

Method for the determination of 346 Residual Pesticides in Milk using LCMS-8045 and GCMS-TQ8040 NX

 Sujit Patil, Yogesh Arote, Chaitanya Atmakuri, Dr. Avinash Gaikwad
 Shimadzu Application Development Centre

User Benefits

- ◆ A modified QuEChERS extraction procedure has been employed for quantifying the pesticides at the desirable concentration levels by using Ultra-fast technologies of LCMS-8045 and GCMS-TQ8040 NX.
- ◆ Shorter run time of analysis increases the productivity and throughput of the LC-MS/MS and GC-MS/MS system.
- ◆ Method employs lower injection volume and lesser flow rate, enhancing column life and stability of assay over longer durations of analysis.

1. Introduction

Milk is an important food in the diet, especially for infants and children. The presence of any contamination in milk is a common food safety concern. Hence great efforts have been taken throughout the dairy industry to ensure the safety of milk. One of the main classes of contaminants in milk is pesticides, which can come from animals ingesting contaminated feed or water. The maximum residue limits for pesticides in milk are often much lower than for general fruits and vegetables.^[1] Therefore, the analysis of pesticides in milk requires a sample preparation method for better matrix removal and analytical instrument methods for increased sensitivity. The aim of this study is to develop a simple and efficient workflow for determining a wide range of pesticides that are broadly controlled in milk worldwide. Based on these requirements, Shimadzu Application Development Center (ADC) has developed and validated a simple, sensitive and high throughput multiclass, multiresidue method for the determination of 346 pesticides in milk by using LCMS-8045 and GCMS-TQ8040 NX. The multi-residue extraction was performed with modified QuEChERS^[2] method for simultaneous quantification of 223 pesticides by LC-MS/MS and 167 pesticides by GC-MS/MS. Out of these, 44 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 compounds regulated	No. of compounds covered in this method
FSSAI	66	53
EU	484	265
APEDA	275	136

2. Materials and Methods

The reference standards were procured from Restek with below catalogue numbers:

LC multi residue pesticides kit – 31971

GC multi residue pesticides kit – 32562



Fig. 1 Shimadzu LCMS-8045

Milk sample procured from local market, was used to prepare matrix-matched calibration standards and fortified samples. The calibration standards were analyzed in the range of 2.5 to 40 µg/L. Calibration curves were generated by external standard method and using weighted regression of 1/C². Fortified samples were prepared in six replicates of each 5 and 10 µg/kg. The compounds marked with asterisk (*) in summary result tables; were present in both LCMS and GCMS standard mixture. Hence their calibration range and spiking levels were two times the concentration levels mentioned above.

Shimadzu LCMS-8045 with Nexera X2 (Figure 1) and GCMS-TQ8040 NX (Figure 2), manufactured by Shimadzu Corporation Japan, were used to quantify residual pesticides in milk sample.

Shimadzu's Method Package™ Ver.3 for LC-MS/MS and Smart Pesticides Database™ Ver.2 for GC-MS/MS enabled quick instrumental method optimization for higher throughput. 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 LC-MS/MS and GC-MS/MS in which modified QuEChERS method was adopted. Initially, the sample was deproteinized with acetonitrile and divided in two parts A and B. Part A was extracted using AR grade anhydrous magnesium sulphate (MgSO₄) salt. After phase separation, upper acetonitrile layer was divided in two parts i.e. A-I and A-II.

For LC-MS/MS analysis, A-I was mixed with part B and subjected to clean up using C18 followed by solvent exchange in mobile phase solution.

For GC-MS/MS, A-II was treated with C18 and MgSO₄. This clean up was followed by solvent exchange in ethyl acetate. The final reconstitution volume was adjusted to avoid dilution of the sample.

