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APPLICATION NOTE 72952

Large scale screening and quantitation of pesticide residues in rice using GC-(EI)-MS/MS

Authors

Subodh Kumar Budakoti, Sarvendra Pratap Singh, and Dasharath Oulkar

Customer Solution Center, Ghaziabad,
Thermo Fisher Scientific, India

Keywords

TraceFinder, pesticide residues, rice, QuEChERS, GC-MS/MS, TSQ 9000, targeted screening, quantification

Goal

The objective of this work is to develop a screening solution followed by quantitation of 159 pesticides in rice by using gas chromatography-triple quadrupole mass spectrometry. The optimized method was validated in accordance with the SANTE guidelines and fulfills the Food Safety and Standards Authority of India (FSSAI) and the European Commission (EC) maximum residue levels (MRLs) for rice.

Introduction

Rice is a staple food in India and for more than 60% of the world's population. Every year, billions of tons of rice are grown and exported, with India being the world largest exporter. To meet the ever-increasing demand for larger quantities of high quality rice, many plant protection products are used. Currently, there are 282 chemicals registered under the Central Insecticide Board and Registration Committee (CIBRC) of India.[1] These are required to reduce pest damage and increase yields but inevitably result in the occurrence of pesticide residues in rice intended for human consumption.

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Therefore, it is necessary to have suitable methods to facilitate effective monitoring of pesticide residues in rice and to ensure compliance with legislative MRLs. The lowest MRLs for pesticides have been set at 0.01 mg/kg by the European Commission (EC) and FSSAI regulations.[2,3]

The aim of this work was to optimize and validate a multi-residue method for monitoring pesticide residues in rice. Extraction of residues was performed by the widely used QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) acetonitrile extraction method.[4] Any residues in the extract were determined using the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system equipped with the ExtractaBrite source and vacuum probe interlock (VPI) technology. The data acquisition and processing were carried out using Thermo Scientific™ TraceFinder™ software. The optimized method was validated according to the SANTE/11813/2017 guidelines.[5]

Experimental

GC-MS/MS analysis

A Thermo Scientific™ TRACE™ 1310 gas chromatograph was coupled to a TSQ 9000 triple quadrupole GC-MS/MS system using electron impact (EI) ionization. The detailed, optimized GC-MS/MS conditions are given in Table 1.

Sample preparation

The rice grains were homogenized by using a heavy-duty homogenizer to get a small particle size of approximately 200–500 µm. The QuEChERS method was used for extraction as described on the next page.

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:	Spit/Spitless (SSL)
Liner:	SSL splitless liner, single taper, deactivated (P/N 453A1925)
Injector mode:	Splitless
Splitless time:	2 min
Injection volume:	1.5 µL
Injector temp.:	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 5 °C/min to 280 °C 10 °C/min to 300 °C, 1.4 min
Mass spectrometry method	
Instrumentation:	TSQ 9000 triple quadrupole mass spectrometer with ExtractaBrite ion source and VPI
Method type:	Acquisition-Timed (SRM mode)
MS transfer line temp.:	310 °C
Ion source temp.:	280 °C
Ionization:	Electron Ionization (EI)

Sample extraction and cleanup:

- Weigh 5 g of homogenized sample into a 50 mL extraction tube.
- For recovery, blank samples (n=6 for each level) were spiked before addition of water and extraction with the full mixture of target pesticides at 0.01 mg/kg and 0.05 mg/kg.
- Add 10 mL of HPLC grade water (containing 1% acetic acid) and leave the sample for 10 min soaking.
- Add 10 mL acetonitrile to the tube.
- Shake vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Add 4 g anhydrous $MgSO_4$ and 1 g sodium acetate to the tube and again mix vigorously for 1 minute on a vortex mixer at 2500 rpm.
- Centrifuge at 5000 rpm for 5 min.
- Transfer the supernatant (1 mL) into the 2 mL microcentrifuge tube containing 150 mg $MgSO_4$, 50 mg PSA.
- Vortex for 1 min at 2500 rpm and centrifuge samples with 10,000 rpm for 5 min.
- Collect the supernatant and transfer into a GC vial for instrumental analysis.
- Prepare a blank (control) extract by following the above protocol for matrix-matched (MM) calibration standards.
- Matrix-matched calibration standards were prepared by spiking pesticides into matrix extracts after extraction. See Table 2.
- The final extract as well as the MM standards were injected into the GC-MS/MS.

Data acquisition and processing

The data acquisition and processing were carried out by 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). The target list of analytes are given in Table 3 in the Appendix with their quantitative and qualitative ion, collision energies, and retention time (min). 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 criteria with user filters as per the SANTE guidelines.

Results and discussion

Sample preparation

Rice is a dry powder and a complex matrix, which has a high content of carbohydrate (80%), proteins (7%), and fiber (1.5%). Since it is a dry and neutral matrix, 10 mL water (1% acetic acid) was used to maintain the moisture content required for liquid-liquid partitioning with acetonitrile. Acidification improved the stability of base sensitive compounds during extraction. The rice matrix did not have any pigment that could interfere during analysis. Hence only PSA was used for cleanup, which offered excellent performance of the method in terms of recovery and precision at 0.1 mg/kg. There is always high matrix enhancement observed in GC-MS/MS analysis and thus the residues cannot be calculated correctly using solvent standards. To harmonize the results, a matrix-matched calibration was preferred in this experiment.

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

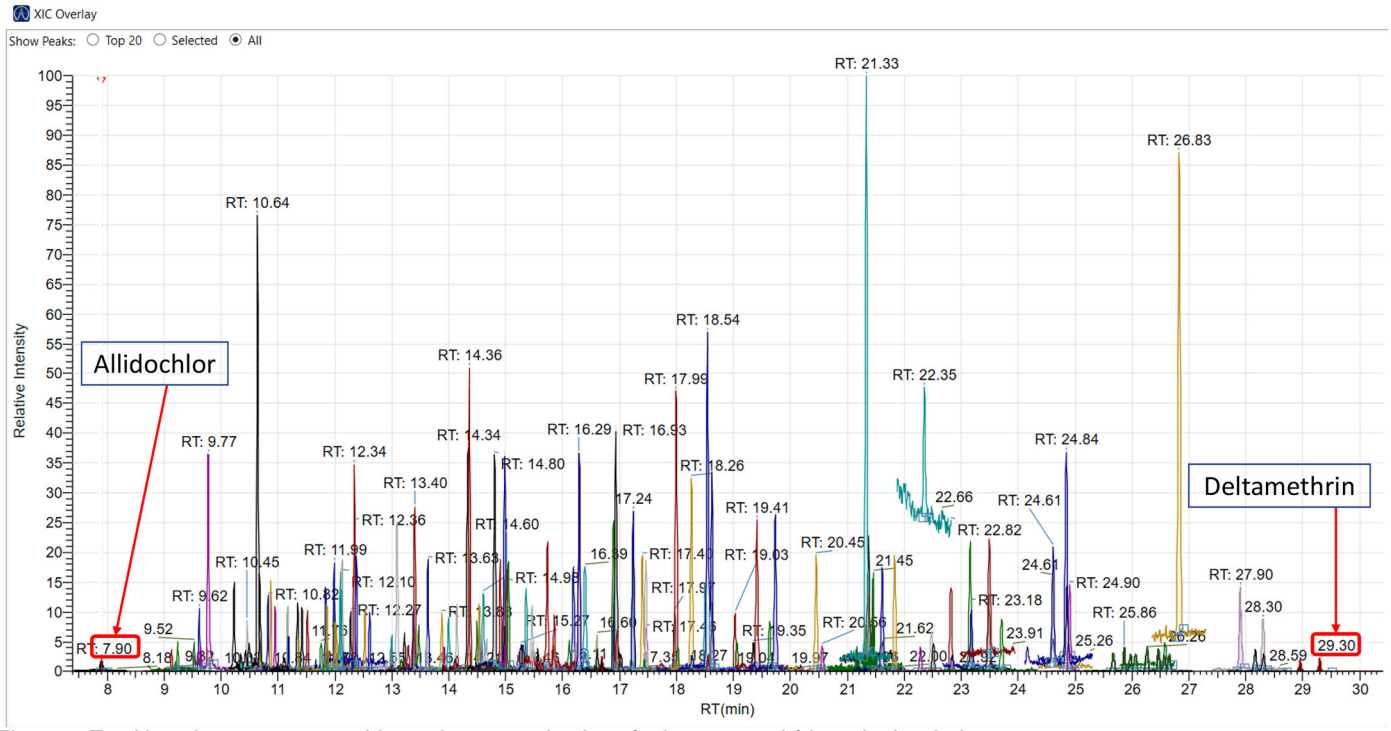

Figure 1. Total ion chromatograms with overlay extraction ions (159 compounds) in a single window

GC-MS/MS analysis

The gas chromatographic method offered excellent separation for the target analytes and absence of isobaric interference from the matrix. The total ion chromatogram (TIC) is shown in Figure 1 for 159 compounds. In this method no internal standard was used.

