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Application News

LCMS™-8050 and GCMS-TQ™8040

Method for the determination of 243 Residual Pesticides in Red Chili Powder using LCMS-8050 and GCMS-TQ8040 NX

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User Benefits

- The method involves study of LOQ on both GC-MS/MS and LC-MS/MS, based on validation parameters like linearity, recovery, repeatability and within-laboratory reproducibility.
- A modified QuEChERS extraction procedure has been employed for quantifying pesticides at trace levels in complex matrix like Red chili powder using Ultra-fast technologies of LCMS-8050 and GCMS-TQ8040 NX.
- LCMS Method Package for Residual Pesticide Ver.3 and GCMS Smart Pesticides Database™ Ver.2 from Shimadzu Corporation enables ease of optimizing instrumental method.

1. Introduction

Red chili powder is the dried, pulverized fruit of varieties of red peppers. It is used as a spice (or spice blend) to add pungency and flavor to culinary dishes. It is widely used in many different cuisines throughout the world. Owing to its high demand worldwide, the intensive use of agrochemicals during its cultivation has given rise to concerns over consumer's exposure to pesticide residues and associated health risks. Hence, it is important for chili powder producers and regulators to ensure that presence of residual pesticides meet compliance criteria. Therefore, Maximum Residual Limits (MRL) are listed by various international regulatory bodies for wide variety of pesticides. Thus, increasing the importance of analytical method for determination of a range of pesticides present in red chili powder.

This study reports a validated method for the determination of 243 pesticides in red chili powder by using LCMS-8050 and GCMS-TQ8040 NX. The multi-residue extraction was performed with modified QuEChERS[1] method for simultaneous quantification of 146 pesticides by LC-MS/MS and 122 pesticides by GC-MS/MS. Out of these, 25 pesticides were common and analyzed by both the techniques. Regulation wise coverage of number of pesticides is shown in Table 1.

Table 1 Coverage of pesticides as per regulations

Compliance / Regulation	No. of pesticides regulated	No. of pesticides covered in this method
FSSAI	57	29
EU	409	182
APEDA	275	118

2. Materials and Methods

The reference standards were procured from Restek Corporation with below catalogue numbers:
LC multi residue pesticides kit – 31971
GC multi residue pesticides kit – 32562
Additionally, some individual reference standards were procured from Sigma-Aldrich. Red chili powder sample was procured from local market and used to prepare matrix-matched calibration standards and fortified samples.

Fig. 1 Shimadzu LCMS™-8050

The calibration standards were analyzed from 0.1 to 10 µg/L for LC-MS/MS and from 1 to 50 µg/L for GC-MS/MS. Calibration curves were generated by external standard method and using weighted regression of $1/C^2$. Fortified samples were prepared in six replicates of each 5, 10 and 25 µg/kg. The compounds marked with asterisk (*) in summary result Table 4 and 5; were present in both LCMS and GCMS standard mixture. Hence their calibration curve range and spiking levels were two times the concentration levels mentioned above.

Shimadzu LCMS-8050 with Nexera™ X2 (Fig. 1) and GCMS-TQ8040 NX (Fig. 2), manufactured by Shimadzu Corporation Japan, were used to quantify residual pesticides in red chili powder sample.

For most of the compounds, 1 target and 2 reference MRM transitions were included in the method.

Shimadzu's data processing software LabSolutions Insight™ was used for data processing, which helped in evaluating validation parameters with ease.

2.1. Sample preparation

This study uses single extraction procedure for GC-MS/MS and LC-MS/MS. For extraction, modified QuEChERS method approach was adopted. Sodium chloride, anhydrous magnesium sulphate ($MgSO_4$) salts were used in optimised proportion to maximize recoveries of pesticides. Acetonitrile was used as extraction solvent.

After extraction, clean up was performed using optimum combination of C-18, GCB (Graphitized carbon black), PSA (Primary secondary amine) and anhydrous $MgSO_4$ to minimise matrix interference, reduce instrument contamination and achieve lower LOQs.

After clean up, the aliquot of acetonitrile was divided in two parts. For GC-MS/MS, one part was reconstituted in ethyl acetate. For LC-MS/MS, the remaining aliquot was reconstituted using methanol : water (60:40 v/v) and filtered through 0.22µm nylon filter. The final reconstitution volume was adjusted such that fortified samples concentration is diluted by twenty times in LC-MS/MS and five times in GC-MS/MS.

All samples were analysed as per conditions shown in Table 2 and 3 for LC-MS/MS and GC-MS/MS, respectively.

Fig. 2 Shimadzu GCMS-TQ™8040 NX

2.2. Analytical Conditions

Table 2 Instrument configuration and Analytical Conditions: LC-MS/MS

System Configuration	
LC-MS/MS	: LCMS-8050
Auto-sampler	: Nexera X2 SIL-30AC
Column	: Restek Raptor™ Biphenyl, (100 mm × 2.1 mmI.D., 2.7 µm)

LC	
Flow rate	: 0.3 mL/min
Mobile phase A	: 2 mM Ammonium formate in water + 0.02% Formic acid
Mobile phase B	: 2 mM Ammonium formate in methanol + 0.02% Formic acid
Gradient program	: 15-55%B (1.0 min to 3.0 min) → 55-100%B (3.0 min to 15.0 min) → 100%B (15.0 min to 17.0 min) → 100-15%B (17.0 min to 17.5 min)
Run time	: 22 min
Injection volume	: 10 µL (Co-injection with water)
Column oven temp	: 35 °C

MS	
Ionization mode	: ESI
Nebulizing gas flow	: 3 L/min
Interface temp.	: 300 °C
Heating gas flow	: 8 L/min
Drying gas flow	: 8 L/min
DL temp.	: 150 °C
Heating block temp.	: 400 °C

Table 3 Instrument configuration and Analytical Conditions: GC-MS/MS

System Configuration	
GC-MS/MS	: GCMS-TQ8040 NX
Auto-injector	: AOC™-20i + s
Column	: SH-Rxi-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm)
Liner	: Topaz Liner, Splitless Single Taper w/Wool

GC	
Injector temp.	: 280 °C
Column oven temp	: 60 °C (1 min), 40 °C/min to 170 °C (0 min), 10 °C/min to 310 °C (7.25 min)
Run time	: 25 min
Injection mode	: Splitless (High pressure at 250 kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 44.7 cm/sec (Constant mode)

MS	
Ionization mode	: EI
Ion source temp.	: 230 °C
Interface temp.	: 300 °C
Solvent cut time	: 3.0 min
Loop Time	: 0.3 sec

3. Result and Discussion

Validation parameters like specificity, linearity, recovery and precision were studied as per SANTE guidelines[2]. Results obtained on LC-MS/MS and GC-MS/MS are shown in Table 4 and 5, respectively.