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-TQ8040 NX

2.2. Analytical Conditions

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

System Configuration	
LC-MS/MS	: LCMS-8045
Auto-sampler	: Nexera X2 SIL 30AC
Column	: Shim-pack™ XR-ODS II, (3.0 mm i.d. X 150 mm , 3 µm)
LC	
Flow rate	: 0.4 mL/min
Mobile phase A	: 2mM Ammonium formate in water + 0.02% Formic acid
Mobile phase B	: 2mM Ammonium formate in methanol + 0.02% Formic acid
Gradient program	: 20-65%B (1.0 mins to 4.0 mins) → 65-100%B (4.0 min to 12.0 min) → 100-100%B (12.0 min to 16.0 min) → 100-20%B (16.0 min to 16.5 min) → 20-20%B (16.5 min to 21.0 min)
Run time	: 21 min
Injection volume	: 5 µL (Co-injection with water)
Column oven temp	: 40 °C
MS	
Ionization	: ESI
Nebulizing gas flow	: 3 L/min
Heating gas flow	: 10 L/min
Drying gas flow	: 10 L/min
Interface temp.	: 300 °C
DL temp.	: 250 °C
Heating block temp.	: 400 °C

3. Result and Discussion

Validation parameters like specificity, linearity, recovery and precision were studied as per SANTE guidelines^[3]. Results obtained on LC-MS/MS and GC-MS/MS are shown in Table 4 and 5, respectively.

3.1. System precision and specificity

System precision was evaluated by calculating variation of the peak area and retention time of six replicates of 10 µg/L pesticide mixture. The % RSD was found to be less than 20 for peak area and retention times were within tolerance limit of ±0.1 min. 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 2.5 to 40 µg/L (5 to 80 µg/L for compounds marked with * in summary results tables 4 and 5). All calibration standards were found within 80 to 120% accuracy as per SANTE guidelines. The linearity graphs of some representative compounds are shown in figure 3 and 4.

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	: Sky Liner, Splitless
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 250kPa)
Injection volume	: 1 µL
Carrier gas	: He
Linear Velocity	: 36.5 cm/sec (Constant mode)
MS	
Interface temp.	: 300 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 3.5 min
Loop Time	: 0.5 sec

3.3. Recovery study

Recovery was evaluated by analysing fortified samples at 5 and 10 µg/kg (20 and 40 µg/kg for * marked compounds in summary result tables 4 and 5) (six replicates at each level) 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 at their LOQ levels.

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 at 5 µg/kg and 10 µg/kg concentration levels. The % RSD for repeatability of six injections at their respective LOQ levels were found to be less than 20%. (Refer tables 4 and 5)

Reproducibility (RSD_R):