The number of scans across a chromatographic peak depends on the dwell time required to monitor a specific SRM transition. In general, for accurate peak integration and, in turn, for reliable quantification of a target compound, a minimum of 10 scans/peak are needed. In this method, the automatic optimized dwell time for all target analytes offered >12 points per peak. Early eluting compounds like trifluralin and etridazole have more than 12 points per peak shown in Figure 2. The optimized instrument conditions provided excellent repeatability and reproducibility. The necessary conditions used in this analysis offered excellent selectivity.

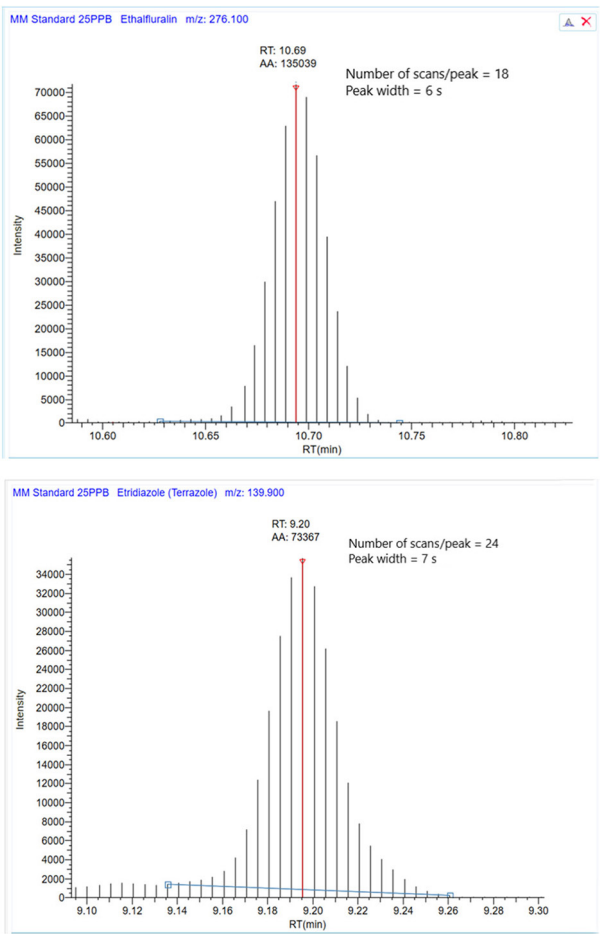

Figure 2. Impact of optimized dwell time on the data points per peak

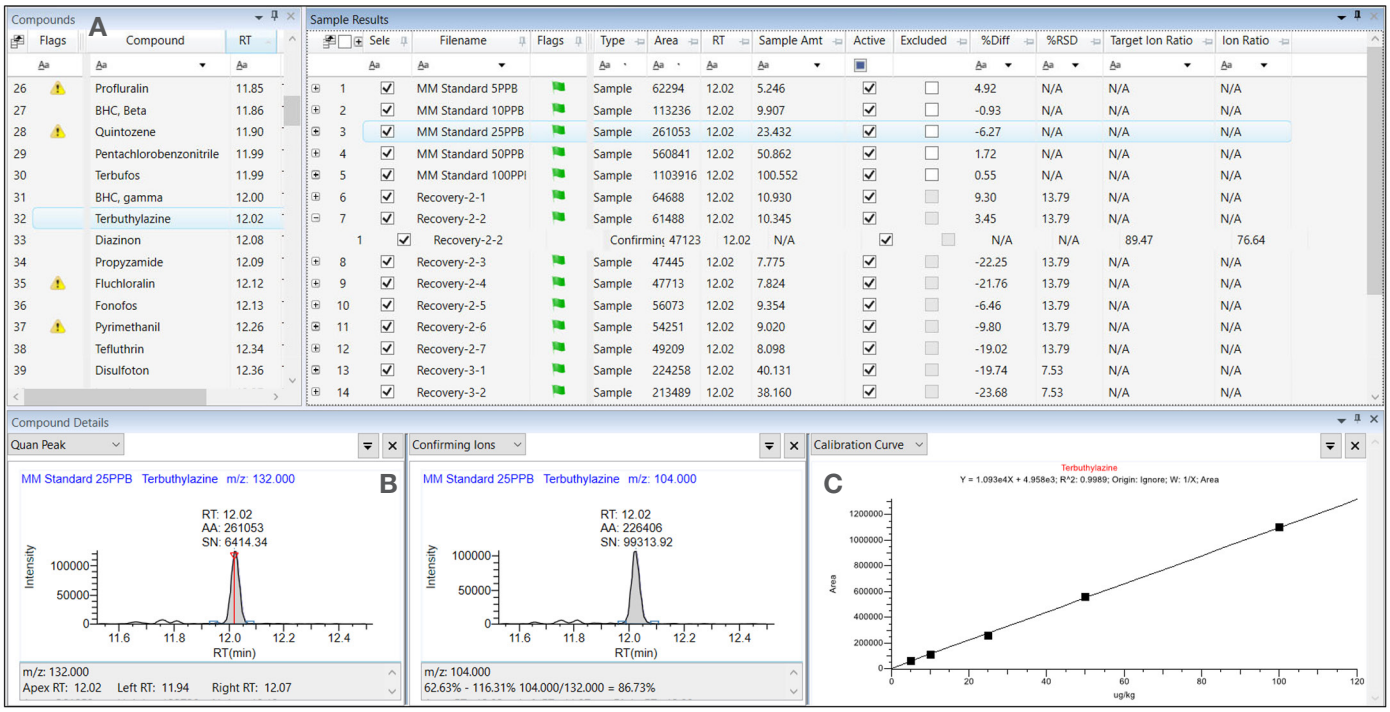

Figure 3. TraceFinder results browser showing the flagging for identification and quantitation . (A) List of target compounds; (B) SRM chromatograms of quan ion and confirmation ion; (C) calibration curve

Identification and quantitation

User-defined parameters for data processing include a minimum of two SRM transitions per analyte, retention time, correlation coefficient, and residuals ion ratio set in the data processing method of TraceFinder software. Based on these parameters, the data was processed and automated flags indicated through color codes whether results passed or failed against acceptance/user-defined criteria such as ion ratio, expected RT, etc. (SANTE guidelines) (Figure 3).

Three SRM transitions (263→109, 125→79, and 125→47) were selected for parathion-methyl to identify. In the extracted ion chromatogram (XIC), there were two adjacent peaks found close to the parathion-methyl. These three analytes have the same transition but different retention times. This different retention offered excellent selectivity and baseline separation between the three analytes.

In Figure 4, the identification of parathion methyl in rice was demonstrated with three transitions *m/z* 263→109 (quantitative), *m/z* 125→79, and *m/z* 125→47 (confirmatory) at the same retention time with 88.52% (59.91–111.26%) and 69.58% (48.99–90.98%) observed ion ratio in rice. The linearity for parathion methyl provided correlation coefficient R^2 >0.997 with <20% RSD residuals. This approach meets the requirement of the SANTE guidelines for identification and quantitation.

