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APPLICATION NOTE 72981

A selective and sensitive method for quantification of pesticide residues in wheat using GC-(EI)-MS/MS

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Keywords

TraceFinder, pesticide residues, wheat, QuEChERS, GC-MS/MS, TSQ 9000, quantitation, triple quadrupole, gas chromatography

Goal

The objective of this application note is to demonstrate a comprehensive analytical solution for selective detection and accurate quantification of 148 pesticide residues in wheat matrix by using gas chromatography-triple quadrupole mass spectrometry. The proposed analytical solution is validated in accordance with the SANTE guidelines, and overall results are checked for compliance with maximum residue levels (MRLs) set by the Food Safety and Standards Authority of India (FSSAI) as well as the European Commission (EC) for wheat.

Introduction

Wheat is one of the most important food crops as it is a staple food of many diets around the world. In India, wheat is second only to rice in cultivated food crops.[1] In addition, India is the second largest cultivator of wheat in the world.[2] The use of crop protection products such as pesticides plays an important role in meeting the growing market demand for higher quantities of high quality wheat. Agrochemicals are used to protect crops and to improve the efficiency of production by controlling disease and reducing losses due to pests. Currently, there are 282 chemicals registered under the

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Central Insecticide Board and Registration Committee (CIBRC), Government of India.[3] Usage of agrochemicals can result in residues in wheat and wheat products with possible risk concerns to human health and the environment. Therefore, it is necessary to implement effective monitoring for the presence of pesticide residues in wheat grain as well as wheat flour. This requires an analytical method that is simple to set up and use, robust, and provides accurate and precise results. The European Commission (EC) and FSSAI have set the maximum residue levels (MRLs) in wheat.[4,5] The extraction of pesticide residues was performed using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method.[6] Pesticide residues in the extracts were detected, identified, and quantified using the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system, in accordance with the SANTE/11813/2017 guidelines.[7] The data acquisition and processing was carried out by using Thermo Scientific™ TraceFinder™ software.

Experimental

GC-MS/MS analysis

A Thermo Scientific™ TRACE™ 1310 gas chromatograph was coupled to a TSQ 9000 triple quadrupole mass spectrometer fitted with the Thermo Scientific™ ExtractaBrite ion source operated in electron ionization (EI) mode. The optimized GC-MS/MS conditions are given in Table 1.

Sample preparation

The wheat grains were procured from a local market and homogenized by using a heavy duty homogenizer to get a small particle size between 250 and 500 µm. The smaller particle size and homogenized sample maximizes the surface area for extraction, therefore making the sample easier to extract.

Table 1. GC-MS/MS instrument conditions

Gas chromatography method	
Instrumentation:	TRACE 1310 GC with Thermo Scientific™ TriPlus™ RSH™ autosampler
Column:	Thermo Scientific™ TraceGOLD™ TG-5SIL MS, 30 m × 0.25 mm ID × 0.25 µm (P/N 26096-1420)
Injector:	Split/Splitless (SSL)
Liner:	SSL splitless liner, single taper, deactivated (P/N 453A1925)
Injector mode:	Splitless
Splitless time:	2 min
Injection volume:	1.0 µL
Injector temperature.:	280 °C
Column flow:	1.20 mL/min
Carrier gas and purity:	Helium (99.999%)
Purge flow:	5.00 mL/min
Split flow:	50.00 mL/min
Total run time:	32.0 min
GC oven program:	90 °C, 5 min 25 °C/min to 180 °C, 0 min hold 5 °C/min to 280 °C, 0 min hold 10 °C/min to 300 °C, 1.4 min
Mass spectrometry method	
Instrumentation:	TSQ 9000 triple quadrupole mass spectrometer with ExtractaBrite ion source and vacuum probe interlock VPI
Acquisition mode:	Timed-SRM (t-SRM)
MS transfer line temperature:	310 °C
Ion source temperature:	280 °C
Ionization:	Electron Ionization (EI)

Extraction and cleanup:

- Weigh 5 g homogenized sample into a 50 mL extraction tube.
- For recovery, blank samples (n=6 for each level) were spiked with the full mixture of target pesticides at 0.005, 0.010, and 0.05 mg/kg before addition of water and extraction solvent.
- Add 15 mL of HPLC grade water (containing 1% acetic acid), vortex for 1 min, and soak for 10 min.
- Add 15 mL acetonitrile and vortex for 1 min at 2500 rpm.
- Add 6 g anhydrous $MgSO_4$ and 1.5 g sodium acetate to the tube and vortex for 1 min at 2500 rpm.
- Centrifuge at 5000 rpm for 5 min.
- Transfer supernatant (1 mL extract) into the 2 mL microcentrifuge tube containing 150 mg $MgSO_4$, 50 mg primary secondary amine (PSA).
- Vortex for 1 min and centrifuge samples with 10,000 rpm for 5 min.
- Transfer an aliquot (0.5 mL) into a GC autosampler vial for analysis.
- Matrix-matched calibration standards:
  - Prepare blank (control) extract by following the above protocol for matrix-matched calibration standards.
  - The matrix-matched calibration standards were prepared as by post extraction spiking (Table 2).
- Final cleaned extract, as well as matrix-matched calibration standards, were injected (1.0 µL) into the GC-MS/MS.

Data acquisition and processing

The data acquisition and processing was carried out by using the instrument conditions in Table 1 and TraceFinder software. The data was acquired in t-SRM mode, which includes two or more transitions per analyte from the compound database (CDB). The target list of analytes along with their MS/MS transition, collision energies, and retention time (min) are given in Table 3 (Appendix). The user defined parameters based on SANTE guideline were set in the master method (data processing), which included the ion ratio (±30%), retention time (±0.1 min), linearity (>0.99 with residuals ±20), recovery at 0.005, 0.010, and 0.050 mg/kg of analytes spiked before extraction (70–120%), and precision (±20%).

Results and discussion

Sample preparation

Milled wheat grain is composed of protein (9–14%), fat (1–2%), carbohydrates (54–62%), fiber (1.7–2.6%), trace moisture, and ash (1.2–1.7%). Since it is a dry and neutral matrix, the addition of acidified water helped to maintain moisture content required for liquid-liquid partitioning. The addition of acetic acid improves the stability of base-sensitive compounds during extraction. The use of PSA to remove acidic matrix components and matrix-matched calibration resulted in excellent recovery and precision even at low level (0.005 mg/kg).

GC-MS/MS analysis

In this method, the automatic optimized dwell time was in the range of 2 to 10 ms per transition, which offered a minimum of 12 points per peak. Here, the matrix-matched calibration standard at the trace level of 0.001 mg/kg showed >12 data points per peak of

Table 2. Matrix-matched calibration standards preparation

Working standard (µg/mL)	Volume taken from working standard (µL)	Extracted matrix (µL)	Final concentration (mg/kg)	Total volume (µL)
2.000	50	950	0.100	1000
1.000	50	950	0.050	1000
0.500	50	950	0.025	1000
0.200	50	950	0.010	1000
0.100	50	950	0.005	1000
0.040	50	950	0.002	1000
0.020	50	950	0.001	1000