Reproducibility experiment for recoveries was performed on six different fortified samples at 5 µg/kg and 10 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 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)	% RSD _D (n=6)
1	Formetanate Hydrochloride	1.885	222>165.1	-16	0.9972	0.005	97.40	101.9	2.12	3.08
2	Propamocarb	2.156	189.2>102.15	-8	0.9995	0.005	99.40	88.98	4.74	3.76
3	Methamidophos	2.641	142.2>93.95	-15	0.9995	0.005	101.44	89.4	2.35	0.98
4	Acephate	3.11	184>143	-10	0.9997	0.005	97.76	91.84	3.32	2.43
5	Omethoate	3.638	214.1>183	-11	0.9998	0.005	106.04	80.49	5.09	4.25
6	Dinotefuran	4.033	203.15>114.15	-13	0.9994	0.005	97.28	82.2	3.27	3.97
7	Aldicarb-sulfoxide	3.948	207.1>89.1	-14	0.9993	0.005	99.04	91.99	3.62	6.34
8	Aldicarb-sulfone (Aldoxycarb)	4.343	240.1>148.15	-14	0.9826	0.005	98.64	92.5	5.77	2.43
9	Butocarboxim-sulfone	4.159	240.1>106.15	-13	0.9973	0.005	99.88	84.04	4.89	3.73
10	Aminocarb	3.993	209>152.05	-5	0.9999	0.005	104.08	76.83	4.39	5.18
11	Oxamyl	4.421	237.1>72.1	-22	0.9962	0.005	100.48	84.78	6.45	2.38
12	Pymetrozine	4.141	218.1>105	-12	0.9955	0.005	99.56	77.02	5.84	4.32
13	Nitenpyram	4.4	271.1>225	-12	0.9951	0.005	101.16	75.89	11.96	7.77
14	Methomyl	4.799	163>106.15	-11	0.9936	0.005	99.36	83.58	9.12	3.7
15	Thiamethoxam	4.861	292>211.1	-13	0.9974	0.005	104.36	86.77	2.28	3.76
16	Monocrotophos	4.921	240.9>193	-12	0.9976	0.005	104.12	78.28	12.93	5.48
17	Dicrotophos	5.321	237.9>72	-26	0.9919	0.005	98.84	91.68	7.77	7.72
18	Dichlorvos	5.329	238>109.1	-21	0.9996	0.010	96.96	80.57	19.98	14.43
19	Carbendazim	5.276	192.1>160.15	-6	0.9996	0.005	104.64	100.6	9.88	4.62
20	Vamidotion	5.804	288.1>146.05	-6	0.9968	0.005	102.04	86.37	3.69	6.34
21	Imidacloprid	5.556	256.1>174.95	-20	1.0000	0.005	100.56	100.2	13.07	7.83
22	Mevinphos*	5.85	225.1>127	-17	0.9983	0.010	98.96	78.9	7.19	3.24
23	Clothianidin	5.652	250>132.05	-16	0.9968	0.005	103.36	78.22	9.05	7.17
24	Acetamiprid	5.874	223.1>126.1	-11	0.9979	0.005	104.16	80.75	4.72	8.04
25	Carbofuran-3-hydroxy (3-Hydroxycarbofuran)	5.826	255>163.15	-19	0.9988	0.005	98.72	76.93	4.63	8.71
26	Fenuron	5.932	165>46.1	-14	0.9991	0.005	102.28	73.39	6.57	6.95