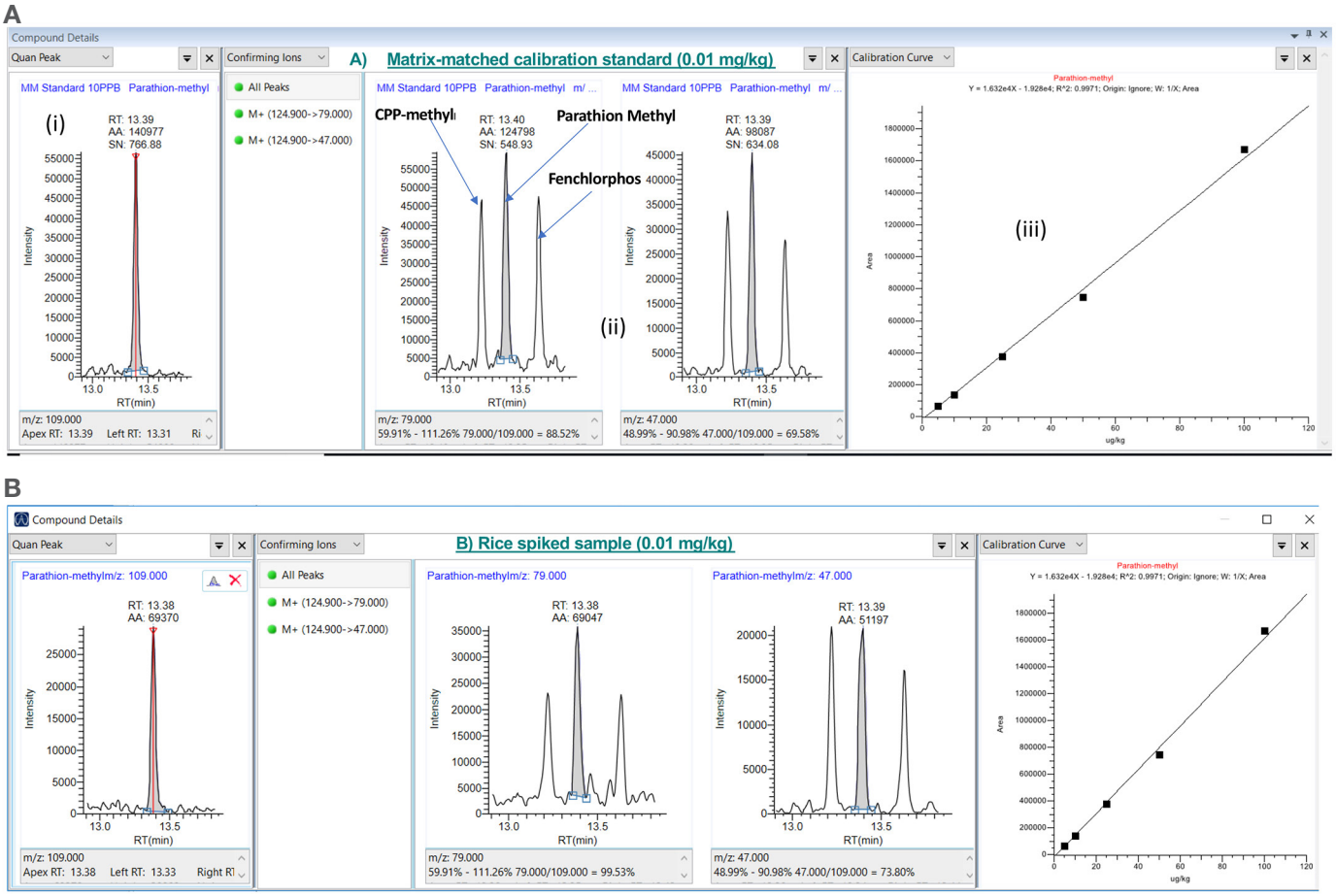

Figure 4. (A) Matrix-matched calibration standard and B) rice spiked sample at 0.01 mg/kg. (i) Extracted ion chromatogram for quantifier ion of parathion-methyl (ii) Identification based on the selectivity of confirmatory ions separated from isobaric interference caused by chlorpyrifos (CPP) methyl and fenchlorphos peaks and confirmed with ion ratio, and (iii) calibration curve over a concentration range of 0.005–0.100 mg/kg.

Method performance

In this method, the linearity was plotted in the range of 0.005 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 the rice matrix. The lower calibration level (0.005 ng/g) showed good sensitivity with ≥15–20 signal-to-noise ratio. But as per the extraction protocol, the sample is diluted twofold. Therefore, the limit of quantitation (LOQ) values observed in the rice matrix were 0.01 mg/kg with acceptable recoveries (70–120%) and precision (<20%). The recovery experiment was carried out at 0.01 (LOQ) and 0.05 (LOQ × 5) mg/kg to demonstrate the method accuracy and precision (n = 6). Average recoveries were observed in the range of 76 to 116% with <15% RSD (Table 2, Appendix), which were within acceptance criteria (recovery 70–120% and precision <20%) of the SANTE guidelines.[5] Few pesticides were considered as default MRLs (0.01 mg/kg set at LOQ) whose MRL is not available in both the EU and FSSAI requirements. Also, the method was tested for repeatability of results obtained in through large batch (n = 50 injections) by simulating a commercial food testing lab schedule. The repeatability of peak area was <15% and <±0.05 min retention time. It reveals that the optimized method offered excellent repeatability in results. Area repeatability for some compounds is shown in Figure 5, and retention time and ion ratio repeatability is shown in Figures 6 and 7.

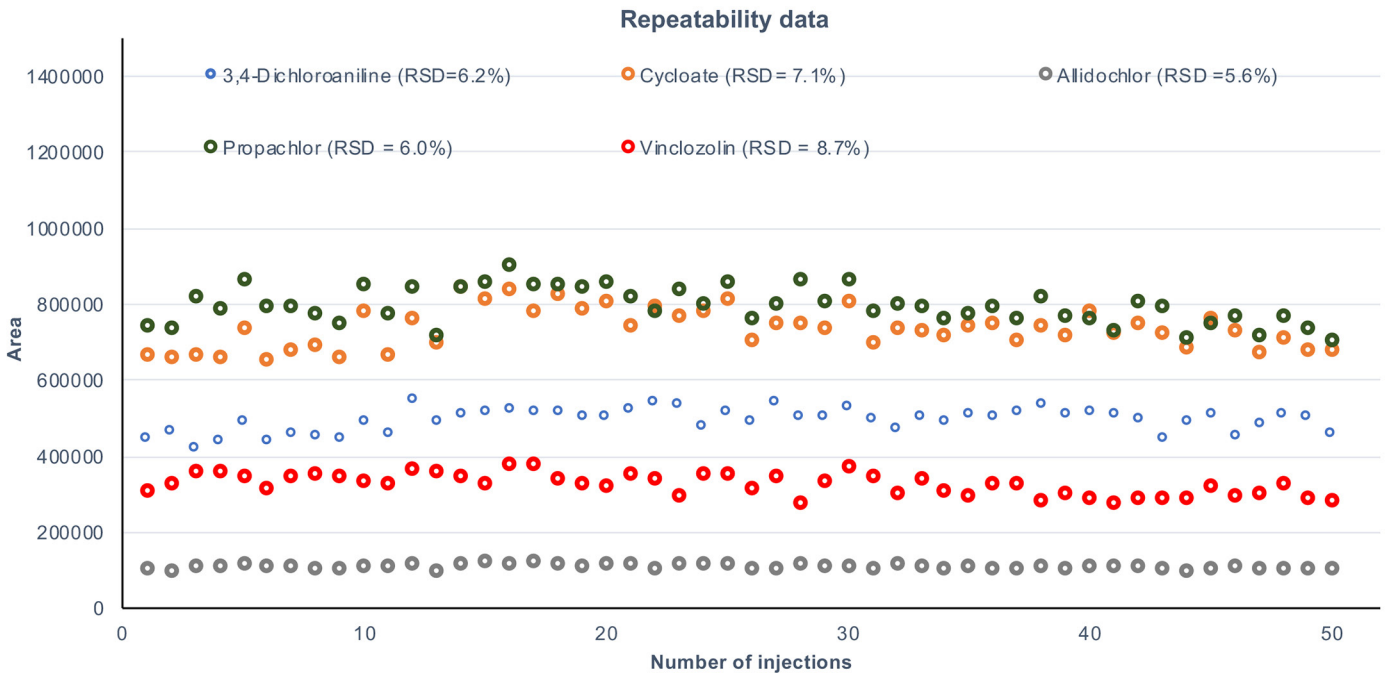

Figure 5. Absolute peak area repeatability for 2,3,5,6 dichloroaniline, cycloate, allidochlor, propachlor, and vinclozolin (n=50). No internal standard correction was applied.