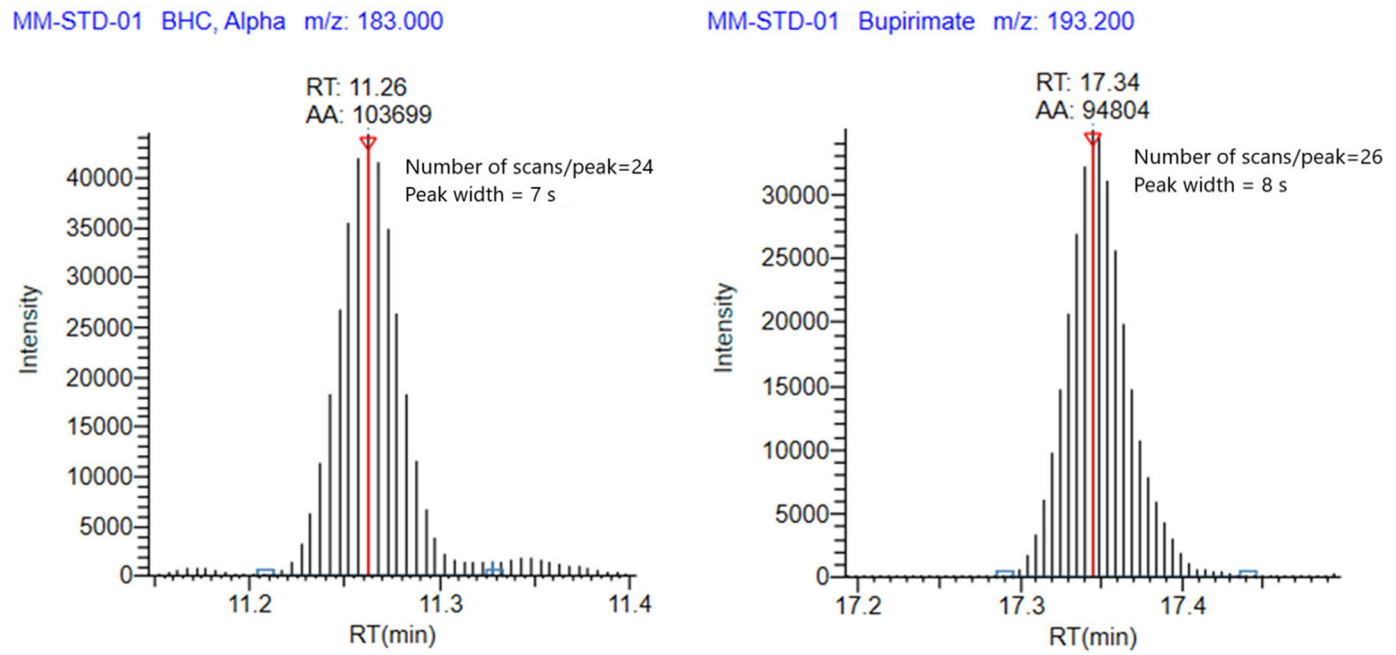

Figure 1. Impact of auto-optimized dwell time on the data points per peak

alpha-BHC and bupirimate, presented in Figure 1. The gas chromatographic method offered excellent separation of target analytes and absence of an isobaric interference from the matrix. In this method no internal standard was used. The total ion chromatogram (TIC) for 148 compounds is shown in Figure 2.

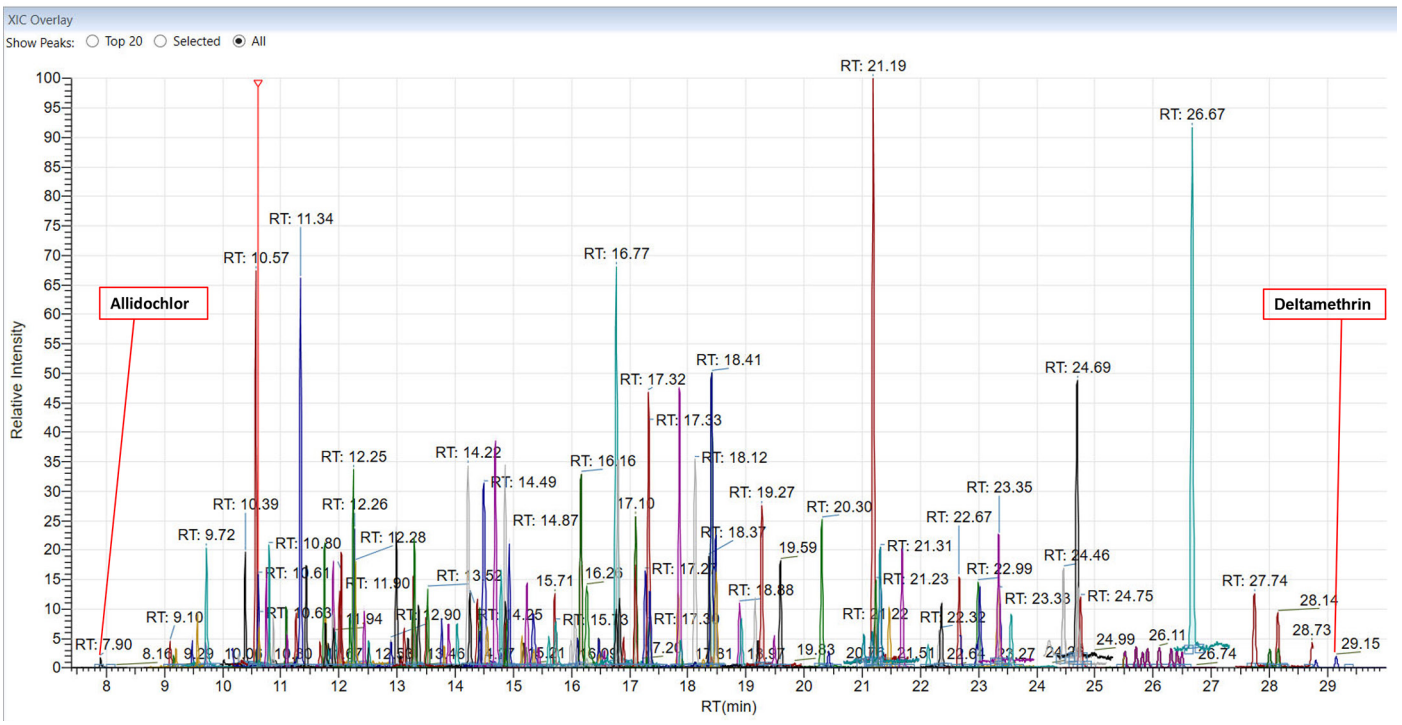

Figure 2. Extracted ion chromatograms of 148 pesticides in wheat showing the quan SRM transition at 0.01 mg/kg. First eluting compound (allidochlor) and last eluting compound (deltamethrin) are shown.

Detection, identification, and quantification

User-defined parameters for data processing were set in the data processing method of TraceFinder software (Master method). Based on these parameters, the data was processed automatically with flagging. These flags indicate through color codes whether results pass or fail the user-defined acceptance criteria taken from the SANTE guidelines. The results that passed user-defined tests (SANTE guidelines) are shown in green (Figure 3).

Two transitions, 282→91 (quantitative) and 282→238 (qualifier), were used for the identification of fluazifop-P-butyl shown in Figure 4. The ion ratios observed for fluazifop-P-butyl in MMS (89.29%) and spiked sample (106.17%) were within 30% (62.49–116.04%) of the calibration standard. The linearity for fluazifop-P-butyl provided correlation coefficients >0.999 with residuals <20%. This approach was applied to all target analytes, and it meets the requirement of the SANTE guidelines in terms of identification and quantitation.

Method performance

Calibration curves over the range of 0.001 to 0.100 mg/kg provided excellent correlation coefficients (>0.99) with <20% residuals for all the target analytes in both solvent as well as in wheat matrix. In wheat, matrix enhancement was observed with >20 % matrix effect. The lowest spiked concentration 0.005 mg/kg (equivalent to 1.66 ng/mL concentration in the final vial) showed good sensitivity with ≥12–20 signal-to-noise ratio, 80–120% recovery, and <20% precision for the majority of target analytes. Therefore, 0.005 mg/kg was considered as the limit of quantitation (LOQ) in wheat matrix. The recoveries were observed in the range of 71% to 110% with <18% RSD for 0.005 (LOQ), 0.010 (LOQ × 2), and 0.05 (LOQ × 10) mg/kg (Table 4, Appendix), which were within acceptance criteria (recovery 70–120% and precision <20%) of SANTE guidelines.[7] Also, the optimized method offered an excellent reatability (%RSD = <15%) for replicate injections (n=38) of spiked matrix (0.010 mg/kg).