27	Trichlorfon	5.98	257>109	-17	0.9986	0.005	99.92	79.45	5.9	7.78
28	Dimethoate	5.951	230>125	-11	0.9997	0.005	99.64	86.13	3.54	4.03
29	Thiacloprid	6.156	253>126.05	-11	0.9983	0.005	95.28	82.18	8.66	6.16
30	Thiabendazole	5.983	202>175	-15	0.9958	0.005	102.48	79.29	10.47	5.45
31	Cymoxanil	6.201	199.1>128.15	-9	0.9935	0.005	95.12	78.52	7.96	6.66
32	Fuberidazole	6.129	184.9>156.15	-17	0.9991	0.005	99.20	74.14	5.66	6.57
33	Tricyclazole*	6.466	190.1>136	-24	0.9980	0.010	99.48	81.1	6.3	4.8
34	Carbetamide	6.799	237.1>192.1	-5	0.9978	0.005	93.80	89.2	8.91	11.33
35	Fenamiphos-sulfoxide	6.94	320.1>233	-21	0.9962	0.005	97.36	136.99	19.37	10.13
36	N-(2,4-Dimethylphenyl)formamide	6.924	150.1>107.1	-21	0.9959	0.005	95.92	76.38	7.33	4.51
37	Propoxur	7.095	209.9>168.15	-9	0.9964	0.005	100.36	68.82	13.68	1.57
38	Thidiazuron	7.117	221>102	-16	0.9984	0.010	93.48	90.87	10.42	12.78
39	Fenthion-sulfoxide	7.159	295>279.9	-19	0.9943	0.005	98.04	121.29	16.3	13.66
40	Carbofuran	7.131	222.1>165	-6	0.9996	0.005	100.36	74.93	10.54	5.03
41	Metribuzin	7.297	215.1>187.1	-18	0.9984	0.010	93.88	63.52	10.57	11.29
42	Imazalil	7.034	297.1>159.05	-23	0.9960	0.005	100.32	80.86	10.39	3.88
43	Hexazinone	7.296	253.2>171.15	-6	0.9991	0.005	100.04	77.27	5.04	7.68
44	Spiroxamine	7.335	298.2>144.2	-10	0.9955	0.005	102.20	72.82	12.8	6.49
45	Ethirimol	7.222	210.2>140.2	-16	0.9966	0.005	95.68	94.39	9.63	9.2
46	Dimethirimol	7.222	210.2>140.1	-22	0.9947	0.005	102.88	89	9.19	7.39
47	Tebuthiuron	7.378	229.1>116	-27	0.9964	0.005	98.20	64.13	11.42	4.53
48	Carbaryl (NAC)	7.403	202.1>145.1	-12	0.9961	0.005	95.08	95.88	6.67	9.76
49	Carboxin	7.454	236.1>143.1	-5	0.9984	0.005	100.12	48.98	11.08	2.41
50	Fluometuron	7.665	233.1>46.2	-18	0.9958	0.005	101.72	84.63	10.74	7.9
51	Monolinuron	7.7	215.1>148	-15	0.9979	0.005	98.32	59.87	18.79	7.77
52	Disulfoton-sulfoxide	7.696	290.8>97	-34	0.9950	0.005	98.48	80.05	6.2	4.74
53	Diuron (DCMU)	7.662	233>46.15	-17	0.9970	0.005	98.76	72.26	18.23	5.02
54	Pirimicarb	7.628	239.2>72	-13	0.9988	0.005	102.88	82.31	8.39	4.14
55	Ethiofencarb	7.646	226.1>107	-21	0.9984	0.005	94.88	50.17	8.33	3.42
56	Phorate-sulfone	7.72	293>96.9	-34	0.9954	0.005	104.16	91.9	12.9	13.26