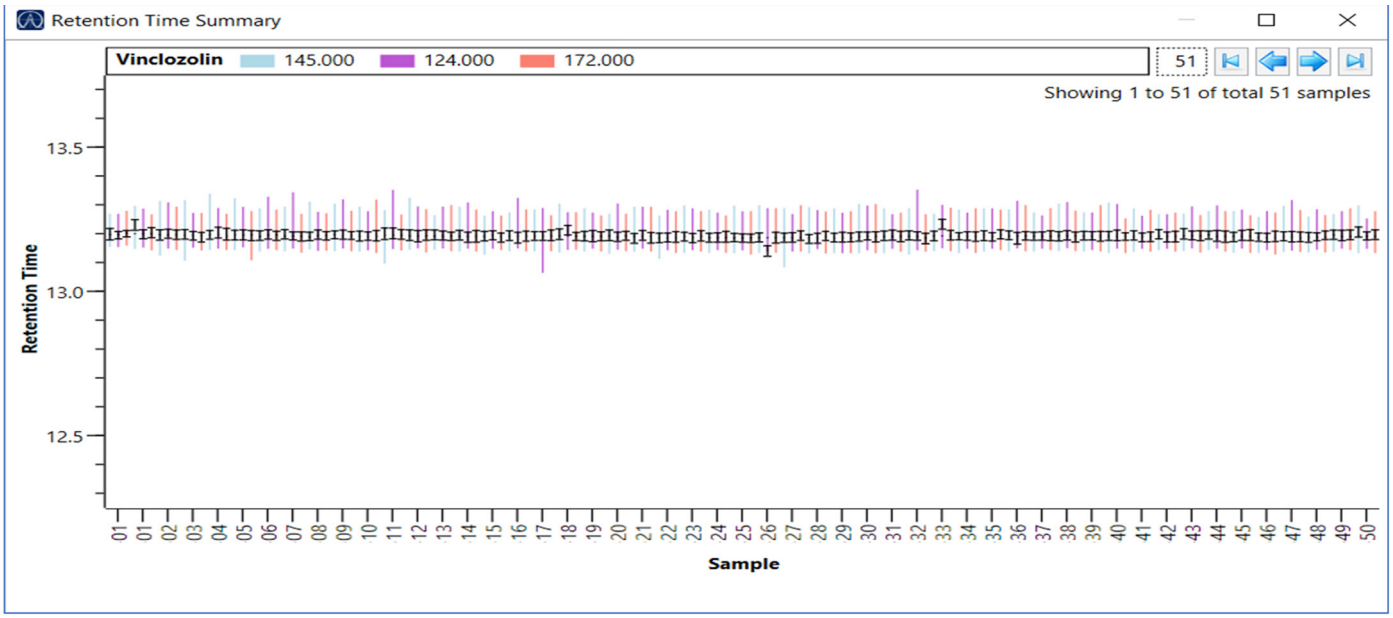

Figure 6. Retention time repeatability for vinclozolin (n = 50)

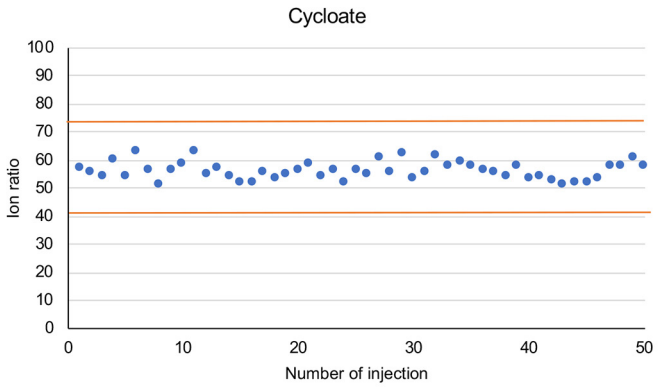

Figure 7. Ion ratio repeatability for cycloate (n=50)

Conclusion

This application note provides a solution for the simultaneous screening and quantitation of 159 pesticide residues in rice using the Thermo Scientific GC-EI-MS/MS system. The use of the QuEChERS method for extraction and cleanup followed by analysis using GC-MS/MS decreased downtime and increased productivity for commercial food testing laboratories. Using this approach, at least 40 injections (standards, samples, blank) could completed in a day (24 hr cycle).

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 improves accuracy and precision of the determination. This validated method data meets the requirement of the SANTE guidelines and complies with the EU as well as FSSAI MRLs requirement by achieving an excellent lower limit of quantitation (LLOQ).

References

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4. Lehotay, S.J.; Maštovská, K.; Lightfield. A.R. Use of buffering and other means to improve the results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. J. AOAC Int. 2005, 88, 615–630; (Note: Basis of the AOAC Method 2007.01).
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Appendix

Table 3 (part 1). List of pesticides with SRM transitions used and validation data (ion ratio, linearity, recovery, and precision) and MRLs (FSSAI and EU)

Pesticide Name	RT (min)	Quantitive ion			Confirmatory ion 1			Ion ratio (IR)		R^2	LOQ (mg/kg)	% Recovery (%RSD)		MRLs (mg/kg)	
		Q1	Q3	CE (V)	Q1	Q3	CE (V)	IR range in MMS	IR in rice 0.01 (mg/kg)			0.01 (mg/kg)	0.05 (mg/kg)	FSSAI	EU
Allidochlor	7.88	132	56.1	8	132	49	24	8.85 - 16.43	13.12	0.9998	0.01	92.9 (11.6)	90.0 (5.9)	-	-
3,4-Dichloroaniline	9.12	160.9	126	10	160.9	99	20	118.51 - 220.10	189.33	0.9997	0.01	100.6 (6.2)	94.1 (9.4)	-	-
Etridiazole (Terrazole)	9.19	211	139.9	20	211	182.9	10	103.40 - 192.02	144.58	0.9973	0.01	101.9 (14.2)	79.3 (6.8)	-	0.05
Pebulate	9.23	128	57	8	203	128	8	12.50 - 23.22	22.02	0.9953	0.01	85.1 (13.2)	88.2 (8.3)	-	-
Methacrifos	9.52	240	180	10	180	93	10	66.15 - 122.86	98.02	0.9976	0.01	88.8 (7.7)	92.2 (8.0)	-	0.01
Chloroneb	9.61	190.9	141	10	190.9	113	14	71.54 - 132.86	95.41	0.9997	0.01	84.5 (10.2)	91.2 (8.8)	-	-
Pentachlorobenzene	9.75	249.8	214.8	16	249.8	143.6	38	20.95 - 38.91	32.98	0.9963	0.01	82.7 (9.1)	85.4 (8.4)	-	-
Ortho-phenylphenol	9.77	170.1	141.1	22	170.1	115	34	58.40 - 108.45	85.83	0.9986	0.01	94.4 (9.6)	86.0 (8.1)	-	-
Tecnazene	10.34	261	203	13	215	179	8	46.38 - 86.13	57.59	0.9989	0.01	94.7 (11.5)	81.6 (6.4)	-	0.01
Propachlor	10.45	176	57	8	120	77	19	108.22 - 200.98	160.2	0.999	0.01	86.1 (6.6)	92.1 (7.2)	-	0.02
Diphenylamine	10.63	169	168	12	169	167	25	29.87 - 55.47	43.63	0.9999	0.01	87.2 (10.8)	92.1 (6.3)	-	0.05
2,3,5,6-Tetrachloroaniline	10.67	231	158	20	231	160	22	63.61 - 118.14	115.18	0.9983	0.01	82.9 (11.4)	92.8 (7.1)	-	-
Cycloate	10.68	154.1	83.1	8	154.1	55.1	18	32.41 - 60.19	42.92	0.9995	0.01	80.1 (7.2)	86.3 (4.3)	-	-
Ethalfluralin	10.7	315.9	276.1	8	276	202	14	96.29 - 178.82	167.34	0.9968	0.01	100.4 (6.8)	96.7 (4.1)	-	0.01

Q1 = Precursor ion, Q3 = Product ion *MRL limit for DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD expressed as DDT),
**MRL limit of endosulfan (sum of endosulfan I, endosulfan II, and endosulfan sulfate). Those compounds without MRL values were considered as the default, i.e., 0.01 mg/kg (set at LOQ).