3.1. Specificity

Specificity of the method was determined by comparing the response of blank sample (reagent and matrix) against reporting level. Response in reagent/matrix blank sample was well within 30 % of the reporting limit and met the acceptance criteria.

3.2. Linearity study

For linearity study, matrix match calibration standards were used. Calibration curve ranged from 0.1 to 10 µg/L for LC-MS/MS and from 1 to 50 µg/L for GC-MS/MS. All calibration standards were found within 80 to 120% accuracy as per SANTE guidelines.

The compounds marked with asterisk (*) in summary result Table 4 and 5 were twice of the concentration levels mentioned above.

The linearity graphs of some representative compounds are shown in Fig. 3 and 4.

3.3. Recovery study

Recovery was evaluated by analysing six replicates of fortified samples at 5, 10 and 25 µg/kg (10, 20 and 50 µg/kg for * marked compounds in Table 4 and 5) against matrix match calibration curve. Mean recoveries for most of the compounds were found within 70-120%. As per SANTE guidelines, all the compounds were found to be reproducible with 20% RSD at their LOQ levels. (Refer to Tables 4 and 5)

3.4. Precision study

For precision, repeatability and within-laboratory reproducibility studies were carried out. Concentrations of fortified samples were back calculated against matrix matched linearity.

Repeatability (RSD_r):

Repeatability experiment was performed by injecting six replicates of 5, 10 and 25 µg/kg concentration levels. The % RSD for repeatability of six injections at their respective LOQ levels were found to be less than 20%. (Refer to Tables 4 and 5)

Reproducibility (RSD_R):

Reproducibility experiment for recoveries was performed on six different fortified samples at 5, 10 and 25 µg/kg concentration levels. The % RSD for recovery of six fortified samples at their respective LOQ levels were found to be less than 20%. (Refer to Tables 4 and 5)

Table 4 Summary results of LC-MS/MS analysis

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
1	Formetanate Hydrochloride	1.657	222.00>165.10	-16	0.9974	0.005	103.25	87.49	6.66	7.28
2	Acephate	1.954	184.00>49.15	-22	0.9968	0.005	94	95.81	4.66	4.02
3	Omethoate	2.509	214.10>125.00	-18	0.9990	0.005	96.8	105.30	2.39	5.80
4	Aldicarb-sulfoxide	2.878	207.10>89.10	-14	0.9981	0.005	95.6	106.87	10.00	9.61
5	Dinotefuran	2.952	203.15>114.15	-13	0.9897	0.005	85.65	87.96	10.42	8.43
6	Aldicarb-sulfone (Aldoxycarb)	3.197	240.10>86.20	-21	0.9931	0.01	100.84	116.20	3.70	7.17
7	Oxamyl	3.253	237.10>72.10	-22	0.9974	0.005	99	113.36	4.04	3.18
8	Methomyl	3.519	163.00>87.90	-10	0.9854	0.005	121.25	120.27	8.17	6.00
9	Thiamethoxam	3.69	292.00>181.10	-22	0.9906	0.01	89.44	106.55	14.95	12.90
10	Monocrotophos	3.784	240.90>127.10	-17	0.9969	0.005	92.25	107.24	9.08	5.78
11	Imidacloprid	4.133	256.10>209.00	-17	0.9951	0.01	91.26	98.81	14.39	14.05
12	Vamidothion	4.307	288.10>146.05	-6	0.9968	0.005	95.05	90.76	6.74	14.07
13	Fenuron	4.312	165.00>72.15	-7	0.9743	0.01	103.92	110.33	6.28	7.30
14	Dimethoate	4.373	230.00>198.90	-4	0.9878	0.005	81.8	90.62	10.13	15.59
15	Acetamiprid	4.379	223.10>126.10	-11	0.9985	0.005	97.45	98.61	14.22	21.91
16	Thiacloprid	4.606	253.00>126.05	-11	0.9673	0.01	89.44	113.35	14.44	16.10
17	Tricyclazole	4.79	190.10>136.00	-24	0.9975	0.005	107.35	96.23	9.50	9.81
18	Aldicarb	4.89	208.20>115.85	-9	0.9954	0.01	97.54	113.93	16.06	14.90
19	Bentazone	5.123	239.00>132.15	25	0.9982	0.01	103.32	102.74	6.18	17.05
20	Carbetamide	5.133	237.10>192.10	-5	0.9943	0.005	110.85	99.31	8.30	16.67
21	Triasulfuron	5.158	402.00>167.15	-18	0.9825	0.01	109.04	88.83	14.35	12.30
22	Carbofuran	5.404	222.10>123.15	-11	0.9861	0.005	118.3	114.03	12.52	10.25
23	Simazine	5.446	202.10>104.00	-25	0.9813	0.025	109.6	102.02	16.55	6.52
24	Hexazinone	5.484	253.20>171.15	-6	0.9842	0.01	96.08	92.07	9.44	7.74
25	Thidiazuron	5.509	221.00>102.00	-16	0.9846	0.01	92.44	83.34	17.45	6.35
26	Tebuthiuron	5.587	229.10>116.00	-27	0.9937	0.005	111.4	99.20	9.82	19.74
27	Pirimicarb	5.611	239.20>182.05	-6	0.9980	0.005	96	107.98	9.20	9.94
28	Carbaryl (NAC)	5.677	202.10>127.00	-25	0.9937	0.025	96.32	96.35	11.37	12.86
29	Carboxin	5.682	236.10>143.10	-5	0.9826	0.01	98.24	96.70	9.40	5.72
30	Monolinuron	5.929	215.10>148.00	-15	0.9911	0.005	102.85	111.87	15.67	13.04
31	Diuron (DCMU)	6.007	233.00>46.15	-17	0.9864	0.005	81.5	100.93	13.80	11.54
32	Chlorotoluron	6.169	213.10>72.15	-7	0.9858	0.01	79.2	99.38	9.35	20.08
33	Metazachlor	6.275	277.90>134.10	-11	0.9821	0.01	103.22	108.87	7.11	4.25
34	Atrazine	6.311	216.10>174.10	-13	0.9915	0.005	84.25	109.65	7.04	10.62
35	Azimsulfuron	6.341	425.10>182.10	-18	0.9992	0.005	97.25	102.95	4.05	8.01
36	Propachlor	6.355	212.10>170.10	-6	0.9707	0.01	80.64	88.49	9.94	5.35
37	Methabenzthiazuron	6.359	222.10>150.10	-32	0.9861	0.005	93.75	103.50	9.54	9.93
38	Metalaxyl-M (Mefenoxam)	6.373	280.20>220.00	-6	0.9931	0.005	86.05	96.46	5.19	9.97
39	Metalaxyl	6.379	280.10>220.00	-6	0.9942	0.005	86.2	107.65	7.85	9.19
40	Prometon	6.385	226.20>142.00	-12	0.9827	0.005	83.6	89.84	7.00	8.53
41	Isoproturon	6.449	207.20>72.15	-8	0.9936	0.005	107.95	114.13	20.89	17.39
42	Terbumeton	6.596	225.90>170.00	-6	0.9977	0.005	105.15	110.86	9.34	8.89
43	Monuron	6.634	199.10>46.10	-17	0.9946	0.005	108.25	96.06	11.08	7.66
44	Forchlorfenuron	6.644	248.10>129.15	-17	0.9676	0.01	85.72	94.25	7.30	7.00
45	Norflurazon	6.644	304.10>283.95	-18	0.9992	0.005	101.2	112.80	13.26	11.12
46	Cycluron	6.647	199.20>88.95	-10	0.9971	0.005	92.8	99.38	12.08	11.20
47	Iodosulfuron-methyl	6.699	508.00>167.00	-21	0.9979	0.005	92.8	86.03	6.79	7.37
48	Desmedipham	6.7	318.00>182.00	-6	0.9943	0.005	109.15	97.42	14.65	8.53
49	Diphenamid	6.701	239.90>134.15	-6	0.9985	0.005	101.35	103.34	11.87	10.50