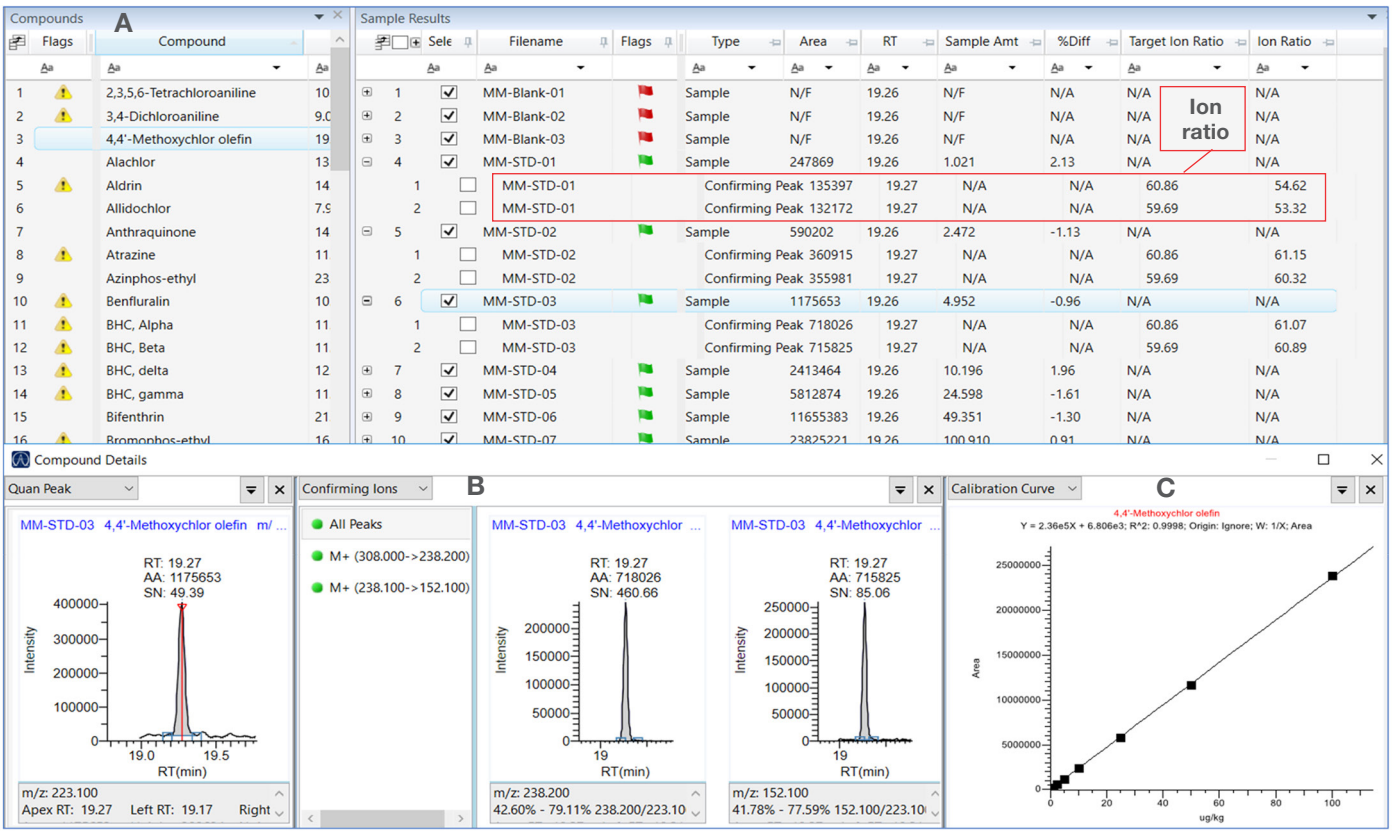

Figure 3. TraceFinder software results browser showing the flagging for detection, identification, and quantification of target analytes in wheat. (A) List of target compounds; (B) SRM chromatograms of quantification ion and qualifier ion; (C) calibration curve

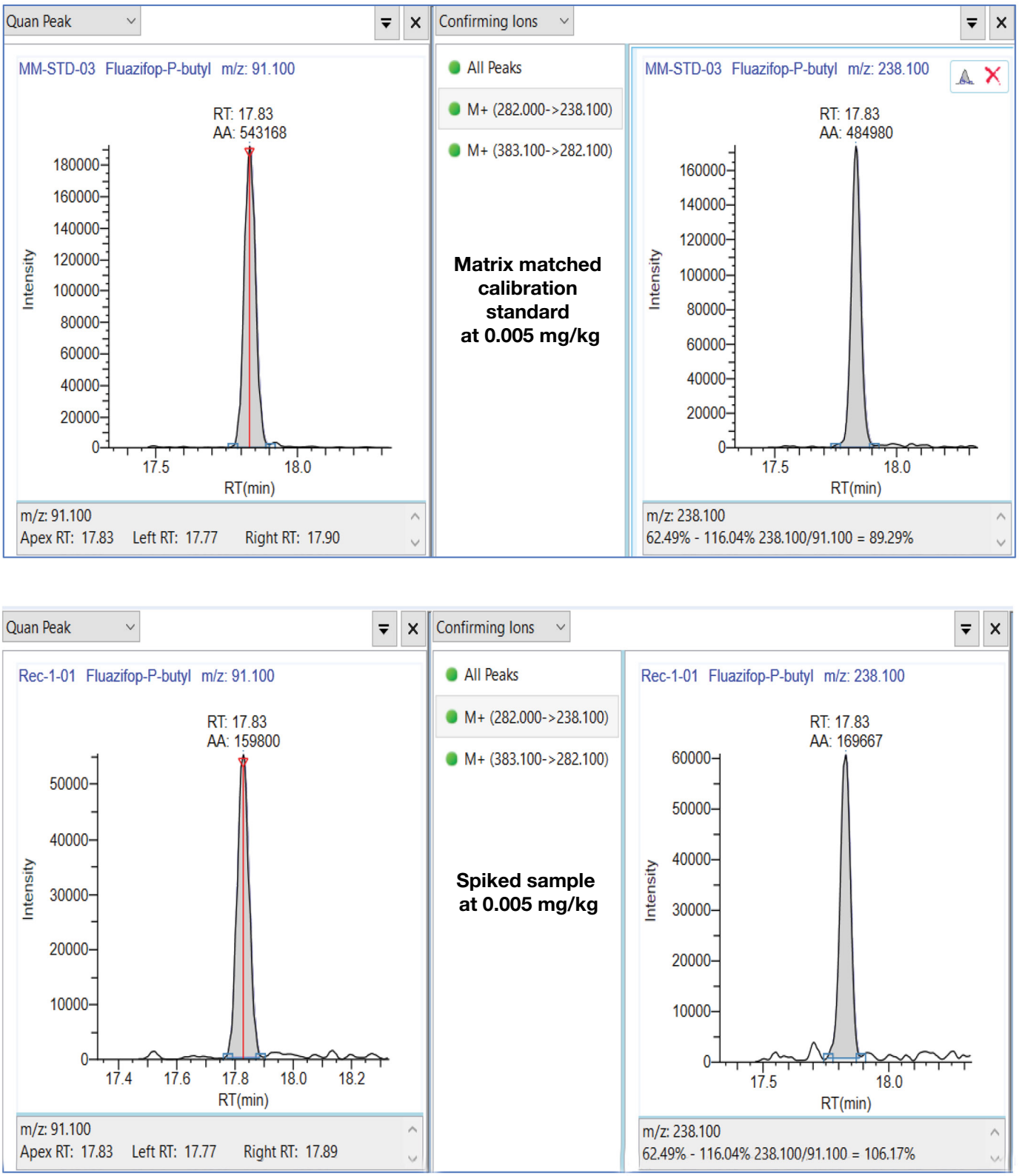

Figure 4. Detection, identification, and quantification of fluazifop-P-butyl in matrix-matched (MM) calibration standard as well as spiked sample at 0.005 mg/kg, based on quantification ion and qualifier ion with their ion ratio

The repeatability data is shown in Figure 5 (area repetability for fluazifop-P-butyl, fluquinconazole, and myclobutanil), Figure 6 (retention time for fluquinconazole), and Figure 7 (ion ratio for fluquinconazole).