Table 3 (part 2). List of pesticides with SRM transitions used and validation data (ion ratio, linearity, recovery, and precision) and MRLs (FSSAI and EU)

Pesticide Name	RT (min)	Quantitive ion			Confirmatory ion 1			Ion ratio (IR)		R^2	LOQ (mg/kg)	% Recovery (%RSD)		MRLs (mg/kg)	
		Q1	Q3	CE (V)	Q1	Q3	CE (V)	IR range in MMS	IR in rice 0.01 (mg/kg)			0.01 (mg/kg)	0.05 (mg/kg)	FSSAI	EU
Benfluralin	10.87	292	264	8	292	206.1	10	27.64 - 51.33	35.06	0.998	0.01	114.7 (2.9)	96.7 (3.9)	-	0.02
Sulfotep	10.95	322	202	10	202	145.9	10	80.99 - 150.40	139.02	0.9984	0.01	106.4 (5.5)	94.7 (7.5)	-	-
Diallate-cis	11.17	234.1	150	18	234	192	12	64.66 - 120.09	92.53	0.9989	0.01	96.8 (5.6)	89.3 (7.6)	-	-
Phorate	11.19	260	75	8	121	65	8	105.65 - 196.20	171.36	0.9957	0.01	103.4 (9.5)	92.6 (7.4)	-	0.02
BHC, Alpha	11.34	218.8	183	8	182.8	146.7	12	38.74 - 71.95	51.09	0.9983	0.01	89.1 (7.8)	94.4 (6.2)	0.05	0.01
Diallate-trans	11.35	234.1	150	18	234.1	192	12	53.61 - 99.55	88.46	0.9969	0.01	94.6 (12.4)	85.6 (10.1)	-	-
Hexachlorobenzene	11.42	283.8	248.8	18	283.8	213.8	30	58.30 - 108.27	89.4	0.9989	0.01	86.4 (8.8)	79.8 (7.4)	-	0.01
Pentachloroanisole	11.52	279.9	236.8	22	264.8	236.9	10	164.43 - 305.37	209.79	0.9998	0.01	86.8 (8.7)	83.5 (8.9)	-	-
Atrazine	11.75	200	122.1	8	215.1	173	8	69.81 - 129.65	103.5	0.998	0.01	93.5 (14.2)	91.7 (5.5)	-	0.05
Clomazone	11.83	204	107	18	125	89	13	49.40 - 91.74	66.32	0.9985	0.01	95.4 (7.7)	97.4 (7.4)	0.01	0.01
Profluralin	11.85	318.1	199	15	330.2	69.1	20	14.90 - 27.67	20.97	0.9961	0.01	111.2 (8.8)	92.8 (6.9)	-	-
BHC, Beta	11.86	218.7	183	8	180.9	145	14	79.33 - 147.32	111.93	0.9996	0.01	90.5 (7.5)	93.0 (3.7)	0.05	0.01
Quintozene	11.9	294.8	236.9	14	213.8	178.9	14	63.79 - 118.47	111.35	0.999	0.01	95.7 (10.0)	82.8 (5.3)	-	0.02
Pentachlorobenzonitrile	11.99	274.8	239.9	18	272.9	237.9	16	41.51 - 77.10	60.81	0.9953	0.01	101.6 (4.8)	87.3 (6.4)	-	-
Terbufos	11.99	230.9	128.9	22	230.9	174.9	12	66.30 - 123.12	89.29	0.9957	0.01	90.5 (8.0)	92.3 (4.9)	-	0.01
BHC, gamma	12.01	218.7	183	8	180.9	145	14	85.50 - 158.79	105.23	0.9995	0.01	89.9 (11.7)	90.5 (5.1)	0.05	0.01
Terbuthylazine	12.02	214.1	132	10	214.1	104	16	62.63 - 116.31	88.21	0.9989	0.01	90.5 (13.8)	85.8 (7.5)	-	0.05
Diazinon	12.08	137.1	84.1	12	137.1	54.1	20	38.87 - 72.19	53.57	0.9998	0.01	98.9 (12.8)	97.1 (4.9)	-	0.01
Propyzamide	12.09	172.9	109	26	172.9	145	14	161.53 - 299.98	244.22	0.9999	0.01	91.1 (3.9)	97.0 (3.9)	-	0.01
Fluchloralin	12.12	306	264	8	264	206.1	8	16.05 - 29.81	19.17	0.9977	0.01	91.8 (15.2)	95.3 (5.6)	-	-
Fonofos	12.13	246	137	6	137	109	6	87.33 - 162.19	124.39	0.9996	0.01	111.8 (3.9)	92.0 (6.7)	-	-
Pyrimethanil	12.26	198.1	117.9	30	198.1	182.9	14	85.59 - 158.96	113.08	0.999	0.01	96.1 (7.4)	94.5 (3.7)	-	0.05
Tefluthrin	12.34	177	127	14	197	141.1	10	33.83 - 62.82	52.85	0.9988	0.01	90.7 (4.4)	93.5 (6.5)	-	0.05
Disulfoton	12.36	185.9	96.9	16	88	59.8	6	437.68 - 812.84	642.01	0.9992	0.01	89.4 (10.8)	93.8 (7.6)	-	0.02
Isazophos	12.37	161	119	8	161	146	6	18.14 - 33.69	31.32	0.9995	0.01	89.1 (9.0)	95.5 (5.4)	-	-
Triallate	12.53	268	226	12	86.1	43.3	6	115.87 - 215.19	175.09	0.9984	0.01	87.4 (6.8)	86.0 (8.1)	-	-
BHC, delta	12.61	218.8	182.9	8	182.8	146.7	14	39.96 - 74.20	60.22	0.9996	0.01	87.2 (8.4)	88.3 (6.5)	0.05	0.01
Endosulfan ether	13.01	240.9	206	14	238.9	204	12	68.69 - 127.56	72.63	0.9934	0.01	87.1 (12.6)	93.2 (6.7)	-	0.05
Pentachloroaniline	13.01	264.8	193.6	18	264.8	202.8	20	39.85 - 74.01	52.17	0.9997	0.01	85.4 (11.2)	98.7 (4.2)	-	0.02
Dimethachlor	13.09	197	148.1	10	134	105.1	12	48.73 - 90.51	68.45	0.9991	0.01	89.1 (8.1)	93.5 (4.3)	-	0.01
Chlorpyrifos-methyl	13.22	285.9	93	20	125	47	12	53.55 - 99.44	66.61	0.9978	0.01	100.2 (10.4)	88.2 (5.4)	-	3
Vinclozolin	13.29	198	145	14	186.8	124	18	72.43 - 134.52	109.01	1	0.01	94.4 (5.4)	95.1 (4.6)	-	0.01
Parathion-methyl	13.38	263	109	12	124.9	79	6	59.91 - 111.26	99.53	0.9971	0.01	106.1 (4.4)	84.6 (7.3)	0.01	0.02
Transfluthrin	13.38	163	143	14	163	91.1	12	74.25 - 137.88	103.01	0.9998	0.01	98.8 (4.2)	90.2 (5.1)	-	-
Alachlor	13.39	188.1	130	32	188.1	160.1	8	151.82 - 281.94	217.66	0.9975	0.01	95.4 (10.9)	89.6 (4.6)	-	0.01
Tolclofos-methyl	13.4	265	250	12	266.8	252	12	26.47 - 49.15	38.33	0.9993	0.01	97.7 (4.3)	93.3 (4.3)	-	-
Propisochlor	13.47	162.1	144.1	8	162.1	120.1	12	88.25 - 163.90	136.86	0.9959	0.01	98.3 (15.1)	90.2 (4.9)	-	0.01
Heptachlor	13.61	99.8	65	12	271.8	236.9	12	152.72 - 283.62	195.83	0.999	0.01	82.6 (12.9)	88.5 (7.7)	-	0.01
Fenchlorfos	13.64	285	270	11	287	272	11	48.25 - 89.60	68.89	0.9997	0.01	101.12 (6.0)	90.9 (5.9)	-	0.01
Pirimiphos-methyl	13.87	290.1	233	8	305.1	180.1	8	73.98 - 137.39	101.92	0.9989	0.01	96.3 (7.1)	90.9 (3.3)	0.5	-
Prodiamine	13.92	321.1	279.1	6	275.1	255.1	8	25.07 - 46.56	43.32	0.9941	0.01	113.3 (4.3)	90.0 (5.9)	-	-
Fenitrothion	13.99	277	260	6	277	109	16	31.92 - 59.29	53.01	0.9976	0.01	109.0 (6.9)	84.3 (2.0)	-	0.05
Pentachlorothioanisole	14.14	295.7	262.9	12	295.7	245.9	30	22.74 - 42.23	29.66	0.9997	0.01	83.5 (7.9)	87.3 (3.2)	-	-
Metolachlor	14.33	238.1	162.2	10	162.1	132.9	14	53.34 - 99.07	79.44	0.9997	0.01	95.6 (4.1)	93.8 (4.1)	-	0.05
Chlorpyrifos-ethyl	14.36	313.9	257.9	12	196.7	168.9	12	61.77 - 114.71	87.24	0.9982	0.01	103.7 (6.1)	95.7 (4.0)	-	0.05