Table 4 Summary results of LC-MS/MS analysis (Contd.)

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
50	Dimethachlor	6.736	256.10>148.00	-25	0.9897	0.005	83.4	103.26	10.53	9.02
51	Chlorantraniliprole	6.813	483.90>452.90	-19	0.9845	0.005	85.3	92.93	10.64	9.72
52	Phenmedipham	6.89	318.10>168.10	-14	0.9989	0.005	96.9	101.46	4.62	11.08
53	Methoprotryne	6.952	272.20>197.95	-13	0.9870	0.005	86.8	99.94	14.82	11.89
54	Clomazone	6.969	239.90>125.00	-11	0.9971	0.005	107.95	99.47	12.02	7.73
55	Ametryn	6.978	228.10>186.00	-9	0.9962	0.005	101.8	103.40	11.41	9.66
56	Fluridone	6.98	330.10>309.00	-31	0.9975	0.005	93.35	97.62	6.79	4.75
57	Sulfosulfuron	6.991	471.00>211.00	-10	0.9957	0.005	94.4	106.03	9.03	5.44
58	Azoxystrobin	7.117	404.00>371.95	-5	0.9887	0.01	93.28	96.87	15.41	15.10
59	Fenobucarb	7.199	208.10>95.00	-10	0.9798	0.01	84.96	113.75	9.73	8.35
60	Diethofencarb	7.22	268.20>226.05	-5	0.9989	0.005	104.4	99.45	5.38	8.65
61	Siduron	7.397	233.20>137.10	-12	0.9962	0.01	96.1	102.39	10.22	9.68
62	Linuron	7.403	248.80>160.00	-18	0.9943	0.005	95.9	109.33	17.45	12.91
63	Fenamidone	7.41	312.10>236.00	-10	0.9784	0.01	99.78	114.81	7.25	7.17
64	Terbuthylazine	7.512	230.10>174.05	-7	0.9881	0.005	84.95	87.64	13.95	13.39
65	Flonicamid	7.526	230.10>174.00	-17	0.9965	0.005	94.05	95.36	6.01	5.33
66	Sebuthylazine	7.53	230.10>174.05	-8	0.9979	0.005	104.2	97.63	5.27	9.18
67	Acibenzolar-S-methyl	7.576	210.90>136.05	-28	0.9957	0.025	105.35	84.95	8.72	16.95
68	Mandipropamid	7.629	412.10>327.90	-10	0.9896	0.005	85.95	92.67	10.65	10.91
69	Fludioxonil	7.661	247.00>180.15	28	0.9881	0.01	104.62	109.90	9.43	10.47
70	Benzthiazuron	7.711	208.10>151.10	-6	0.9768	0.01	111.42	99.87	13.63	11.76
71	Paclobutrazol*	7.776	294.10>70.10	-22	0.9952	0.01	87.95	90.79	9.89	8.14
72	Flutolanil*	7.777	324.10>242.00	-20	0.9966	0.01	89.7	99.24	9.65	15.11
73	Fluopicolide	7.798	383.00>172.85	-13	0.9860	0.005	120.65	111.13	17.42	13.85
74	Dimethomorph (E, Z)	7.84	388.10>301.00	-11	0.9925	0.005	103.3	112.29	12.12	14.77
75	Methoxyfenozide	7.85	369.20>312.95	-9	0.9861	0.005	82.4	110.30	6.66	8.34
76	Propyzamide	7.934	256.00>190.00	-14	0.9897	0.005	96	95.54	14.13	14.73
77	Prometryn	7.946	242.10>158.00	-12	0.9897	0.005	112.9	89.76	20.70	14.52
78	Triadimefon*	7.962	294.10>196.95	-15	0.9994	0.01	99.5	98.79	8.29	8.33
79	Mefenacet	8.085	299.00>148.15	-6	0.9932	0.005	114.7	95.32	9.00	7.66
80	Isazofos	8.106	314.10>120.00	-18	0.9896	0.005	115.65	96.92	9.74	15.99
81	Isoxathion	8.143	314.00>97.05	-40	0.9936	0.005	101.1	84.70	9.71	11.16
82	Triazophos	8.156	314.10>162.05	-9	0.9975	0.005	100.6	102.06	7.79	12.09
83	Iprovalicarb	8.172	321.20>119.15	-6	0.9987	0.005	102.2	82.16	12.17	9.23
84	Butafenacil	8.257	492.10>330.85	-14	0.9966	0.005	89.65	93.63	9.06	9.57
85	Triadimenol (isomer)	8.275	296.10>70.05	-22	0.9959	0.005	110.15	108.92	12.32	10.02
86	Spirotetramat	8.31	374.10>216.00	-23	0.9968	0.005	101.1	97.67	12.49	9.19
87	Naled (Dibrom)	8.423	395.80>127.15	-21	0.9899	0.005	118.05	102.27	11.86	15.70
88	Flufenacet	8.471	364.10>152.05	-9	0.9966	0.005	107.1	93.17	10.68	12.98
89	Fenarimol*	8.53	331.00>268.00	-23	0.9863	0.02	93.46	93.57	14.96	17.49
90	Tetraconazole	8.621	372.00>159.00	-30	0.9938	0.01	94.52	102.06	12.25	10.71
91	Alachlor	8.65	270.10>162.15	-21	0.9991	0.005	100.65	102.77	14.31	8.93
92	Metolachlor	8.685	284.10>176.10	-26	0.9933	0.005	87.1	109.64	6.17	6.99
93	Epoxiconazole	8.717	330.00>101.10	-44	0.9889	0.005	88.3	105.27	14.51	18.14
94	Cyazofamid	8.778	325.00>108.10	-15	0.9958	0.005	110.3	103.92	10.35	11.62
95	Bupirimate*	8.79	317.20>166.00	-19	0.9990	0.01	103.25	106.29	7.61	4.31
96	Fenbuconazole	8.93	337.10>70.10	-22	0.9965	0.005	100	107.08	17.63	11.20
97	Rotenone	8.934	395.10>192.00	-24	0.9906	0.01	103.42	114.40	19.60	14.32
98	Tebufenozide	9.021	353.20>133.10	-11	0.9968	0.005	96.55	106.47	9.80	6.12