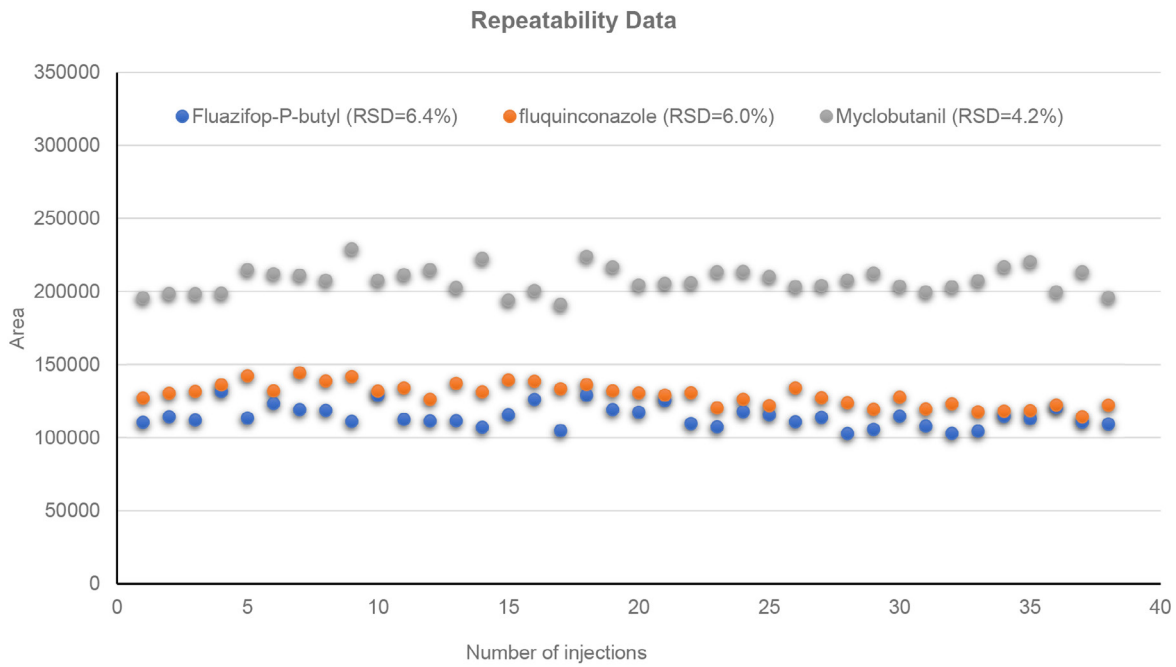

Figure 5. SRM quantitative ion peak area repeatability for fluazifop-P-butyl, fluquinconazole, and myclobutanil (n=38) at 0.010 mg/kg

