine	0.8	167.1	85.1	26	125.1	24	98	8	91	4.3	25.66	14.30–26.55
Desmedipham	6.73	318.1	154	35	182	19	99	7.3	116	7	6.78	3.66–6.80
Dicrotophos	2.78	238.1	112.1	17	193	13	87	8.5	78	5.5	87.93	55.76–103.55
Diethofencarb	6.75	268.1	124	43	226.1	13	87	8.5	80	16.1	27.76	18.81–34.92
Difenoconazole	7.76	406.1	251.1	41	253.1	31	110	7.5	106	4.2	46.76	31.05–57.66
Dimethoate	4.15	230	125	29	199	13	82	4.3	78	2.9	60.67	40.66–75.51
Dimethomorph Isomer	6.38	388.1	165.1	45	301	29	71	4.8	81	6.1	44.17	28.85–53.58
Dimethomorph Isomer _1	6.6	388.101	165.1	45	301	29	71	0	94	3.3	49.44	33.81–62.80
Dimoxystrobin	7.41	327.1	116	29	205	23	76	3.2	71	7.3	7.44	4.52–8.39
Dioxacarb	4.11	224.1	123	21	167	11	88	7.5	78	7.2	74.32	44.01–81.74
Diuron	6.21	233.1	72	37	72.1	33	76	6	74	3.7	22.36	13.69–25.42
Emamectin-benzoate b1a	8.54	886.5	82.1	65	158.1	49	79	14.6	76	13.2	8.59	6.96–12.92
Epoxiconazole	7.1	330	101.1	65	121.1	49	100	16.2	91	9.7	59.59	37.18–69.05
Eprinomectin	8.65	914.6	154.2	49	186.2	25	94	18	82	7.8	27.87	18.25–33.89
Etaconazole	7.17	328.1	159	31	205	23	105	5.5	103	2.2	2.85	1.71–3.18
Ethirimol	4.2	210.2	98.1	39	140.1	31	83	3	75	3.4	76.74	56.25–104.47
Etoxazole	8.9	360.1	57.2	51	141	59	101	8.3	111	2.7	1.15	0.83–1.53
Fenamidone	6.96	312.1	92	35	236.1	21	70	10.1	114	4.2	38.77	27.03–50.20
Fenarimol	6.9	331	81	55	268	35	74	18.7	114	14.2	13.15	8.15–15.14
Fenazaquin	9.69	307.1	147	25	161.1	27	84	7.9	82	7.6	85.96	68.15–126.57
Fenbuconazole	7.31	337	70	39	124.9	55	72	11	93	8.2	65.94	36.82–68.38
Fenpropimorph	7.23	304	117	65	147	39	76	14.6	74	7.6	68.99	50.73–94.21
Fenpyroximate	8.92	422	135.1	53	366.1	23	95	1.7	90	1.2	0.38	0.21–0.40
Fenuron	3.8	165.1	46	29	72.1	45	89	1.4	85	5.2	83.34	66.25–123.04
Fipronil	7.7	435	250	35	330	20	109	7.4	87	1.8	30.69	20.43–37.94
Fluazinam	8.63	465	91	43	148.9	43	95	12.7	104	12.5	35.83	32.61–60.56
Flubendiamide	7.63	683.1	274.1	41	408	9	85	6.7	83	7.5	44.85	26.35–48.94
Fluometuron	5.95	233.1	46	37	72.1	35	84	9.8	85	4.1	10.61	7.15–13.28
Fluoxastrobin	7.46	459.2	188	47	427.2	23	79	9.1	70	6.9	31.65	25.34–47.07
Flusilazole	7.34	316.1	165.1	37	247.1	21	71	9.7	93	6.4	69.95	50.86–94.46
Flutolanil	7.38	324.1	242.1	35	262.1	31	73	7	81	14	14.52	8.23–15.28
Flutriafol	6.49	302.1	70.1	59	123	37	82	13	71	7.1	16.78	10.51–19.52
Forchlorfenuron	6.05	248	93.1	49	129.1	25	71	4.5	81	1.4	16.66	12.96–24.07
Formetanate HCl	1.05	222.1	120	37	165	23	115	4.7	106	3.2	8.99	5.06–9.39

*Quantitation ion, **Confirmatory ion

Table 2 (part 3). Target list pesticides with their SRM transitions and validation data at 0.01 and 0.05 mg/kg in wheat