Table 4 Summary results of LC-MS/MS analysis (Contd.)

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
99	Picoxystrobin	9.033	368.00>145.10	-12	0.9977	0.005	105.35	99.91	5.79	9.11
100	Flusilazole*	9.046	316.10>247.00	-13	0.9981	0.01	93.9	95.22	8.97	8.62
101	Diflubenzuron	9.094	311.00>158.10	-16	0.9971	0.01	93.56	122.80	15.75	13.02
102	Fenoxycarb	9.119	302.10>88.00	-12	0.9948	0.005	91.55	97.60	5.57	8.62
103	Iprobenfos	9.132	289.10>204.95	-6	0.9962	0.01	92.86	98.16	7.80	10.56
104	Spinosyn A	9.16	732.60>142.20	-29	0.9960	0.005	94.45	37.05	11.81	10.03
105	Dimoxystrobin	9.195	327.10>116.10	-12	0.9990	0.005	103.25	100.78	19.86	16.95
106	Diclobutrazol (stereo isomer)	9.372	328.00>70.10	-22	0.9847	0.005	117.55	84.51	15.18	13.21
107	Tolylfluanid	9.398	364.00>237.95	-15	0.9490	0.01	74.5	96.12	14.16	7.80
108	Penconazole*	9.464	284.10>70.05	-17	0.9984	0.01	101.2	91.17	4.49	7.38
109	Anilofos	9.55	368.00>199.05	-5	0.9978	0.005	93.8	98.11	19.08	17.26
110	Benalaxyl	9.566	326.20>148.10	-11	0.9981	0.005	99.45	98.75	7.72	4.39
111	Diazinon	9.644	305.10>169.10	-12	0.9972	0.005	105.25	110.87	8.00	4.43
112	Chlorfenvinphos (E, Z)	9.724	359.00>155.15	-13	0.9863	0.005	85.15	112.07	11.48	11.37
113	Bromfenvinfos	9.927	404.90>155.10	-13	0.9680	0.01	96.66	102.14	13.73	8.26
114	Pyraclostrobin	9.952	388.00>194.10	-8	0.9997	0.005	98.85	93.48	7.81	8.68
115	Spinetoram J	10	748.50>142.15	-26	0.9965	0.005	97.75	36.47	6.49	17.96
116	Prochloraz	10.007	376.00>307.95	-8	0.9995	0.005	98.15	95.92	8.78	5.89
117	Pirimiphos-methyl	10.042	306.10>164.10	-18	0.9802	0.01	109.72	95.91	3.69	4.40
118	Pyraclofos	10.077	361.10>256.90	-22	0.9974	0.005	99.9	95.93	5.42	6.59
119	Phorate	10.078	261.00>75.15	-11	0.9893	0.01	86.58	89.86	14.74	8.52
120	Phosalone	10.094	368.00>182.00	-17	0.9825	0.01	101.36	83.34	13.45	15.50
121	Benzoximate	10.108	364.10>198.95	-10	0.9809	0.01	96.76	97.17	4.66	6.80
122	Pyrazophos	10.135	374.10>222.05	-12	0.9978	0.005	106.3	93.53	17.27	13.10
123	Triflumuron	10.139	359.00>156.05	-17	0.9813	0.01	88.98	85.79	13.23	10.69
124	Bitertanol (diastereo isomers)	10.165	338.00>99.15	-15	0.9954	0.005	107.2	101.81	14.56	9.51
125	Clofentezine	10.176	303.00>102.10	-37	0.9979	0.01	92.36	85.16	17.82	13.56
126	Orbencarb	10.207	258.10>125.10	-11	0.9964	0.005	90.85	95.88	10.02	8.89
127	Thiobencarb	10.213	257.80>125.10	-11	0.9850	0.005	101.8	100.13	6.21	8.99
128	Metrafenone	10.238	409.00>209.10	-6	0.9973	0.005	93.6	104.10	6.92	7.78
129	Pencycuron	10.336	329.10>125.00	-10	0.9831	0.01	99.16	86.19	3.59	9.46
130	Diniconazole	10.431	326.10>70.05	-25	0.9946	0.005	94	112.94	9.33	8.30
131	Trifloxystrobin	10.681	409.10>205.90	-10	0.9976	0.025	100.44	83.68	9.50	6.97
132	Pretilachlor	10.85	312.20>252.05	-7	0.9986	0.01	97.58	90.18	3.49	10.69
133	Clethodim (isomer)	10.961	360.10>164.15	-21	0.9982	0.005	103.25	84.64	13.91	10.58
134	Hexaflumuron	10.977	458.80>439.00	11	0.9738	0.01	100.06	78.72	9.12	9.78
135	Emamectin B1a	11.047	886.40>82.05	-55	0.9972	0.005	99.55	44.86	17.06	10.58
136	Profenofos	11.184	375.00>304.70	-19	0.9787	0.005	92.75	79.45	5.22	20.15
137	Fluazifop-butyl	11.327	384.20>281.90	-7	0.9915	0.005	95.9	99.33	10.63	17.17
138	Furathiocarb	11.34	383.20>195.00	-10	0.9928	0.005	115.3	98.66	13.61	10.65
139	Buprofezin	11.547	306.20>201.05	-6	0.9990	0.005	103.35	80.38	4.01	5.72
140	Pirimiphos-ethyl	11.561	334.10>198.15	-12	0.9947	0.005	88.5	92.70	10.25	7.93
141	Chlorpyrifos-oxon	11.66	334.00>197.85	-30	0.9970	0.005	94.3	90.65	3.75	2.61
142	Piperonyl-butoxide*	11.671	356.20>177.00	-3	0.9981	0.01	100.7	91.52	3.04	2.71
143	Pyriproxyfen*	11.921	322.10>78.05	-54	0.9995	0.01	102	76.68	7.37	8.50
144	Hexythiazox	12.109	353.10>228.00	-11	0.9991	0.005	97.5	72.86	11.23	7.99
145	Quinoxyfen	12.114	308.00>197.00	-31	0.9985	0.005	96.45	61.93	7.42	11.95
146	Ivermectine	15.074	892.40>307.20	-28	0.9815	0.005	78.6	71.03	15.26	17.02