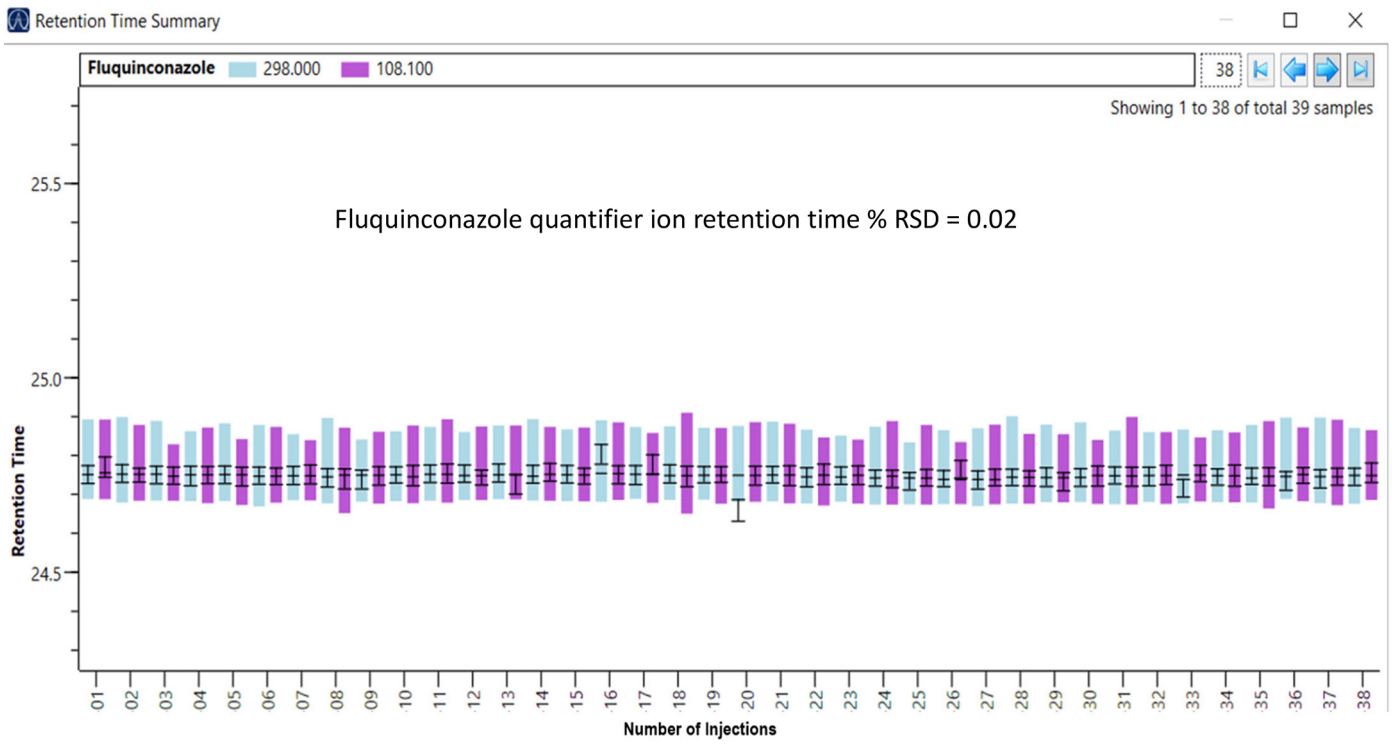

Figure 6. Retention time repeatability for fluquinconazole (n=38) at 0.010 mg/kg

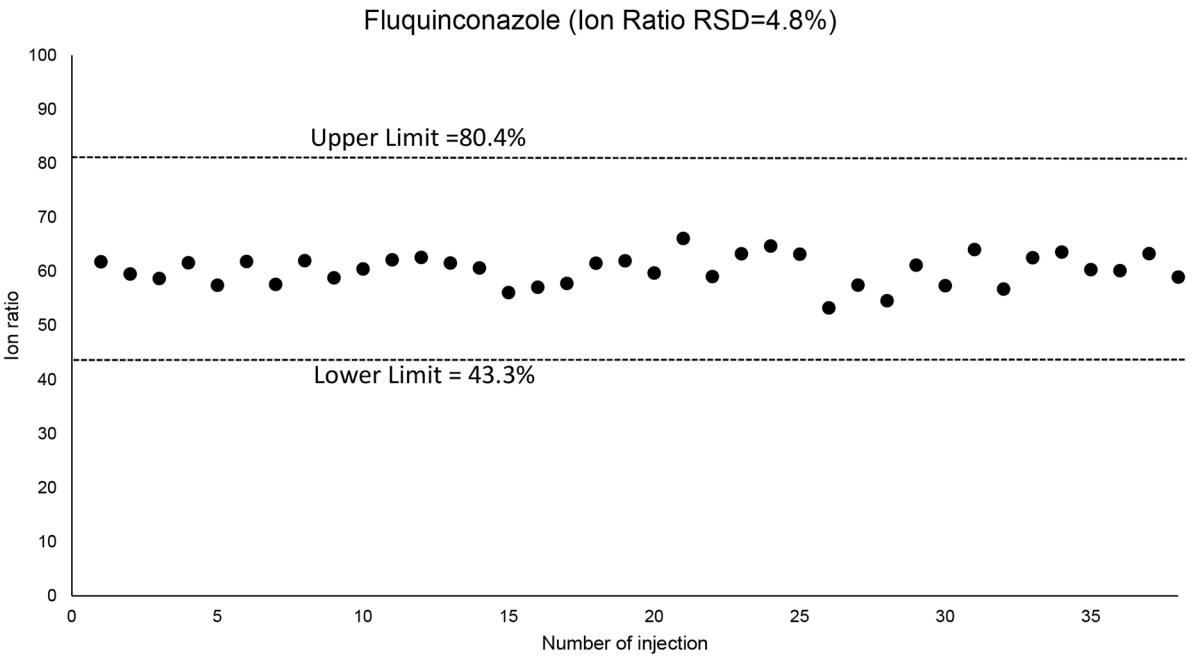

Figure 7. Ion ratio repeatability for fluquinconazole (n=38) at 0.010 mg/kg

Conclusion

The developed analytical solution, based on the use of a Thermo Scientific GC-EI-MS/MS system provides quantitative results for 148 pesticides in wheat matrix in a single injection. The results are compliant with the SANTE guidelies and can meet the testing requirements for compliance with the EU and FSSAI MRLs. The GC-MS/MS determination combined with the use of the QuEChERS approach for extraction and cleanup can provide the high throughput required by commercial food testing laboratories. By following this approach at least 38 injections (standards, samples, blank) could be completed in a day (24 hr cycle). The optimized analytical solution results showed that GC separations in combination with t-SRM windows allowed maintaining the number of transitions monitored in a single injection by auto-optimized dwell time without compromising data quality. Other features such as the VPI NeverVent™ technology, which allows changing of the ion source and column without venting the system, and the reduced downtime from the robustness of the ExtractaBrite ion source further enhance productivity. Features such as auto-SRM and automatic dwell time assignment reduce the time required for method development and improve accuracy and precision of the determination. The analytical solution is validated in accordance with the requirements of the SANTE guidelines. Also, this method complies with the EU and FSSAI MRL requirements by achieving an excellent lower limit of quantitation (LOQ).

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Appendix

Table 3 (part 1). List of pesticides with their SRM transitions, collision energies (V), and retention time (min)

Name of compound	RT (min)	Quantifier ion			Qualifier ion		
		Q1	Q3	CE (V)	Q1	Q3	CE (V)
2,3,5,6-Tetrachloroaniline	10.59	231	158	20	231	160	22
3,4-Dichloroaniline	9.09	160.9	99	20	160.9	90	18
4,4'-Methoxychlor olefin	19.26	238.1	223.1	10	308	238.2	12
Alachlor	13.28	188.1	160.1	8	188.1	130	32
Aldrin	14.42	330	298.9	10	262.7	192.9	28
Allidochlor	7.9	132	56.1	8	132	49	24
Anthraquinone	14.49	180	152	12	208	180	10
Atrazine	11.67	200	122.1	8	215.1	173	8
Azinphos-ethyl	23.55	160	77	16	132	51	26
Benfluralin	10.8	292	264	8	292	206.1	10
BHC, Alpha	11.26	218.8	183	8	182.8	146.7	12
BHC, Beta	11.77	180.9	145	14	218.7	183	8
BHC, delta	12.52	218.8	182.9	8	182.8	146.7	14
BHC, gamma	11.91	218.7	183	8	180.9	145	14
Bifenthrin	21.18	181	165.9	10	181	179	12
Bromophos-ethyl	16	302.7	284.8	14	96.9	65	16
Bromophos-methyl (Bromophos)	14.87	330.8	315.8	14	328.9	313.8	14
Bromopropylate	21.22	340.8	185	14	184.9	156.9	12
Bupirimate	17.35	273.1	193.2	8	208.1	140.1	12
Carbophenothion	19.2	342	157	10	157	45	12
Carfentrazon-ethyl	19.15	340.1	312.1	10	311.9	150.7	18
Chlorbenside	16.05	125	99	16	125	62.8	28
Chlordane alpha-cis	16.09	372.8	265.8	20	376.6	268	20
Chlordane gamma-trans	16.47	374.7	265.9	20	372.7	263.7	20
Chlorfenson	16.81	174.9	111	10	111	75	14
Chlorobenzilate	18.12	139	111	12	139	74.9	26
Chloroneb	9.55	190.9	113	14	190.9	141	10
Chlorpyrifos-ethyl	14.25	196.7	168.9	12	313.9	257.9	12
Chlorpyrifos-methyl	13.13	285.9	93	20	125	47	12
Chlorthal-dimethyl (Dacthal)	14.43	300.7	222.9	22	300.7	272.9	12
Chlorthiophos	18.48	324.9	269	12	296.9	268.9	8
Chlozolinate	15.34	331	259	8	259	187.9	12
Clomazone	11.74	125	89	13	204	107	18
Cycloate	10.6	154.1	83.1	8	154.1	55.1	18
Cyfluthrin peak 1	25.51	163	127.1	6	206	151.1	18
Cyfluthrin peak 2	25.7	163	127	6	206	151.1	18
Cyfluthrin peak 3	25.81	163	127	6	226	206.1	12
Cyfluthrin peak 4	25.9	163	127	6	226	206.1	12
Cyhalothrin I (lambda)	23.02	207.9	180.9	8	197.1	141.1	10
Cypermethrin peak 1	26.1	163	127.1	6	163	91.1	12
Cypermethrin peak 2	26.31	163	127	6	180.9	151.9	20
Cypermethrin peak 3	26.41	163	127	6	180.9	152.2	20
Cypermethrin peak 4	26.5	163	127.1	6	180.9	152.2	20
Cyprodinil	15.22	224.1	196.9	20	224.1	208	18
DDD p,p*	18.42	235	165.1	20	236.8	165	20
DDD, o, p*	17.32	235	165.1	20	236.8	165	20
DDE o,p*	16.17	246	176.1	28	317.8	246	20
DDE p, p*	17.1	246	176.1	28	317.8	248	18
DDT o,p*	18.47	235	165.1	20	236.8	165	20
Deltamethrin	29.5	252.8	172	8	252.8	92.9	16
Diallate-*cis*	11.09	234.1	150	18	234	192	12
Diallate-*trans*	11.27	234.1	150	18	234.1	192	12
Diazinon	11.99	137.1	84.1	12	137.1	54.1	20
Dichlorobenzophenone, 4, 4	14.69	139	111	12	139	74.9	26
Dimethachlor	12.99	197	148.1	10	134	105.1	12
Diphenamid	14.9	239.1	167.1	8	239.1	72.1	10
Endosulfan ether	12.9	240.9	206	14	238.9	204	12
Endosulfan peak 1**	16.47	240.6	205.9	14	194.7	125	22
Endosulfan peak 2**	18.26	240.6	205.8	12	194.7	159	8
Endosulfan sulfate**	19.48	271.7	236.8	12	238.7	203.9	12
EPN	21.16	169	77	22	157	77	22
Esfenvalerate	28.14	167	125	10	125	89.3	18
Ethalfluralin	10.63	276	202	14	315.9	276.1	8
Ethion	18.36	230.9	128.9	22	153	97	10
Etofenprox	26.67	163.1	135.1	10	163.1	107.1	16
Etridiazole (Terrazole)	9.16	182.8	139.9	14	211	139.9	20
Fenarimol	23.34	139	111	14	219	107	10
Fenchlorfos	13.52	285	270	11	287	272	11
Fenitrothion	13.89	277	260	6	277	109	16
Fenson	14.86	141	77	8	141	50.9	30
Fenvalerate	27.74	167	125	10	125	89	18
Fluazifop-P-butyl	17.83	282	91.1	18	282	238.1	16
Fluchloralin	12.03	306	264	8	264	206.1	8
Fluquinconazole	24.74	340	298	16	340	108.1	36
Flusilazole	17.33	233	164.9	16	233	151.9	14
Flutolanil	16.77	173	145	14	281	173	10
Fluvalinate peak 1	28	250	55.1	16	250	199.9	18
Fluvalinate peak 2	28.14	250	55.1	16	250	199.9	18
Fonofos	12.04	246	137	6	246	109	14
Heptachlor	13.5	271.8	236.9	12	99.8	65	12
Heptachlor epoxide	15.44	352.8	262.9	16	354.7	264.9	12
Iodofenfos	16.77	376.8	361.8	16	125	47	12
Isazophos	12.27	161	119	8	118.9	76	18
Isodrin	15.17	192.9	123	28	146.8	111.1	10
Isopropalin	14.94	280.1	238.2	8	280.1	180.2	10
Leptophos	22.35	171	77.1	18	171	51	38
Metazachlor	15.17	209	132.1	16	133.1	132.1	12
Methacrifos	9.48	180	93	10	240	180	10
Methoxychlor	21.46	227.1	169.1	22	227.1	141.1	32
Metolachlor	14.23	162.1	132.9	14	238.1	162.2	10
MGK-264 A	14.87	164	93.1	10	164	98.1	10
MGK-264 B	15.22	164	98	10	164	80	24
Myclobutanil	17.27	179	125	14	179	90	28