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)	% RSD _r (n=6)
57	Phorate-sulfoxide	7.729	277>198.9	-10	0.9987	0.005	100.00	102.47	11.09	6.72
58	Thiofanox	7.78	241.2>184	-12	0.9998	0.005	101.20	67.67	17.7	9.16
59	Flutriafol (isomer)	7.817	302.1>70.05	-22	0.9983	0.005	102.56	83.22	11.18	12.03
60	Chlorotoluron	7.884	213.1>72.15	-7	0.9988	0.005	105.36	79.22	8.62	8
61	Simetryn	7.874	214.1>96	-20	0.9959	0.005	100.04	86.38	10.32	3.54
62	Metobromuron	7.987	259>170	-19	0.9980	0.005	93.76	68.26	13.9	13.53
63	Isoproc carb	7.944	194.1>95	-11	0.9998	0.010	101.96	48.72	12.62	3.73
64	Metazachlor	7.988	277.9>210.05	-6	0.9994	0.005	98.88	88.23	4.01	7.65
65	Metalaxyl (Mefenoxam) *	8.092	280.2>220	-6	0.9982	0.005	106.92	70.68	9.59	2.58
66	Atrazine	8.12	216.1>174.1	-13	1.0000	0.005	97.84	92.17	12.77	9.97
67	Forchlorfenuron	8.132	248.1>129.15	-17	0.9981	0.005	102.76	94.08	9.97	7.26
68	Lenacil	8.174	234.9>153.15	-6	0.9978	0.010	94.36	105.99	13.98	18.56
69	Propachlor	8.132	212.1>94	-27	0.9886	0.010	102.04	41.42	17.72	5.31
70	Desmedipham	8.096	318>182	-6	0.9996	0.005	92.72	85.82	13.78	15.56
71	Isoproturon	8.149	207.2>46.1	-12	0.9992	0.005	103.40	82.58	7.39	4.93
72	Norflurazon	8.171	304.1>283.95	-18	0.9954	0.005	96.40	77.32	11.12	10.66
73	Diphenamid	8.373	239.9>167.1	-18	0.9972	0.005	97.80	76.79	9.61	11.23
74	Fluridone	8.362	330.1>309	-31	0.9935	0.005	102.40	83.53	13.86	9.74
75	Secbumeton	8.344	226.2>142.1	-12	0.9992	0.005	98.52	74.76	9.78	11.24
76	Cycluron	8.434	199.2>46.2	-16	0.9973	0.005	94.00	76.59	19.52	5.88
77	Azoxystrobin	8.431	404>371.95	-5	0.9954	0.005	99.08	87.6	9.87	4.21
78	Mexacarbate	8.426	223.1>151.15	-14	0.9975	0.005	93.60	100.94	9.15	7.9
79	Fenpropimorph	8.219	304.2>117	-55	0.9989	0.005	104.68	60.12	19.55	10.65
80	Terbumeton	8.543	225.9>170	-6	0.9971	0.005	99.76	71.9	7.65	11.83
81	Furalaxyl	8.651	302.1>95	-17	0.9957	0.005	101.80	73.43	10.06	4.4
82	Azinphos-methyl	8.529	318>160.15	-7	0.9770	0.010	102.68	85.35	19.46	5.03
83	Phosmet	8.533	318>159.9	-8	0.9976	0.010	99.16	85.53	11.9	6.28
84	Mandipropamid	8.821	412.1>327.9	-10	0.9999	0.005	98.72	90.79	10.91	6.83
85	Clomazone	8.707	239.9>125	-11	0.9992	0.010	94.08	44.28	14.31	7.88
86	Methoprotryne	8.734	272.2>240	-13	0.9954	0.005	95.80	83.55	12.6	10.18
87	Ethiprole	8.792	397>350.9	-22	0.9849	0.005	93.64	98.02	8.77	19.33
88	Ethofumesate	8.775	304.1>287	-11	0.9970	0.010	108.24	80.01	16.84	13.73
89	Diethofencarb	8.749	268.2>124.15	-31	0.9975	0.005	99.76	75.32	12.86	9.11
90	Dodine	8.803	228.3>43.05	-30	0.9970	0.005	95.36	89.47	14.15	14.25
91	Fenobucarb	8.757	208.1>95	-10	0.9972	0.010	105.20	46.62	14.08	8.07
92	Nuarimol	8.815	315.1>251.95	-22	0.9992	0.005	96.64	74.68	11.42	16.14
93	Ametryn	8.81	228.1>186	-9	0.9948	0.005	100.28	77.41	6.06	8.61
94	Fenamidone	8.881	312.1>236	-10	0.9956	0.005	103.96	62.29	17.55	9.59
95	Boscalid	9.007	343>306.95	-11	0.9975	0.010	97.72	92.47	6.23	7.86
96	Siduron	8.935	233.2>137.1	-12	0.9890	0.010	105.00	96.63	12.72	12.65
97	Linuron*	8.974	248.8>160	-18	0.9976	0.005	100.20	65.51	11.3	10.14
98	Dimethomorph (E, Z)	9.186	388.1>165.1	-34	0.9964	0.005	107.80	80.39	11.85	14.29
99	Methiocarb	9.03	226.1>169.05	-10	0.9984	0.005	95.52	84.38	11.92	11.73
100	Flutolanil*	9.044	324.1>261.9	-14	0.9983	0.005	101.56	79.83	3.73	7.7
101	Propanil	9.06	218>161.95	-14	0.9990	0.010	107.56	95.6	17.97	14.71
102	Paclobutrazol*	9.183	294.1>70.1	-22	0.9976	0.005	98.40	78.25	18.49	6.9
103	Terbuthylazine	9.162	230.1>174.05	-7	0.9997	0.005	94.32	80.17	10.86	14.6
104	Methoxyfenozide	9.132	369.2>312.95	-9	0.9984	0.005	91.96	87	9.13	11.8
105	Malathion	9.2	348.1>127.05	-17	0.9989	0.005	99.52	59.18	11.27	16.18
106	Pyrimethanil*	9.195	200.1>107	-24	0.9896	0.005	96.08	67.21	10.43	13.09
107	Promecarb	9.203	208.1>109.1	-10	0.9958	0.005	95.52	59.59	6.72	7.52
108	Benzthiazuron	9.197	208.1>151.1	-6	0.9994	0.010	98.92	64.05	10.15	6.46
109	Mepronil	9.284	270.2>228	-9	0.9997	0.005	104.12	72.36	11.91	10.24
110	Myclobutanil*	9.325	289.1>70.05	-22	0.9998	0.005	99.16	87.58	9.28	9.41
111	Pyridaphenthion	9.336	341.1>204.9	-22	0.9994	0.005	104.40	74.99	16.06	6.52
112	Triadimefon*	9.386	294.1>69	-22	0.9926	0.005	98.84	85.22	6.38	11.07