Compound	RT (min)	Q1 (*m/z*)	Q3* (*m/z*)	CE (V)	Q3** (*m/z*)	CE (V)	0.01 mg/kg		0.05 mg/kg		Ion ratio at 0.05 (mg/kg)	Ion ratio range
							% Rec	% RSD	% Rec	% RSD		
Fuberidazole	2.5	185	65	57	157	37	85	10.7	73	2.8	79.34	67.44–125.24
Furalaxyl	6.76	302.1	95	39	242.1	21	94	6.3	70	3.1	112.20	93.94–174.46
Furathiocarb	8.43	383.1	195.1	25	252.1	17	96	3.2	106	3	45.80	33.92–63.00
Hexaconazole	7.44	314.1	70	55	159	33	98	9.5	110	11.9	111.35	68.64–127.48
Imazalil	6.82	297	159	29	201	25	89	6.2	112	7.8	17.11	13.80–25.64
Imidacloprid	4.09	256	175.1	25	209.1	21	72	16.2	88	3	75.30	45.42–84.35
Indoxacarb	8.15	528	203	47	218	35	90	19.9	84	13.3	19.48	19.41–36.04
Ipconazole	7.68	334.2	70	37	125	47	88	15.8	99	9.7	18.67	14.64–27.20
Iprovalicarb	6.83	321.2	119	47	203.1	13	98	7	90	9.9	68.61	48.76–90.56
Isoprocarb	6.2	194.1	95	21	137	13	95	13.1	77	9.9	15.69	10.07–18.69
Isoproturon	6.14	207.2	46.1	35	72.1	29	72	6.9	87	3.7	13.11	9.00–16.71
Ivermectin	9.78	892.6	307.3	31	569.5	19	82	13.4	79	6.3	72.36	43.20–80.22
Linuron	6.85	249.1	160	25	182.1	21	97	10.5	77	15.4	91.88	57.96–107.64
(Monceren) Pencycuron	7.95	329.1	125	31	218.1	23	94	14.8	118	18.6	0.44	0.33–0.62
Mandipropamid	7.08	412.1	328.1	19	356.1	15	83	3.9	72	10.1	25.18	20.26–37.62
Mefenacet	7.06	299	120.1	35	148.1	21	96	2.7	89	7.2	69.30	45.64–84.75
Mepanipyrim	7.36	224	77	55	106	35	97	7.3	117	4	70.22	53.63–99.59
Mepronil	7.26	270.1	119.1	31	228	21	113	3.3	88	7.6	34.18	26.85–49.87
Metalaxyl	6.02	280.1	192.2	25	220.2	19	93	5.1	98	4.7	40.83	27.85–51.71
Metconazole	7.51	320.1	70	43	125	53	95	15.3	86	11	19.74	14.20–26.37
Methabenzthiazuron	5.98	222.1	150.3	45	165.2	21	78	6.6	93	3.7	12.79	9.36–17.38
Methamidophos	0.63	142	94	19	125	19	71	4.7	88	1.4	35.65	21.31–39.57
Methoprotryne	5.72	272.2	198	31	240.2	27	91	3.8	96	3.4	56.79	42.47–78.88
Methoxyfenozide	7.25	369.1	149.1	21	313.2	11	90	2.5	82	3.2	63.81	45.72–84.91
Mevinphos Isomer	3.91	225.101	127.1	21	193.2	11	78	5.3	77	5.1	54.54	32.42–60.21
Mevinphos Isomer _1	4.55	225.102	127.1	21	193.2	11	99	10.2	90	6.3	18.26	13.39–24.86
Mexacarbate	1.88	223.2	151	31	159.1	21	77	2.6	80	1.6	62.83	44.10–81.91
Monocrotophos	1.94	224.1	98	17	127.1	21	98	9.7	94	3.7	104.91	96.77–179.71
Monolinuron	6.07	215.1	99	47	126.1	23	92	10.1	117	5.8	28.97	20.62–38.29
Moxidectin	9.74	640.4	498.5	17	528.5	13	75	9.8	91	8.8	34.15	26.06–48.40
Myclobutanil	6.99	289	70	41	125	39	87	17.4	76	5.1	84.36	52.63–97.74
Nitenpyram	2.22	271	126	35	225.2	17	102	8.2	117	4.9	36.06	27.10–50.32
Omethoate	0.85	214	124.9	31	182.8	17	78	8.7	79	3.9	62.05	43.51–80.80
Oxadixyl	5.26	279.1	132.1	43	219.1	15	82	5.1	82	2.4	26.46	19.53–36.27
Paclobutrazol	6.65	294	70	49	125	41	84	10.4	103	10.3	38.85	31.58–58.65
Penconazole	7.57	284.1	70	37	159	35	96	7.8	117	5.2	42.96	28.27–52.49
Phenmedipham	6.72	301.2	107.9	44	168	12	98	6.3	82	4.1	26.05	17.35–32.21
Picoxystrobin	7.62	368	145	29	205	13	77	3.6	88	8	73.40	52.74–97.95
Piperonyl butoxide	8.37	356.2	119.1	47	177.2	13	80	7.3	96	4.4	16.15	14.15–26.27
Pirimicarb	3.89	239.2	72.1	33	182.1	21	72	3.9	75	1.2	63.57	43.94–81.61
Prochloraz	7.56	376	70	43	308	15	73	7.8	85	4.1	3.05	2.18–4.05
Prometon	5.32	226.1	86	39	142	33	83	4	83	3.5	67.26	45.73–84.93
Prometryne	6.41	242.2	158.1	33	200.1	25	80	3	80	2.7	62.39	44.22–82.13
Propamocarb	1.13	189.2	102	25	144	19	117	5.8	107	5	15.12	8.86–16.46
Propiconazole	7.65	342.1	69	39	159	31	90	11.3	119	4.2	0.81	0.69–1.27
Pyracarbolid	5.78	218.1	97	37	125	25	67	3.7	77	1.7	7.76	5.78–10.73