Table 5 Summary results of GC-MS/MS analysis

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
1	Dichlorvos	4.327	185.00>93.00	14	0.9960	0.025	102.55	58.96	19.27	10.14
2	4-Bromo 2-Chloro Phenol	4.508	208.00>63.10	30	0.9962	0.025	103.05	94.55	9.63	9.20
3	Mevinphos*	5.217	192.00>127.00	12	0.9949	0.010	93	88.57	12.64	6.77
4	Etridiazole	5.422	210.90>182.90	10	0.9999	0.025	99	66.29	17.38	8.61
5	Pebulate	5.440	128.10>57.00	6	0.9914	0.025	101.75	115.63	14.39	7.51
6	Methacrifos	5.631	208.00>180.00	8	0.9932	0.025	99.5	86.17	10.12	10.50
7	Pentachlorobenzene	5.921	249.90>214.90	18	0.9987	0.025	97.7	68.22	13.27	10.39
8	Tecnazene	6.376	260.90>202.90	14	0.9956	0.025	97.2	76.85	14.03	11.10
9	Propoxur	6.404	110.10>64.00	18	0.9950	0.025	106.3	57.16	10.35	14.27
10	Propachlor	6.443	176.10>57.00	8	0.9752	0.005	93.2	90.74	9.11	16.52
11	Ethalfluralin	6.630	276.00>202.00	18	0.9962	0.005	104.6	102.49	10.04	15.14
12	2,3,5,6-Tetrachloroaniline	6.649	228.90>158.00	18	0.9988	0.005	99.4	83.06	11.14	8.39
13	Trifluralin	6.731	306.10>264.10	8	0.9980	0.005	94.8	104.17	12.79	10.20
14	Benfluralin	6.772	292.10>264.00	8	0.9972	0.005	99	83.23	9.16	12.22
15	Chlorpropham	6.761	213.10>127.10	14	0.9975	0.025	97.35	98.98	9.39	6.76
16	Sulfotep	6.830	322.00>202.00	10	0.9948	0.005	96	99.40	8.62	18.83
17	Di-allate	7.036	234.10>150.00	20	0.9971	0.005	94.2	88.74	13.46	9.34
18	Phorate II	7.048	231.00>129.00	24	0.9868	0.025	102.35	83.13	18.39	9.32
19	alpha-BHC	7.196	218.90>182.90	8	0.9989	0.005	97.4	70.31	16.03	19.69
20	Hexachlorobenzene	7.271	283.80>248.80	24	0.9985	0.005	100.2	83.20	15.63	8.80
21	Pentachloroanisole	7.332	264.80>236.80	16	0.9818	0.010	88.2	92.76	15.33	8.89
22	Dicloran	7.386	206.00>176.00	10	0.9978	0.025	98.4	73.96	18.53	11.62
23	Quintozene	7.635	294.80>236.80	16	0.9867	0.025	103.45	66.02	13.92	16.78
24	Phorate	7.687	231.00>129.00	24	0.9865	0.005	91.6	88.46	10.70	12.41
25	Terbufos	7.687	231.00>128.90	26	0.9937	0.005	93.2	81.94	11.79	14.48
26	Pentachlorobenzonitrile	7.704	274.80>239.80	18	0.9979	0.005	93.6	104.43	13.78	8.39
27	Fluchloralin	7.786	306.00>264.00	8	0.9906	0.010	86.8	106.53	19.29	15.82
28	Propyzamide	7.785	172.90>144.90	18	1.0000	0.005	99.6	131.77	14.23	17.58
29	Fonofos	7.791	246.00>137.10	6	0.9873	0.005	97.4	91.29	13.47	9.60
30	Tefluthrin	7.926	177.00>127.10	16	0.9985	0.005	97.6	84.51	12.16	8.32
31	Pyrimethanil*	7.925	198.10>118.10	28	0.9972	0.010	94.4	81.20	16.33	17.75
32	Isazofos	7.957	257.00>162.00	8	0.9806	0.025	101.25	94.98	12.84	10.51
33	Propentamphos	7.628	236.10>194.00	6	0.9976	0.010	93.4	120.16	19.75	10.40
34	Tri-allate	8.091	268.10>184.00	20	0.9972	0.005	93.4	94.14	13.23	15.93
35	Endosulfan ether	8.443	240.90>205.90	16	0.9876	0.005	101.8	111.11	12.62	19.76
36	Dimethachlor	8.494	197.10>148.10	10	0.9850	0.005	113.2	75.80	8.33	11.39
37	Acetochlor	8.547	223.10>132.10	22	0.9697	0.025	83.1	83.78	18.89	16.85
38	Dimethoate	8.582	125.00>47.00	14	0.9866	0.025	103.65	82.05	15.61	16.57
39	Vinclozolin	8.638	285.00>212.00	12	0.9835	0.025	107.3	105.09	12.08	14.20
40	Transfluthrin	8.678	163.10>143.10	16	0.9949	0.010	104	109.16	17.60	16.52
41	Propanil	8.575	160.90>99.00	24	0.9968	0.010	99.4	107.29	15.28	17.53
42	Tolclofos-methyl	8.706	264.90>249.90	14	0.9815	0.010	95.4	104.71	6.30	13.10

Table 5 Summary results of GC-MS/MS analysis (Contd.)