Table 3 (part 2). List of pesticides with their SRM transitions, collision energies (V), and retention time (min)

Name of compound	RT (min)	Quantifier ion			Qualifier ion		
		Q1	Q3	CE (V)	Q1	Q3	CE (V)
Nitralin	20.42	316.2	274	8	274	216	8
Nitrofen	17.88	202	139	24	283	202	10
Nonachlor-*cis*	16.55	408.6	300	18	262.8	192.8	28
Nonachlor-*trans*	18.4	408.7	300	18	236.8	142.9	24
Oxadiazon	17.09	174.9	112	12	174.9	76	28
Oxyfluorfen	17.3	252	146	30	252	169.8	28
Paclobutrazol	16.27	236	125	12	236	167	10
Parathion-methyl	13.29	263	109	12	124.9	47	12
Pebulate	9.14	161	128	6	128	57	8
Penconazole	15.29	248	157	22	158.9	89	28
Pendimethalin	15.16	252.1	162	8	252.1	191.3	8
Pentachloroaniline	12.9	264.8	193.6	18	264.8	202.8	20
Pentachloroanisole	11.43	264.8	236.9	10	266.8	238.9	10
Pentachlorobenzene	9.69	249.8	214.8	16	249.8	143.6	38
Pentachlorobenzonitrile	11.9	274.8	239.9	18	274.8	204.9	28
Pentachlorothioanisole	14.03	295.7	262.9	12	295.7	245.9	30
Permethrin peak 1	24.45	183.1	168	12	183.1	153	12
Permethrin peak 2	24.7	183	165.1	10	183	153	14
Perthane (Ethylan)	17.85	223.1	167	12	223.1	179	20
Phorate	11.1	260	75	8	121	65	8
Phosalone	22.26	182	74.8	30	182	111	14
Piperonyl butoxide	20.3	176.1	131.1	12	176.1	103.1	22
Pirimiphos-ethyl	14.78	304	168.1	12	318.1	166.1	12
Pirimiphos-methyl	13.77	305.1	180.1	8	290.1	233	8
Pretilachlor	16.89	202.1	174.2	8	238.1	146.1	10
Prodiamine	13.81	321.1	279.1	6	275.1	255.1	8
Profluralin	11.76	318.1	199	15	330.2	69.1	20
Propachlor	10.39	120	77	19	176	57	8
Propisochlor	13.36	162.1	120.1	12	162.1	144.1	8
Propyzamide	12	172.9	109	26	172.9	145	14
Prothiofos	16.84	308.9	239	14	266.7	220.9	18
Pyrazophos	23.35	221	193.1	8	231.9	204.1	10
Pyridaben	24.69	147.1	117.1	20	147.1	132.1	12
Pyrimethanil	12.18	198.1	117.9	30	198.1	157.6	18
Pyriproxyfen	22.67	136.1	96	10	136.1	78	20
Quinalphos	15.6	157.1	102	22	146	118.1	10
Quintozene	11.81	294.8	236.9	14	213.8	178.9	14
Sulfotep	10.87	322	202	10	265.9	145.9	15
Sulprofos	18.9	322	156.1	10	156	108	30
Tebufenpyrad	21.68	276.1	171	10	318.1	131.1	14
Tecnazene	10.27	261	203	13	215	179	8
Tefluthrin	12.25	177	127	14	177	137	16
Terbufos	11.89	230.9	128.9	22	230.9	174.9	12
Terbuthylazine	11.93	214.1	132	10	214.1	104	16
Tetradifon	22.13	159	111	20	159	74.8	32
Tetramethrin peak 1	21.02	164	107.1	12	164	77.1	24
Tetramethrin peak 2	21.3	164	107.1	12	164	77.1	24
Tolclofos-methyl	13.29	265	250	12	266.8	252	12
Transfluthrin	13.27	163	143	14	127	91.1	8
Triadimefon	14.55	208	180.8	8	208	126.7	12
Triadimenol	15.72	168.2	70	10	128	65	18
Triallate	12.43	268	183.9	18	268	226	12
Triazophos	18.93	161	134.1	8	161	106.1	12
Trifluralin	10.74	306.1	264.1	8	306.1	159.7	20
Vinclozolin	13.19	198	145	14	212	172	14

Table 4 (part 1). Method validation data for all target analytes (ion ratio, linearity, recovery, and precision)