*Quantitation ion, **Confirmatory ion

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Table 2 (part 4). Target list pesticides with their SRM transitions and validation data at 0.01 and 0.05 mg/kg in wheat

Compound	RT (min)	Q1 (*m/z*)	Q3* (*m/z*)	CE (V)	Q3** (*m/z*)	CE (V)	0.01 mg/kg		0.05 mg/kg		Ion ratio at 0.05 (mg/kg)	Ion ratio range
							% Rec	% RSD	% Rec	% RSD		
Pyraclostrobin	7.83	388	163	31	194	17	95	8.7	99	4.8	90.75	53.33–99.04
Pyridaben	9.23	365	147	33	309	19	102	3.6	86	2.3	72.05	51.26–95.19
Pyrimethanil	6.24	200	82	37	107	33	89	9.8	91	1.9	35.12	23.15–42.99
Pyriproxyfen	8.57	322	96	21	185	31	98	5.7	119	4	16.80	12.06–22.39
Quinoxyfen	8.91	308.1	162.1	63	197.1	45	101	10.3	115	4.5	81.77	58.93–109.45
Secbumeton	5.38	226.2	100	37	170.1	25	89	3	97	1.7	24.42	16.87–31.32
Siduron	6.61	233.3	94	31	137.2	23	71	6.4	70	4.3	72.21	48.44–89.96
Simetryn	5.04	214	124	29	144	29	84	7	74	0.8	50.31	33.36–61.95
Spinetoram	8.44	748.5	98.1	65	142.2	43	73	4.1	78	2.9	21.08	12.51–23.23
Spinosad (Spinosyn A)	7.85	732.5	98.1	65	142.2	39	77	3.4	73	5.5	16.95	10.48–19.47
Spinosad (Spinosyn D)	8.24	746.8	98.3	65	142.4	41	93	15.3	119	4.7	18.11	11.88–22.06
Spiromesifen	9.21	371.2	255.2	31	273.2	11	89	8.1	109	2.9	8.35	6.52–12.11
Spirotetramat	6.8	374.2	302.2	27	330.2	23	85	6.7	72	4.5	83.68	46.20–85.80
Spiroxamine	7.3	298.2	100.1	43	144.2	29	77	3.1	78	2.9	30.46	21.33–39.61
Tebufenozide	7.59	353.2	133	23	297.2	11	86	8	71	2.1	80.60	53.42–99.20
Tebufenpyrad	8.28	334	117	47	145	37	71	13.2	78	6.7	31.64	17.35–32.21
Tebuthiuron	5.05	229.1	116.1	37	172.4	25	86	13.4	102	2.6	20.24	14.72–27.33
Terbumeton	5.38	226.1	100	41	170.1	23	73	2.4	85	0.9	8.43	5.95–11.05
Terbutryn	6.51	242.1	68.1	61	186.1	25	73	5.9	78	2.4	23.24	17.25–32.03
Tetraconazole	7.18	372.1	70	47	159	35	89	5	79	7.7	5.10	3.08–5.72
Thiabendazole	2.41	202.1	131.2	45	175.1	35	78	15.3	101	3	66.91	49.66–92.22
Thiacloprid	5.03	253	99	59	126	29	71	11.3	78	3.6	6.74	4.98–9.26
Thiamethoxam	2.86	292	181	31	211	17	79	3	83	2.1	15.29	8.44–15.67
Thidiazuron	5.28	221.1	102.1	23	127.9	23	99	17.3	81	3.5	26.81	15.24–28.30
Thiophanate-methyl	5.5	343	151.1	31	311	17	79	7.3	98	2.1	3.57	2.04–3.80
Triadimefon	7.06	294	197.1	21	225	19	102	19.3	79	4.6	39.40	23.94–44.46
Triadimenol	6.65	296.1	70	33	227.1	17	80	13.8	91	5.7	12.95	9.58–17.79
Tricyclazole	5	190	136	39	163	33	73	7.6	90	3.4	60.43	46.03–85.48
Trifloxystrobin	8.2	409	186	21	206	19	82	6.1	70	4.9	25.53	18.78–34.87
Triflumizole	7.85	346.1	73	27	278.1	17	70	12.5	79	10.6	1.20	0.91–1.69
Vamidothion	3.86	288	118	37	146	17	75	1.7	81	1.4	8.17	5.62–10.44
Zoxamide	8.21	336.1	159	55	187	29	78	17.9	71	5.5	19.03	13.46–25.00

*Quantitation ion, **Confirmatory ion

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