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
43	Parathion-methyl	8.697	125.00>47.00	12	0.9716	0.010	96.6	92.61	10.88	13.73
44	Fenchlorphos	8.871	284.90>239.90	26	0.9980	0.010	104.6	95.83	10.05	15.18
45	Heptachlor	8.884	271.80>236.90	20	0.9976	0.005	99.8	101.74	14.05	10.02
46	Fenitrothion	9.099	277.00>260.00	6	0.9938	0.010	99.8	95.34	9.75	14.78
47	Malathion	9.210	173.10>99.00	14	0.9912	0.025	101.45	86.49	13.43	10.93
48	Metolachlor (S-Metolachlor)	9.357	238.10>133.10	26	0.9988	0.005	102.8	101.97	19.59	17.21
49	Chlorpyrifos	9.365	313.90>257.90	14	0.9887	0.010	85.6	94.10	13.71	14.60
50	Fenthion	9.439	278.00>109.00	20	0.9979	0.010	99.4	116.86	13.07	17.30
51	Tridemorph	9.471	128.10>70.00	12	0.9774	0.010	100	106.39	14.27	15.77
52	Chlorthal-dimethyl	9.475	298.90>220.90	24	0.9956	0.005	100.4	114.43	17.21	18.24
53	Aldrin	9.505	262.90>193.00	28	0.9715	0.025	91.45	109.47	19.20	16.10
54	Triadimefon*	9.597	208.10>127.00	14	0.9891	0.020	95.2	103.54	14.66	13.86
55	Pirimiphos ethyl	9.708	318.10>166.10	12	0.9853	0.010	109.6	100.43	12.89	14.36
56	4,4'-Dichlorobenzophenone	9.677	139.00>75.00	26	0.9864	0.010	84.8	104.46	10.31	12.04
57	Bromophos	9.781	330.90>315.90	14	0.9949	0.025	97.6	77.88	12.51	7.96
58	Cyprodinil*	10.076	224.10>197.10	22	0.9840	0.020	108.2	114.33	10.25	12.07
59	Fipronil*	10.113	368.90>214.90	30	0.9863	0.010	89.4	131.31	8.06	18.38
60	Penconazole*	10.158	248.10>157.10	26	0.9835	0.020	83.4	99.89	14.45	15.48
61	Bromophos-ethyl	10.518	358.90>302.90	16	0.9989	0.010	103.2	111.24	10.08	19.51
62	o,p'-DDE	10.621	246.00>176.00	30	0.9943	0.005	90.6	84.51	12.26	14.84
63	Chlorbenside	10.581	125.00>89.00	16	0.9922	0.010	89	94.30	14.68	12.03
64	Paclobutrazol*	10.736	236.10>167.00	10	0.9717	0.020	84.8	115.76	9.96	14.78
65	cis-Chlordane	10.842	374.80>265.90	26	0.9924	0.025	91.3	107.06	18.00	19.25
66	Flutriafol*	10.925	219.10>123.10	14	0.9887	0.050	103.6	117.03	10.70	8.60
67	Flutolanil*	10.958	173.00>95.00	26	0.9971	0.010	104.6	98.43	19.03	16.55
68	Prothiofos	11.024	309.00>238.90	14	0.9874	0.010	92.2	112.39	13.22	12.31
69	Pretilachlor	11.063	262.10>202.10	10	0.9696	0.025	109.15	94.86	16.72	17.76
70	Chlorfenson	11.005	175.00>111.00	12	0.9840	0.005	98.4	113.94	17.72	13.38
71	Isoprothiolane	11.039	290.10>118.00	14	0.9978	0.025	104.75	135.73	17.10	19.85
72	p,p'-DDE	11.198	246.00>176.00	30	0.9962	0.025	104.55	104.67	7.48	16.02
73	Bupirimate*	11.631	273.10>193.10	8	0.9252	0.010	61	141.06	15.72	17.56
74	Flusilazole*	11.667	233.10>165.10	14	0.9730	0.050	80.8	89.39	14.45	17.94
75	o,p'-DDD	11.344	235.00>165.00	24	0.9985	0.010	100	121.17	19.16	16.34
76	Nitrofen	11.694	202.00>139.00	24	0.9911	0.025	102.8	84.14	17.74	16.00
77	Chlorobenzilate	11.845	251.00>139.00	14	0.9897	0.005	93.6	119.83	17.87	12.08
78	Ethion	11.967	230.90>129.00	24	0.9921	0.005	89.4	109.60	13.65	17.36
79	Chlorthiophos	12.016	324.90>268.90	14	0.9939	0.025	91.4	91.31	13.07	15.17
80	p,p'-DDD & o,p'-DDT	12.022	235.00>165.00	24	0.9992	0.005	97.4	98.60	6.25	4.03
81	Sulprofos	12.264	322.00>139.00	14	0.9813	0.025	97.7	92.13	11.61	12.34
82	Carfentrazone-ethyl*	12.426	340.10>312.10	14	0.9963	0.010	101	89.63	12.16	6.95
83	Trifloxystrobin	12.441	222.10>190.10	4	0.9929	0.025	101.3	125.17	13.06	15.86

Table 5 Summary results of GC-MS/MS analysis (Contd.)