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)	% RSD _D (n=6)
113	Triazophos	9.447	314.1>162.05	-9	0.9989	0.005	107.04	86.55	5.79	8.35
114	Propyzamide	9.442	256>190	-14	0.9985	0.005	97.52	62.92	16.71	11.46
115	Butafenacil	9.458	492.1>348.85	-15	0.9992	0.005	96.56	86.09	9.02	9.95
116	Isoxathion	9.455	314>97.05	-40	0.9969	0.005	102.88	75.94	7.00	7.30
117	Fluoxastrobin	9.481	458.8>188.1	-35	0.9988	0.005	100.84	98.34	8.65	10.63
118	Mefenacet	9.506	299>192	-11	0.9930	0.005	109.12	82.54	5.70	7.18
119	Isazofos	9.490	314.1>162.05	-12	0.9916	0.005	95.24	82.37	6.51	15.48
120	Spinosyn A	9.368	732.6>142.2	-29	0.9994	0.005	100.76	73.22	6.23	3.56
121	Chloroxuron	9.609	291.1>72.15	-8	0.9911	0.010	102.32	93.26	18.64	11.70
122	Tetraconazole	9.668	372>159	-30	0.9986	0.005	97.80	92.06	19.23	8.68
123	Fenhexamid	9.716	302.1>97.1	-24	0.9989	0.005	97.52	77.97	16.16	10.53
124	Cyproconazole	9.694	292.1>70.05	-22	0.9860	0.010	103.12	97.57	4.98	5.63
125	Fluquinconazole*	9.707	376>349	-20	0.9989	0.010	101.84	86.09	16.87	19.70
126	Hydramethylnon	9.582	495.1>151	-55	0.9967	0.005	104.40	68.87	8.38	7.16
127	Naled (Dibrom)	9.716	395.8>127.15	-21	0.9954	0.010	102.12	105.58	9.12	17.27
128	Flufenacet	9.752	364.1>124.05	-34	0.9967	0.005	101.84	80.20	7.74	4.21
129	Terbutryn	9.752	242.1>68	-43	0.9944	0.005	98.68	94.60	10.53	4.04
130	Azinphos-ethyl	9.791	346>97	-33	0.9997	0.005	94.08	90.03	13.20	6.57
131	Triticonazole	9.836	318.1>70.15	-22	0.9992	0.005	100.44	82.05	14.27	8.71
132	Cyazofamid	9.878	325>108.1	-15	0.9996	0.005	91.92	75.89	10.13	17.23
133	Mepanipyrim	9.910	224.1>106.05	-26	0.9947	0.005	93.32	75.92	10.22	5.62
134	Fenbuconazole	9.953	337.1>125.05	-29	0.9876	0.010	102.72	96.59	6.72	6.54
135	Epoxiconazole	9.928	330>101.1	-44	0.9962	0.010	96.40	92.91	13.59	8.90
136	Etaconazole	9.970	328.1>159	-28	0.9994	0.005	100.76	67.96	19.78	13.40
137	Flusilazole*	10.113	316.1>165.1	-26	0.9992	0.010	94.84	78.24	12.77	7.93
138	Picoxystrobin	10.091	368>205	-5	0.9964	0.005	101.40	84.18	11.44	13.14
139	Rotenone	10.145	395.1>213	-23	0.9954	0.010	96.16	100.26	18.17	12.84
140	Spinosyn D	9.957	746.6>142.1	-30	0.9978	0.005	105.08	87.07	14.97	12.35
141	Fenamiphos	10.102	304.1>233.9	-12	0.9984	0.010	99.88	97.02	15.20	11.27
142	Diflubenzuron	10.163	311>158.1	-16	0.9994	0.005	103.00	79.56	10.40	12.17
143	Bupirimate*	10.141	317.2>108	-26	0.9985	0.010	99.16	83.30	8.19	3.49
144	Spinetoram J	10.047	748.5>142.15	-26	0.9954	0.005	103.36	85.50	6.96	7.01
145	Metolachlor	10.212	284.1>176.1	-26	0.9897	0.005	103.56	62.73	9.54	6.38
146	Fenoxycarb	10.215	302.1>116.15	-11	0.9981	0.005	98.20	83.25	6.93	7.10
147	Tetrachlorvinphos (CVMP)	10.348	366.9>127.15	-16	0.9896	0.005	93.80	87.56	14.04	12.21
148	Dimoxystrobin	10.376	327.1>205	-6	0.9988	0.005	97.00	72.65	12.28	10.35
149	Neburon	10.414	274.8>87.95	-17	0.9903	0.005	103.08	72.44	14.34	11.47
150	Diclobutrazol (stereo isomer)	10.465	328>70.1	-22	0.9988	0.005	102.16	66.91	14.48	19.50
151	Kresoxim-methyl	10.548	314.1>267	-8	0.9996	0.005	98.84	91.54	8.27	13.33
152	Bromuconazole	9.639	377.9>159.05	-26	0.9987	0.005	98.36	88.47	9.23	19.27
153	Tolyfluanid	10.521	364>237.95	-15	0.9935	0.005	92.72	46.07	17.62	18.76
154	Edifenphos	10.578	311>282.95	-9	0.9991	0.005	99.96	71.69	19.51	12.55
155	Quinalphos	10.593	299.1>163	-22	0.9991	0.005	102.00	78.66	6.78	9.68
156	Famoxadone	10.599	391.9>331.25	-10	0.9992	0.005	105.28	89.26	12.64	16.06
157	Tebuconazole*	10.651	308.2>70.05	-22	0.9991	0.010	98.84	79.27	9.47	9.10
158	Penconazole*	10.655	284.1>70.05	-17	0.9932	0.010	98.68	76.02	10.39	12.98
159	Benalaxyl	10.741	326.2>91.05	-22	0.9986	0.005	103.00	72.08	10.00	8.66
160	Chlorfenvinphos (E, Z)	10.742	359>155.15	-13	0.9908	0.005	105.52	75.55	8.10	7.36
161	Spinetoram L	10.642	760.6>142.1	-31	0.9959	0.005	101.68	73.59	18.31	9.02
162	Dimethachlor	10.806	256.1>148	-25	0.9993	0.005	101.44	64.44	17.77	2.82
163	Fenthion	10.812	278.9>247	-14	0.9995	0.010	103.24	74.26	18.76	16.47
164	Emamectin B1a	10.711	886.4>158.2	-31	0.9990	0.005	103.08	83.78	9.30	8.34
165	Pyraclostrobin	10.896	388>164.05	-19	0.9996	0.005	95.64	86.48	7.74	6.46
166	Propiconazole (stereo isomer)	10.837	342>158.9	-28	0.9977	0.005	101.52	99.04	10.63	11.90
167	Triflumuron	10.885	359>156.05	-17	0.9988	0.005	93.48	82.87	9.81	8.25
168	Zoxamide	10.909	336>186.95	-18	0.9943	0.005	101.44	81.24	13.98	6.80