Name of compound	Ion ratio (IR)		R^2	LOQ (mg/kg)	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		0.05 mg/kg (n=6)	
	IR range	IR in wheat 0.005 (mg/kg)			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
2,3,5,6-Tetrachloroaniline	63.50 - 117.93	81.0	0.9991	0.005	88	18	86	2	91	4
3,4-Dichloroaniline	53.82 - 99.96	76.7	0.9987	0.005	84	8	83	6	79	6
4,4'-Methoxychlor olefin	42.60 - 79.11	61.2	0.9998	0.005	95	5	99	3	91	3
Alachlor	38.22 - 70.99	61.1	0.9992	0.005	100	6	99	3	92	1
Aldrin	36.43 - 67.65	52.6	0.9997	0.005	97	10	105	5	95	3
Allidochlor	8.21 - 15.26	11.5	0.9992	0.005	102	3	94	7	89	2
Anthraquinone	67.43 - 125.24	98.1	0.9986	0.005	87	9	91	8	87	2
Atrazine	55.00 - 102.14	80.8	0.9992	0.005	90	9	95	7	89	6
Azinphos-ethyl	41.13 - 76.39	47.3	0.9997	0.005	104	6	90	3	79	3
Benfluralin	26.58 - 49.37	43.4	0.9986	0.005	99	6	95	4	89	3
BHC, Alpha	47.73 - 88.65	64.3	0.999	0.005	91	13	96	5	94	2
BHC, Beta	55.52 - 103.10	68.0	0.9988	0.005	98	8	97	8	92	4
BHC, delta	47.12 - 87.52	55.5	0.9997	0.005	103	10	104	6	89	4
BHC, gamma	96.47 - 179.15	121.4	0.9994	0.005	94	7	94	6	95	3
Bifenthrin	5.31 - 9.86	8.1	0.9999	0.005	90	4	87	3	84	2
Bromophos-ethyl	32.32 - 60.02	47.7	0.9985	0.005	102	12	94	12	86	4
Bromophos-methyl (Bromophos)	41.08 - 76.28	56.4	0.9992	0.005	100	9	91	7	84	4
Bromopropylate	105.67 - 196.25	187.0	0.9998	0.005	97	6	93	2	88	2
Bupirimate	14.82 - 27.53	23.3	0.9997	0.005	100	6	99	5	95	4
Carbophenothion	126.96 - 235.79	179.9	0.9993	0.005	94	10	93	4	83	4
Carfentrazon-ethyl	11.58 - 21.51	19.6	0.9989	0.005	97	5	93	7	84	2
Chlorbenside	46.48 - 86.31	68.0	0.9987	0.005	79	9	76	8	72	2
Chlordane alpha-cis	29.83 - 55.40	44.0	0.9972	0.005	85	7	87	12	83	6
Chlordane gamma-trans	51.74 - 96.08	83.8	0.9996	0.005	89	11	86	9	83	5
Chlorfenson	28.63 - 53.16	42.4	0.9996	0.005	102	6	100	4	93	1
Chlorobenzilate	28.93 - 53.73	39.6	0.9999	0.005	99	4	98	3	94	2
Chloroneb	72.32 - 134.31	128.4	0.9983	0.005	101	3	99	7	95	3
Chlorpyrifos-ethyl	63.17 - 117.31	92.9	0.9995	0.005	99	9	100	4	93	6
Chlorpyrifos-methyl	56.76 - 105.41	68.0	0.9997	0.005	108	7	96	7	91	4
Chlorthal-dimethyl (Dacthal)	60.68 - 112.68	88.5	0.9998	0.005	112	5	99	4	93	3
Chlorthiophos	60.94 - 113.18	71.8	0.9999	0.005	95	6	91	4	80	2
Chlozolinate	55.35 - 102.79	68.8	0.997	0.005	105	7	94	9	87	8
Clomazone	50.68 - 94.12	71.3	0.9997	0.005	105	4	101	10	92	4
Cycloate	41.19 - 76.49	50.8	0.999	0.005	96	4	91	5	90	2
Cyfluthrin peak 1	25.38 - 47.13	37.3	0.9995	0.0013	100	8	94	4	87	2
Cyfluthrin peak 2	18.17 - 33.75	28.1	0.9998	0.0013	95	4	91	5	86	2
Cyfluthrin peak 3	50.11 - 93.06	67.4	0.9995	0.0013	98	7	98	4	88	3
Cyfluthrin peak 4	39.47 - 73.30	63.0	0.9997	0.0013	92	9	93	4	87	4
Cyhalothrin I (lambda)	43.61 - 65.41	54.9	0.9998	0.005	97	5	94	2	89	3
Cypermethrin peak 1	63.54 - 117.99	100.5	0.999	0.0013	105	5	89	5	87	2
Cypermethrin peak 2	34.44 - 63.97	46.7	0.9999	0.0013	100	10	95	8	87	4
Cypermethrin peak 3	135.50 - 251.65	200.2	0.9995	0.0013	97	6	94	5	86	2
Cypermethrin peak 4	16.81 - 31.22	24.3	0.9988	0.0013	106	5	95	6	89	4
Cyprodinil	340.38 - 632.14	535.1	0.9987	0.005	89	5	95	7	91	6
DDD p,p*	46.03 - 85.48	59.2	0.9993	0.005	107	4	100	2	91	3
DDD, o, p*	47.81 - 88.78	64.7	0.9995	0.005	110	2	104	3	92	3
DDE o,p*	19.80 - 36.76	24.8	0.9999	0.005	95	5	92	4	85	2

Table 4 (part 2). Method validation data for all target analytes (ion ratio, linearity, recovery, and precision)

Name of compound	Ion ratio (IR)		R^2	LOQ (mg/kg)	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		0.05 mg/kg (n=6)	
	IR range	IR in wheat 0.005 (mg/kg)			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
DDE p, p*	33.70 - 62.59	52.1	0.9998	0.005	84	7	84	4	80	4
DDT o,p*	46.08 - 85.58	72.6	0.9993	0.005	81	7	77	4	72	2
Deltamethrin	68.95-128.05	103.3	0.9973	0.005	112	3	96	2	81	1
Diallate-cis	54.90 - 101.96	77.9	0.9978	0.005	94	15	89	7	96	4
Diallate-trans	53.06 - 98.54	71.4	0.9975	0.005	104	12	91	11	97	7
Diazinon	38.93 - 72.30	62.9	0.9988	0.005	104	9	98	3	96	6
Dichlorobenzophenone, 4, 4	30.51 - 56.66	41.6	0.9998	0.005	99	6	94	3	89	3
Dimethachlor	66.63 - 123.75	99.6	0.9999	0.005	101	4	97	4	91	3
Diphenamid	34.32 - 63.73	44.8	0.999	0.005	103	7	99	4	92	3
Endosulfan ether	70.35 - 130.65	109.4	0.9997	0.005	112	3	106	7	93	6
Endosulfan peak 1**	27.03 - 50.20	29.1	0.999	0.005	91	10	95	9	87	5
Endosulfan peak 2**	71.93 - 133.59	118.0	0.9983	0.005	87	19	92	13	90	4
Endosulfan sulfate**	18.41 - 34.19	22.6	0.9996	0.005	85	14	81	9	77	6
EPN	51.69 - 95.99	83.7	0.9991	0.005	102	9	98	6	94	5
Esfenvalerate	15.98 - 29.68	26.5	0.9992	0.005	104	4	94	3	87	3
Ethalfluralin	44.64 - 82.90	62.9	0.9988	0.005	93	11	94	5	92	4
Ethion	65.07 - 120.85	109.0	0.9997	0.005	98	6	99	6	91	3
Etofenprox	58.42 - 108.50	84.7	0.9999	0.005	95	3	90	2	86	1
Etridiazole (Terrazole)	69.56 - 129.18	100.0	0.9994	0.005	114	2	93	11	82	3
Fenarimol	53.69 - 99.72	76.1	0.9999	0.005	104	5	96	4	89	4
Fenchlorfos	45.42 - 84.35	56.2	0.9993	0.005	104	9	100	8	88	3
Fenitrothion	37.88 - 70.36	58.1	0.9995	0.005	114	5	109	5	91	5
Fenson	13.85 - 25.73	18.6	0.9997	0.005	98	3	98	3	94	3
Fenvalerate	16.79 - 31.17	21.9	0.9996	0.005	104	5	96	3	86	2
Fluazifop-P-butyl	62.49 - 116.04	87.0	0.9998	0.005	97	12	99	7	94	3
Fluchloralin	19.94 - 37.03	25.0	0.9976	0.005	98	6	94	4	88	3
Fluquinconazole	42.98 - 79.82	68.9	0.9998	0.005	97	6	91	4	87	2
Flusilazole	46.24 - 85.87	60.6	0.9988	0.005	110	5	101	10	92	5
Flutolanil	29.90 - 55.53	43.7	0.9996	0.005	107	3	100	1	94	3
Fluvalinate peak 1	51.69 - 95.99	77.4	0.9985	0.0025	105	6	96	5	90	2
Fluvalinate peak 2	53.71 - 99.74	80.9	0.9986	0.0025	117	2	106	6	89	2
Fonofos	62.06 - 115.25	109.0	0.9995	0.005	97	1	97	9	91	2
Heptachlor	45.89 - 85.23	73.1	0.9993	0.005	96	8	89	6	83	4
Heptachlor epoxide	52.08 - 96.72	87.2	0.9989	0.005	101	7	99	11	89	10
Iodofenfos	15.98 - 29.67	21.8	0.9995	0.005	104	8	93	7	77	4
Isazophos	9.12 - 16.94	12.6	0.9987	0.005	90	9	93	8	94	5
Isodrin	44.45 - 82.54	68.9	0.9993	0.005	95	13	98	8	86	4
Isopropalin	11.53 - 21.41	16.8	0.9988	0.005	96	5	98	3	84	4
Leptophos	31.59 - 58.68	39.7	0.9996	0.005	89	6	83	5	76	3
Metazachlor	94.20 - 174.94	132.6	0.9996	0.005	105	8	98	5	87	2
Methacrifos	55.12 - 102.36	91.1	0.9996	0.005	99	12	96	6	93	5
Methoxychlor	67.68 - 125.69	91.8	0.9974	0.005	97	4	78	4	71	1
Metolachlor	70.20 - 130.37	105.3	0.9997	0.005	97	5	96	3	93	2