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	LOQ mg/kg	% Accuracy at LOQ	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
84	4,4'-methoxychlor olefin	12.441	308.00>238.10	16	0.9968	0.005	100.6	79.43	12.40	9.15
85	Propiconazole	12.731	173.00>109.00	28	0.9902	0.010	87.4	106.06	17.97	13.00
86	Endosulfan sulfate	12.650	271.80>236.90	18	0.9923	0.005	94.2	83.74	14.18	10.02
87	p,p'-DDT	12.677	235.00>165.00	24	0.9999	0.010	101.2	79.90	13.41	13.44
88	2,4'-Methoxychlor	12.761	227.10>121.10	16	0.9991	0.005	96.6	89.89	7.82	6.36
89	Hexazinone	12.846	171.10>71.00	16	0.9976	0.005	94.2	82.86	15.79	13.25
90	Dicofol	12.976	139.00>75.10	27	0.9848	0.025	95.35	108.16	12.07	9.65
91	Diclofop-methyl	12.926	340.00>253.00	14	0.9808	0.010	98.2	113.66	15.91	9.45
92	Piperonyl butoxide*	13.047	176.10>131.10	12	0.9993	0.010	97.8	93.14	8.56	6.43
93	Epoxiconazole	13.230	192.00>138.00	14	0.9952	0.005	98.8	105.34	14.14	8.20
94	Bifenthrin	13.481	181.10>179.10	12	0.9512	0.025	109.25	108.34	13.93	13.80
95	EPN	13.569	169.10>77.00	22	0.9683	0.010	100.8	82.69	12.93	17.33
96	Phosmet	13.547	160.00>133.00	14	0.9474	0.025	94.6	45.19	14.20	20.40
97	Bromopropylate	13.603	340.90>182.90	18	0.9944	0.005	96	109.94	6.58	13.89
98	Bifenazate	13.647	300.10>258.10	8	0.9781	0.010	99.8	100.29	8.24	17.41
99	Methoxychlor	13.673	227.10>169.10	24	0.9982	0.010	94	102.74	12.34	7.97
100	Fenpropathrin	13.690	265.10>210.10	12	0.9987	0.010	102.8	101.40	18.75	15.70
101	Tebufenpyrad*	13.797	333.10>171.10	20	0.9986	0.010	94.8	95.03	6.62	8.52
102	Tetradifon	14.093	355.90>159.00	18	0.9893	0.010	94.4	99.74	13.02	19.74
103	Phosalone	14.188	182.00>102.00	14	0.9940	0.025	100.7	62.93	6.57	7.30
104	Pyriproxyfen*	14.319	136.10>96.00	14	0.9926	0.010	97.4	108.06	13.31	7.55
105	Azinphos-methyl	14.845	160.10>77.00	20	0.9965	0.010	93.2	94.61	10.23	13.53
106	Acrinathrin	14.595	289.10>93.00	14	0.9334	0.025	116.2	107.65	13.35	17.08
107	Mirex	14.617	271.80>236.80	18	0.9974	0.005	93.2	75.89	7.60	6.05
108	Fenarimol*	14.757	251.00>139.00	14	0.9967	0.010	98.8	103.66	8.79	4.05
109	trans-Permethrine	15.392	163.10>127.10	6	0.9995	0.025	98.85	84.33	7.68	9.75
110	Cyfluthrin-2	15.811	226.10>206.10	14	0.9761	0.005	82.2	128.57	12.64	10.78
111	Cypermethrin-2	16.125	163.10>127.10	6	0.9960	0.025	101.95	89.54	3.83	12.52
112	Flucythrinate-1	16.285	157.10>107.10	12	0.9989	0.005	98	98.83	6.27	7.01
113	Flucythrinate-2	16.483	157.10>107.10	12	0.9953	0.005	95	87.57	12.16	10.76
114	Fluridone	16.710	328.10>259.00	24	0.9929	0.005	89.2	84.97	5.38	11.56
115	Fenvalerate	17.007	225.10>119.10	20	0.9865	0.025	94.95	90.58	5.90	8.93
116	tau-Fluvalinate	17.114	250.10>55.00	18	0.9964	0.010	93	70.09	14.36	17.44
117	Difenoconazole	17.465	323.00>265.00	14	0.9933	0.005	94.4	93.43	10.58	6.05
118	Indoxacarb	17.579	264.00>176.00	14	0.9750	0.025	92.35	83.30	12.49	15.13
119	Deltamethrin (Tralomethrin deg.)	17.736	252.90>171.90	8	0.9992	0.005	100.2	110.89	10.59	19.18
120	Azoxystrobin	17.904	344.10>329.10	16	0.9921	0.010	88.2	117.46	12.21	13.86
121	Dimethomorph	18.045	301.10>165.10	14	0.9982	0.005	94.2	87.31	4.19	7.98
122	Famoxadone	18.113	330.10>224.10	10	0.9996	0.005	97.8	96.09	5.68	11.37

At LOQ level, out of total compounds, mean recoveries of 141 on LC-MS/MS and 107 on GC-MS/MS were found to be within 70-120 %. Whereas 4 compounds on LC-MS/MS and 7 compound on GC-MS/MS showed recoveries less than 70 %. Only 1 compound in LC-MS/MS and 8 compounds in GC-MS/MS displayed higher recoveries than 120%. As per SANTE guidelines, recoveries of all the compounds were found to be reproducible with less than 20 % RSD at their LOQ levels. (Refer to Table 4 and 5)

The method successfully achieved 5 µg/kg LOQ on LC-MS/MS for 97 compounds and on GC-MS/MS for 41 compounds. LOQ of 10 µg/kg could be achieved for 84 compounds (44 on LC-MS/MS and 39 on GC-MS/MS). 5 compounds showed LOQ of 20 µg/kg (1 on LC-MS/MS and 4 on GC-MS/MS) and 40 compounds showed LOQ of 25 µg/kg (4 on LC-MS/MS and 36 on GC-MS/MS). Only 2 compounds LOQ was found to be 50 µg/kg on GC-MS/MS (Refer to Table 4 and 5). Representative chromatograms of a few compounds at their LOQ levels are shown in Fig. 3 and 4.