Q1 = Precursor ion, Q3 = Product ion *MRL limit for DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD expressed as DDT),
**MRL limit of endosulfan (sum of endosulfan I, endosulfan II, and endosulfan sulfate). Those compounds without MRL values were considered as the default, i.e., 0.01 mg/kg (set at LOQ).

Table 3 (part 3). List of pesticides with SRM transitions used and validation data (ion ratio, linearity, recovery, and precision) and MRLs (FSSAI and EU)

Pesticide Name	RT (min)	Quantitive ion			Confirmatory ion 1			Ion ratio (IR)		R^2	LOQ (mg/kg)	% Recovery (%RSD)		MRLs (mg/kg)	
		Q1	Q3	CE (V)	Q1	Q3	CE (V)	IR range in MMS	IR in rice 0.01 (mg/kg)			0.01 (mg/kg)	0.05 (mg/kg)	FSSAI	EU
Fenthion	14.49	278	169	14	278	109	18	151.40 - 281.18	231.08	0.9997	0.01	98.7 (10.2)	88.4 (5.7)	-	0.01
Aldrin	14.51	330	298.9	10	262.7	192.9	28	29.02 - 53.90	44.35	0.9988	0.01	97.8 (7.2)	93.0 (5.8)	-	0.01
Chlorthal-dimethyl (Dacthal)	14.53	300.7	222.9	22	300.7	272.9	12	59.74 - 110.95	74.11	0.9991	0.01	101.3 (10.9)	97.8 (4.5)	-	0.01
Parathion (ethyl)	14.57	291	109	12	109	81	10	59.86 - 111.17	95.27	0.9997	0.01	108.7 (4.5)	89.1 (4.0)	-	0.05
Anthraquinone	14.59	208	151.7	22	208	180	10	88.79 - 164.89	144.27	0.9998	0.01	110.6 (5.2)	100.8 (4.3)	-	0.01
Triadimefon	14.66	208	111	20	208	180.8	8	120.27 - 223.36	147.28	0.9992	0.01	104.7 (4.7)	98.4 (5.8)	-	0.01
Dichlorobenzophenone, 4, 4	14.8	139	111	12	139	74.9	26	27.35 - 50.79	41.7	0.9994	0.01	98.5 (4.1)	91.5 (3.3)	-	-
Pirimiphos-ethyl	14.9	304	168.1	12	318.1	166.1	12	29.21 - 54.26	45.52	0.9995	0.01	85.9 (6.6)	94.8 (2.0)	-	-
Bromophos-methyl (Bromophos)	14.98	330.8	315.8	14	328.9	313.8	14	43.30 - 80.41	65.65	0.9994	0.01	94.4 (7.4)	89.0 (3.9)	-	-
Fenson	14.98	141	77	8	77	51	14	24.22 - 44.99	33.86	0.9999	0.01	94.9 (7.7)	95.5 (3.3)	-	-
MGK-264 A	15.01	164	93.1	10	164	98.1	10	40.11 - 74.49	63.21	0.999	0.01	97.2 (11.0)	98.6 (3.4)	-	-
Diphenamid	15.01	239.1	167.1	8	166.8	152	16	117.91 - 218.98	161.34	0.999	0.01	86.3 (6.0)	91.4 (3.3)	-	-
Isopropalin	15.05	280.1	238.2	8	280.1	180.2	10	9.56 - 17.75	17.69	0.996	0.01	116.1 (2.1)	90.0 (2.9)	-	-
Pendimethalin	15.27	252.1	162	8	252.1	161	14	38.80 - 72.06	56.01	0.9985	0.01	116.5 (1.8)	88.7 (7.4)	0.05	0.05
Isodrin	15.3	192.9	123	28	192.9	157.2	20	37.48 - 69.60	62.6	0.9957	0.01	98.8 (13.9)	85.9 (11.1)	-	-
Cyprodinil	15.32	224.1	196.9	20	224.1	208	18	352.84 - 655.27	601.75	0.9973	0.01	106.3 (6.2)	94.9 (6.6)	-	0.02
MGK-264 B	15.34	164	98	10	164	67.1	8	91.41 - 169.77	126	0.9926	0.01	84.9 (10.8)	94.8 (2.4)	-	-
Metazachlor	15.36	209	132.1	16	133.1	132.1	12	86.78 - 161.17	135.62	0.9996	0.01	94.7 (5.3)	89.1 (2.5)	-	0.02
Penconazole	15.46	248	192	12	248	157	22	90.40 - 167.89	118.49	0.9981	0.01	99.8 (9.3)	96.9 (4.3)	-	0.05
Chlozolinate	15.47	331	259	8	259	187.9	12	37.80 - 70.20	44.1	0.9983	0.01	106.9 (12.6)	86.6 (4.8)	-	0.01
Heptachlor epoxide	15.57	352.8	262.9	16	354.7	264.9	12	46.96 - 87.21	60.59	0.999	0.01	101.8 (9.2)	93.0 (4.3)	-	0.01
Quinalphos	15.73	146	118.1	10	157.1	102	22	13.28 - 24.67	18.01	0.9995	0.01	104.8 (8.0)	95.0 (3.8)	0.01	-
Procymidone	15.85	283	96.1	8	95.9	53	16	46.39 - 86.15	72.05	0.9992	0.01	85.7 (7.8)	92.7 (3.5)	-	-
Triadimenol	15.85	128	65	18	168.2	70	10	207.66 - 385.66	377.96	0.9987	0.01	101.3 (12.2)	90.7 (6.4)	-	-
Bromophos-ethyl	16.12	302.7	284.8	14	96.9	65	16	30.37 - 56.41	45.75	0.9964	0.01	93.5 (6.4)	93.6 (4.5)	-	0.01
Chlorbenside	16.19	125	89	16	125	99	16	34.90 - 64.81	50.97	0.9998	0.01	90.9 (5.7)	89.2 (2.9)	-	0.01
Chlordane alpha-*cis*	16.23	374.7	265.8	20	372.8	265.8	20	72.10 - 133.90	91.68	0.9995	0.01	92.5 (11.9)	94.7 (4.4)	-	-
DDE o,p	16.29	246	176.1	28	317.8	248	18	23.76 - 44.13	32.86	0.9997	0.01	91.9 (8.1)	86.1 (3.9)	-	0.05
Paclobutrazol	16.38	236	125	12	236	167	10	21.14 - 39.26	31.66	0.9993	0.01	99.7 (1.9)	93.8 (3.1)	-	-
Chlordane gamma-*trans*	16.61	374.7	265.9	20	372.7	263.7	20	54.79 - 101.74	92.32	0.9999	0.01	83.1 (11.3)	94.7 (2.5)	-	-
Endosulfan I	16.62	240.6	205.9	14	194.7	125	22	31.89 - 59.22	32.73	0.9984	0.01	92.5 (14.9)	94.9 (6.6)	-	0.05**
Nonachlor-*cis*	16.69	408.6	300	18	262.8	192.8	28	25.37 - 47.12	35.43	0.9986	0.01	88.0 (12.6)	89.7 (6.0)	-	-
Flutolanil	16.89	281	173	10	173	145	14	158.69 - 294.71	222.13	0.9995	0.01	108.4 (2.6)	98.0 (3.4)	-	2
Iodofenfos	16.9	376.8	361.8	16	125	79	6	18.36 - 34.09	30.52	0.9986	0.01	102.2 (6.0)	88.6 (4.0)	-	-
Chlorfenson	16.93	174.9	111	10	111	75	14	21.16 - 39.29	29.55	0.9997	0.01	93.8 (5.5)	94.2 (2.1)	-	0.01
Prothiofos	16.96	266.7	220.9	18	266.7	238.9	8	142.35 - 264.37	195.48	0.9997	0.01	98.8 (12.1)	89.6 (3.4)	-	-
Pretilachlor	17.02	238.1	146.1	10	202.1	174.2	8	85.32 - 158.44	90.83	0.9967	0.01	113.1 (14.4)	94.3 (5.9)	0.05	-
Oxadiazon	17.23	174.9	112	12	174.9	76	28	23.56 - 43.75	31.82	0.9997	0.01	91.5 (6.1)	94.7 (3.0)	0.03	0.05
DDE p, p	17.24	246	176.1	28	317.8	246	20	40.23 - 74.72	53.82	0.9997	0.01	87.3 (5.7)	85.1 (2.9)	-	0.05*
Myclobutanil	17.39	179	125	14	179	151.7	8	19.50 - 36.21	25.65	0.9998	0.01	97.2 (7.5)	94.1 (2.3)	-	0.02
Dieldrin	17.43	262.8	227.8	16	262.8	190.9	30	123.93 - 230.15	223.86	0.9964	0.01	100.4 (13.0)	95.1 (6.3)	-	0.01
Oxyfluorfen	17.44	252	146	30	252	169.8	28	52.87 - 98.19	59.05	0.9996	0.01	97.55 (11.5)	90.5 (8.7)	0.05	0.05
Flusilazole	17.46	233	164.9	16	233	151.9	14	42.03 - 78.06	64.01	0.9994	0.01	106.2 (6.6)	99.9 (4.1)	-	0.01
Bupirimate	17.47	208.1	140.1	12	273.1	193.2	8	385.47 - 715.87	542.73	0.9957	0.01	94.7 (9.3)	98.2 (7.8)	-	0.05
DDD, o, p	17.47	235	199	14	235	165.1	20	191.04 - 354.79	303.93	0.9991	0.01	94.9 (2.3)	94.1 (3.7)	-	0.05*