Table 4 (part 3). Method validation data for all target analytes (ion ratio, linearity, recovery, and precision)

Name of compound	Ion ratio (IR)		R^2	LOQ (mg/kg)	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		0.05 mg/kg (n=6)	
	IR range	IR in wheat 0.005 (mg/kg)			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
MGK-264 A	32.72 - 60.77	44.0	0.9991	0.005	82	10	90	8	93	7
MGK-264 B	37.29 - 69.24	64.3	0.9986	0.005	105	8	100	12	92	4
Myclobutanil	19.61 - 36.42	30.9	0.9995	0.005	99	9	99	6	93	4
Nitralin	22.10 - 41.04	25.4	0.9947	0.010	-	-	114	5	91	2
Nitrofen	32.57 - 60.48	49.1	0.9993	0.005	113	4	98	5	97	5
Nonachlor-cis	26.10 - 48.48	28.9	0.9967	0.005	102	5	87	15	77	5
Nonachlor-trans	21.69 - 40.29	26.9	0.9994	0.005	101	11	98	13	81	3
Oxadiazon	28.50 - 52.92	49.8	0.9994	0.005	112	8	103	6	93	4
Oxyfluorfen	53.25 - 98.90	63.0	0.9996	0.005	113	4	106	11	96	5
Paclobutrazol	21.80 - 40.48	26.2	0.9997	0.005	103	5	97	4	93	3
Parathion-methyl	63.66 - 118.23	85.0	0.9986	0.005	108	10	103	8	92	4
Pebulate	114.46 - 212.57	157.8	0.9975	0.005	94	6	94	9	87	10
Penconazole	30.65 - 56.92	51.6	0.9994	0.005	98	8	99	7	92	5
Pendimethalin	26.93 - 50.01	32.0	0.9968	0.005	91	14	100	9	96	4
Pentachloroaniline	38.61 - 71.70	59.4	0.9993	0.005	99	9	97	7	90	3
Pentachloroanisole	41.34 - 76.77	59.9	0.9998	0.005	94	10	87	8	83	2
Pentachlorobenzene	22.64 - 42.05	36.9	0.9996	0.005	95	6	87	7	80	2
Pentachlorobenzonitrile	39.34 - 73.06	72.4	0.9992	0.005	102	10	93	5	92	3
Pentachlorothioanisole	38.85 - 72.15	49.0	0.9996	0.005	97	6	91	8	76	4
Permethrin peak 1	63.80 - 118.48	79.5	0.9997	0.005	90	7	85	5	80	3
Permethrin peak 2	60.63 - 112.60	77.9	0.9999	0.005	85	9	83	7	81	3
Perthane (Ethylan)	50.43 - 93.66	62.7	0.9999	0.005	102	4	95	4	89	3
Phorate	145.12 - 269.50	208.7	0.999	0.005	90	11	84	5	82	5
Phosalone	147.72 - 274.33	182.8	0.9998	0.005	94	10	85	3	82	2
Piperonyl butoxide	53.77 - 99.86	79.2	0.9998	0.005	98	3	98	3	93	2
Pirimiphos-ethyl	26.56 - 49.33	40.4	0.9984	0.005	101	7	100	6	91	4
Pirimiphos-methyl	73.16 - 135.87	93.5	0.9996	0.005	109	7	106	6	95	4
Pretilachlor	47.07 - 87.42	72.3	0.9994	0.005	111	11	107	10	90	4
Prodiamine	29.13 - 54.09	44.3	0.9991	0.005	109	7	101	8	91	5
Profluralin	14.76 - 27.41	19.9	0.9948	0.005	97	16	99	8	87	8
Propachlor	43.75 - 81.25	70.8	0.9998	0.005	92	8	92	5	89	2
Propisochlor	53.49 - 99.33	74.2	0.9997	0.005	114	4	103	5	94	4
Propyzamide	148.54 - 275.85	193.5	0.9993	0.005	104	4	104	7	94	3
Prothiofos	44.39 - 82.43	52.0	0.9995	0.005	99	10	94	9	84	4
Pyrazophos	37.07 - 68.84	47.8	0.9999	0.005	99	11	91	3	85	2
Pyridaben	42.86 - 79.59	59.7	0.9996	0.005	84	12	81	3	83	1
Pyrimethanil	27.91 - 51.83	41.9	0.9993	0.005	99	11	98	5	94	4
Pyriproxyfen	68.33 - 126.89	105.4	0.9999	0.005	95	3	96	3	91	2
Quinalphos	302.07 - 560.98	416.3	0.9982	0.005	106	9	110	7	95	5
Quintozene	72.93 - 135.44	112.5	0.9977	0.005	101	12	98	10	86	4
Sulfotep	61.93 - 115.01	81.2	0.9993	0.005	110	8	108	6	97	3
Sulprofos	29.74 - 55.23	47.1	0.9989	0.005	85	6	82	7	73	2

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Table 4 (part 4). Method validation data for all target analytes (ion ratio, linearity, recovery, and precision)

Name of compound	Ion ratio (IR)		R^2	LOQ (mg/kg)	0.005 mg/kg (n=6)		0.01 mg/kg (n=6)		0.05 mg/kg (n=6)	
	IR range	IR in wheat 0.005 (mg/kg)			% Rec	% RSD	% Rec	% RSD	% Rec	% RSD
Tebufenpyrad	30.61 - 56.86	48.2	0.9992	0.005	92	4	95	6	93	2
Tecnazene	53.89 - 100.09	67.1	0.9993	0.005	112	9	104	6	91	5
Tefluthrin	23.05 - 42.81	32.3	0.9999	0.005	103	6	102	5	95	3
Terbufos	68.28 - 126.81	84.5	0.9993	0.005	99	8	95	5	91	4
Terbuthylazine	66.07 - 122.70	107.7	0.9993	0.005	110	3	115	2	96	5
Tetradifon	53.42 - 99.21	90.8	0.9995	0.005	91	8	93	7	89	3
Tetramethrin peak 1	70.00 - 104.99	86.8	0.9999	0.0025	104	12	105	8	95	3
Tetramethrin peak 2	60.82 - 91.23	62.8	0.9997	0.0025	99	8	98	5	91	3
Tolclofos-methyl	29.64 - 55.04	40.5	0.9984	0.005	102	5	102	2	92	4
Transfluthrin	63.35 - 117.66	76.4	0.9993	0.005	100	8	93	6	95	3
Triadimefon	37.35 - 69.36	46.9	0.9972	0.005	109	8	99	7	95	4
Triadimenol	24.53 - 45.56	26.4	0.9998	0.005	102	5	99	8	93	2
Triallate	46.43 - 86.23	69.7	0.9991	0.005	98	7	95	7	90	3
Triazophos	38.56 - 71.61	57.9	0.9999	0.005	101	4	96	6	83	3
Trifluralin	10.77 - 20.01	18.6	0.999	0.005	97	10	92	8	86	2
Vinclozolin	62.43 - 115.94	80.3	0.9993	0.005	105	4	106	6	100	4

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