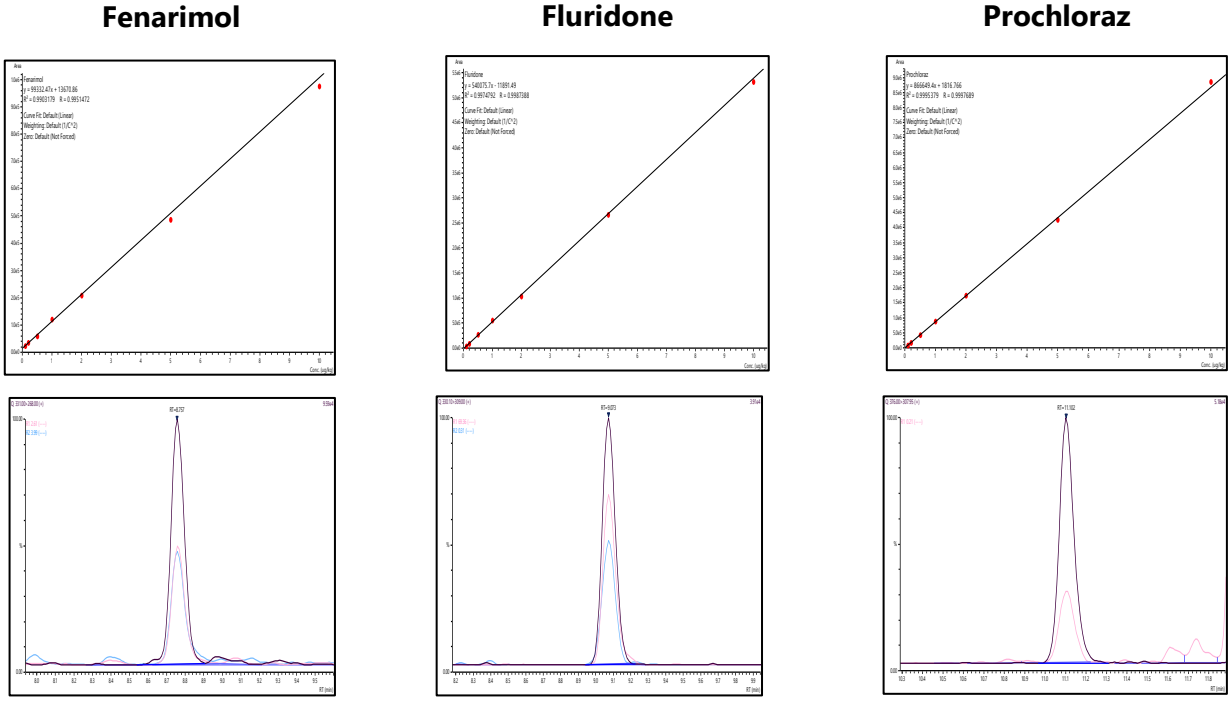

Fig. 3 Representative linearity graphs and chromatograms at LOQ level for LC-MS/MS compounds

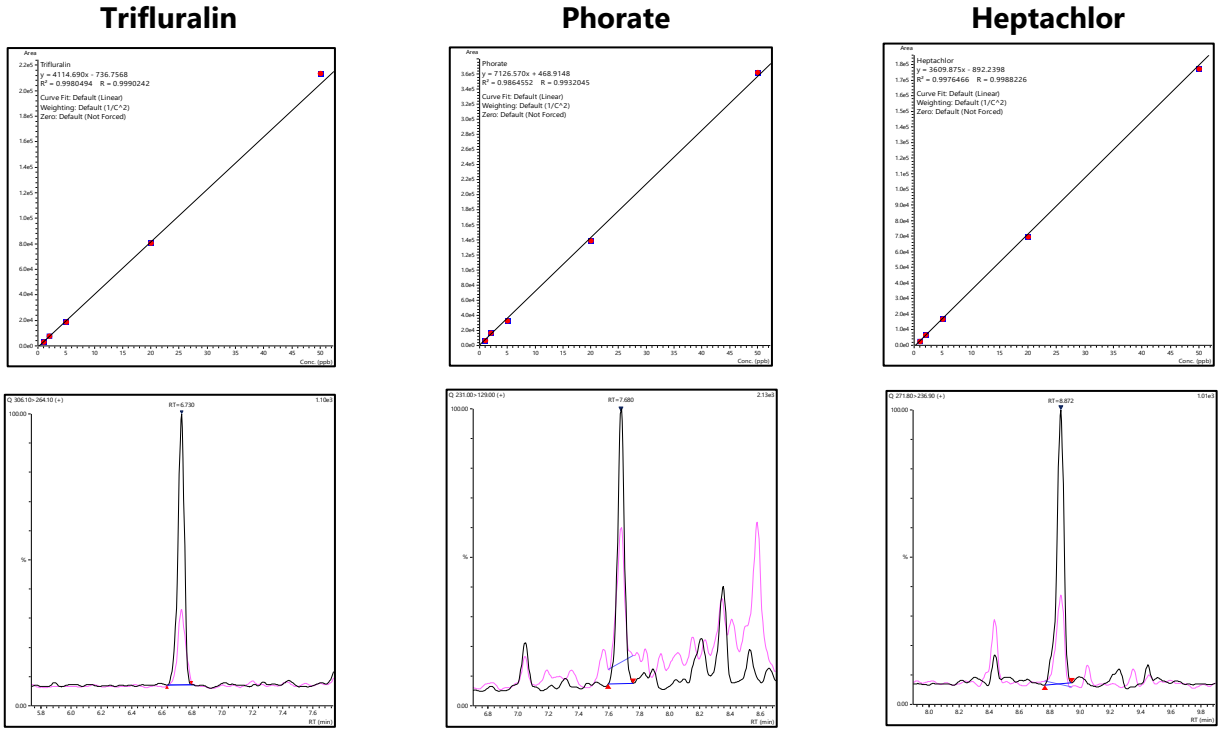

Fig. 4 Representative linearity graphs and chromatograms at LOQ level for GC-MS/MS compounds

4. Conclusion

A simple, sensitive and rapid method has been developed to quantify 243 pesticides by LC-MS/MS and GC-MS/MS in red chili powder sample. Quantification of pesticides in red chili powder is highly challenging due to the complexity of matrix. Hence, a modified QuEChERS extraction technique was used for sample preparation.

The method developed on Shimadzu LC-MS/MS and GC-MS/MS proved to be highly sensitive and reproducible as most of the compounds showed good RSD_r and RSD_R (as per SANTE guidelines) at trace levels.

This highlights the reliability of the method and enables its use in testing laboratories for multi-residue analysis of red chili samples.

5. References

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