Q1 = Precursor ion, Q3 = Product ion *MRL limit for DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD expressed as DDT), **MRL limit of endosulfan (sum of endosulfan I, endosulfan II and endosulfan sulfate). Those compounds without MRL values were considered as the default, i.e., 0.01 mg/kg (set at LOQ).

Table 3 (part 4). List of pesticides with SRM transitions used and validation data (ion ratio, linearity, recovery, and precision) and MRLs (FSSAI and EU)

Pesticide Name	RT (min)	Quantitive ion			Confirmatory ion 1			Ion ratio (IR)		R^2	LOQ (mg/kg)	% Recovery (%RSD)		MRLs (mg/kg)	
		Q1	Q3	CE (V)	Q1	Q3	CE (V)	IR range in MMS	IR in rice 0.01 (mg/kg)			0.01 (mg/kg)	0.05 (mg/kg)	FSSAI	EU
Chlorfenapyr	17.77	248.9	112	24	248.9	137.1	18	75.61 - 140.42	121.07	0.9963	0.01	83.4 (14.8)	98.8 (12.8)	-	0.02
Fluazifop-P-butyl	17.97	383.1	282.1	14	282	91.1	18	60.50 - 112.36	91.02	0.9991	0.01	107.6 (4.4)	93.4 (1.3)	-	0.01
Perthane (Ethylan)	17.99	223.1	167	12	223.1	179	20	48.00 - 89.14	59.03	1	0.01	96.0 (3.7)	94.7 (1.2)	-	-
Nitrofen	18.03	202	139	24	283	202	10	41.88 - 77.77	59.17	0.9996	0.01	99.5 (6.0)	75.1 (4.8)	-	0.01
Endrin	18.06	280.8	245.3	8	245	173	22	163.53 - 303.70	246.23	0.9928	0.01	94.9 (15.7)	83.0 (10.4)	-	0.01
Chlorobenzilate	18.26	139	111	12	139	74.9	26	26.53 - 49.27	39.81	0.9999	0.01	95.4 (2.2)	93.7 (1.4)	-	0.02
Endosulfan II	18.41	240.6	205.8	12	194.7	159	8	72.13 - 133.95	82.35	0.9992	0.01	84.56 (11.7)	95.0 (6.5)	-	0.05**
Ethion	18.51	230.9	174.9	12	230.9	128.9	22	77.37 - 143.68	110.56	0.999	0.01	108.9 (5.4)	97.7 (2.2)	-	0.01
DDD p,p	18.55	235	165.1	20	236.8	165	20	43.42 - 80.63	63.04	0.9989	0.01	95.4 (3.8)	97.5 (2.7)	-	0.05*
Nonachlor-trans	18.55	408.7	300	18	236.8	142.9	24	18.12 - 33.65	31.64	0.999	0.01	96.9 (13.6)	87.8 (7.0)	-	-
DDT o,p	18.62	235	165.1	20	236.8	165	20	41.78 - 77.59	68.48	0.9995	0.01	95.7 (3.8)	91.7 (2.5)	-	0.05*
Chlorthiophos	18.63	268.9	205	14	324.9	269	12	115.11 - 213.78	160.75	0.9995	0.01	87.9 (3.9)	78.0 (1.9)	-	-
Endrin Aldehyde	18.87	249.8	214.9	24	173	138.1	16	72.31 - 134.29	91.66	0.9979	0.01	103.6 (10.0)	84.0 (9.9)	-	-
Sulprofos	19.03	322	156.1	10	156	141	14	81.74 - 151.80	128.31	0.9995	0.01	104.8 (2.3)	92.7 (3.1)	-	-
Triazophos	19.06	161	106.1	12	161	134.1	8	133.28 - 247.52	183.26	0.9998	0.01	94.9 (5.5)	92.4 (3.1)	0.6	0.02
Carfentrazon-ethyl	19.3	311.9	150.7	18	340.1	312.1	10	564.06 - 1047.54	772.8	0.9961	0.01	106.8 (6.4)	99.0 (4.3)	-	0.05
Carbophenothion	19.35	342	157	10	157	45	12	98.12 - 182.22	129.09	0.9984	0.01	108.9 (5.0)	91.7 (5.3)	-	-
4,4'-Methoxychlor olefin	19.41	238.1	223.1	10	308	238.2	12	52.46 - 97.42	75.75	0.9996	0.01	101.8 (5.3)	91.5 (2.1)	-	-
Endosulfan sulfate	19.63	271.7	236.8	12	238.7	203.9	12	15.53 - 28.83	22.11	0.9998	0.01	77.7 (4.5)	81.3 (4.1)	-	0.05**
DDT p,p	19.74	235	165.1	22	236.8	165	22	45.54 - 84.57	59.93	0.9997	0.01	88.6 (5.5)	71.6 (1.7)	-	0.05*
Piperonyl butoxide	20.44	176.1	131.1	12	176.1	103.1	22	53.90 - 100.10	78.6	0.9997	0.01	111.7 (2.3)	92.7 (2.4)	-	-
Nitralin	20.56	316.2	274	8	274	216	8	17.83 - 33.11	26.61	0.9998	0.01	97.3 (4.1)	72.6 (3.5)	-	-
Bifenthrin	21.34	181	165.9	10	181	179	12	5.62 - 10.45	7.8	0.9996	0.01	88.5 (3.1)	97.5 (0.8)	0.05	0.01
EPN	21.37	169	141	8	169	77	22	25.53 - 47.40	34.59	0.9997	0.01	98.9 (6.3)	80.2 (3.4)	-	-
Bromopropylate	21.39	340.8	185	14	184.9	156.9	12	106.70 - 198.16	143.03	0.9999	0.01	99.1 (3.2)	97.4 (0.9)	-	0.01
Methoxychlor	21.61	227.1	169.1	22	227.1	141.1	32	63.93 - 118.73	84.49	0.9994	0.01	91.7 (3.3)	74.5 (1.8)	-	0.01
Tebufenpyrad	21.83	276.1	171	10	318.1	131.1	14	34.25 - 63.61	53.25	0.9994	0.01	83.3 (3.6)	88.6 (1.5)	-	0.01
Tetradifon	22.28	159	111	20	159	131	10	145.70 - 270.58	210.52	0.9999	0.01	87.9 (5.3)	87.5 (1.1)	-	0.01
Phenothrin	22.35	123.1	81.1	8	123.1	41.1	24	13.63 - 25.30	18.32	0.9995	0.01	102.6 (7.4)	87.9 (1.7)	-	0.05
Phosalone	22.47	182	74.8	30	182	111	14	159.26 - 295.76	234.54	0.9998	0.01	89.1 (3.7)	79.3 (2.5)	-	0.01
Leptophos	22.51	171	124.3	10	171	77.1	18	173.67 - 322.53	239.86	0.9994	0.01	89.2 (5.5)	76.0 (1.6)	-	-
Pyriproxyfen	22.81	136.1	96	10	136.1	78	20	67.93 - 126.16	98.24	0.9994	0.01	86.4 (3.8)	87.0 (1.6)	-	0.05
Mirex	23.15	272	236.8	14	273.8	238.8	14	8.08 - 15.01	12.17	0.999	0.01	77.4 (2.4)	78.5 (1.3)	-	-
Fenarimol	23.48	139	111	14	219	107	10	57.96 - 107.64	96.12	0.9998	0.01	91.5 (5.1)	87.7 (2.5)	-	0.02
Pyrazophos	23.49	221	193.1	8	231.9	204.1	10	36.87-68.47	53.68	0.9999	0.01	101.7 (2.6)	89.7 (1.4)	-	0.01
Azinphos-ethyl	23.7	160	77	16	132	77	12	84.23-156.43	108.38	0.9999	0.01	94.5 (2.7)	77.0 (3.4)	-	0.05
Permethrin peak 1	24.61	183.1	153	12	183.1	168	12	83.67-155.39	118.37	0.9996	0.01	78.3 (4.7)	90.1 (1.0)	-	0.05
Permethrin peak 2	24.85	183	165.1	10	183	168.1	10	72.93 - 135.44	104.48	0.9999	0.01	86.8 (8.5)	89.6 (2.0)	-	
Pyridaben	24.84	147.1	117.1	20	147.1	119.1	8	48.97 - 90.94	74.59	0.9998	0.01	95.9 (5.0)	87.0 (2.6)	-	0.05
Fluquinconazole	24.9	340	298	16	340	108.1	36	39.34 - 73.07	58.16	0.9999	0.01	89.5 (4.8)	88.3 (2.5)	-	0.05
Cyfluthrin peak 1	25.67	163	127.1	6	163	65.1	26	11.02 - 20.47	18.01	0.9997	0.01	88.7 (9.3)	88.9 (1.5)	-	0.02
Cyfluthrin peak 2	25.87	163	127	6	163	91.1	12	60.16 - 111.72	94.98	0.9999	0.01	83.9 (5.1)	92.8 (2.2)	-	
Cyfluthrin peak 3	25.97	163	127	6	226	206.1	163	54.40 - 101.02	66.05	0.9953	0.01	112.1 (4.3)	93.4 (10.9)	-	
Cyfluthrin peak 4	26.06	163	127	6	226	206.1	12	42.80 - 79.49	66.05	0.9999	0.01	88.5 (5.2)	91.2 (2.6)	-	

Q1 = Precursor ion, Q3 = Product ion *MRL limit for DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD expressed as DDT), **MRL limit of endosulfan (sum of endosulfan I, endosulfan II and endosulfan sulfate). Those compounds without MRL values were considered as the default, i.e., 0.01 mg/kg (set at LOQ).

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Table 3 (part 5). List of pesticides with SRM transitions used and validation data (ion ratio, linearity, recovery, and precision) and MRLs (FSSAI and EU)

Pesticide Name	RT (min)	Quantitive ion			Confirmatory ion 1			Ion ratio (IR)		R^2	LOQ (mg/kg)	% Recovery (%RSD)		MRLs (mg/kg)	
		Q1	Q3	CE (V)	Q1	Q3	CE (V)	IR range in MMS	IR in rice 0.01 (mg/kg)			0.01 (mg/kg)	0.05 (mg/kg)	FSSAI	EU
Cypermethrin peak 1	26.26	163	127.1	6	163	91.1	12	65.22 - 121.13	110.1	0.9987	0.01	90.6 (8.8)	87.5 (4.7)	2	2
Cypermethrin peak 2	26.46	163	127	6	163	91.1	12	63.52 - 117.97	96.45	0.9988	0.01	85.6 (9.9)	86.5 (3.4)		
Cypermethrin peak 3	26.56	180.9	152.2	20	163	127	6	32.91 - 61.12	35.01	0.9993	0.01	90.4 (7.4)	88.7 (2.3)		
Cypermethrin peak 4	26.64	163	127.1	6	180.9	152.2	20	56.17 - 104.32	85.6	0.9992	0.01	90.7 (13.0)	86.1 (4.4)		
Etofenprox	26.83	163.1	135.1	10	163.1	107.1	16	55.91 - 103.84	80.49	0.9997	0.01	89.9 (1.1)	91.8 (0.9)	-	0.5
Fenvalerate	27.9	167	125	10	125	89	18	13.18 - 24.47	17.81	0.9998	0.01	86.7 (5.0)	88.7 (2.0)	-	0.02
Fluvalinate peak 1	28.16	250	55.1	16	250	199.9	18	51.24 - 95.17	74.81	0.9981	0.01	103.4 (6.5)	89.4 (3.0)	-	-
Fluvalinate peak 2	28.3	250	55.1	16	250	200	18	46.06 - 85.54	71.08	0.9998	0.01	112.4 (2.2)	104.4 (3.0)	-	-
Esfenvalerate	28.29	167	125	10	125	89.3	18	11.86 - 22.03	20.43	0.9996	0.01	89.3 (5.8)	85.9 (3.5)	-	0.02
Deltamethrin-1	29.1	252.8	172	8	252.8	92.9	16	68.48 - 127.18	97.83	0.9997	0.01	98.1 (3.1)	83.8 (2.1)	2	1
Deltamethrin-2	29.9	252.8	92.9	16	181	152.1	22	173.73-322.65	224.35	0.9999	0.01	99.1 (3.2)	97.4 (0.9)		

Q1 = Precursor ion, Q3 = Product ion *MRL limit for DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD expressed as DDT), **MRL limit of endosulfan (sum of endosulfan I, endosulfan II and endosulfan sulfate). Those compounds without MRL values were considered as the default, i.e., 0.01 mg/kg (set at LOQ).

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