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	% Accuracy at LOQ	Recovery at LOQ (%)	Precision	
						mg/kg			% RSD _R (n=6)	% RSD _r (n=6)
169	Cyprodinil*	10.866	226.1>93	-37	0.9951	0.010	99.96	74.05	15.44	8.42
170	Bromfeninfos	10.939	404.9>155.1	-13	0.9999	0.005	103.40	76.28	5.67	7.97
171	Bitertanol (diastereo isomers)	11.033	338>99.15	-15	0.9967	0.005	96.68	94.93	14.44	12.44
172	Fonofos	11.013	247>109.1	-21	0.9989	0.005	101.32	34.18	15.08	7.86
173	Prochloraz*	11.071	376>307.95	-8	0.9980	0.010	98.04	71.08	12.06	5.03
174	Pyraclufos	11.021	361.1>256.9	-22	0.9994	0.005	105.32	75.23	10.33	8.04
175	Metconazole	11.050	320.1>70.15	-23	0.9998	0.005	98.96	81.45	13.78	9.82
176	Phosalone	11.090	368>182	-17	0.9999	0.005	94.44	86.44	9.28	8.10
177	Pyrazophos	11.191	374.1>194	-28	0.9988	0.010	101.60	90.49	14.07	8.38
178	Indoxacarb	11.234	528.1>150.1	-24	0.9991	0.010	95.36	91.40	14.30	6.73
179	Pirimiphos-methyl	11.300	306.1>164.1	-18	0.9989	0.010	98.80	56.72	16.38	3.65
180	Clofentezine	11.344	303>138.15	-15	0.9997	0.005	103.76	57.31	5.49	7.32
181	Diniconazole	11.369	326.1>70.05	-25	0.9958	0.005	105.36	87.58	17.13	15.49
182	Novaluron	11.380	493>158	-18	0.9994	0.005	102.56	93.45	17.77	12.35
183	Difenoconazole (isomer)	11.369	406.1>250.9	-20	0.9997	0.005	100.72	85.53	12.31	7.13
184	Trifloxystrobin	11.409	409.1>186.1	-6	0.9987	0.005	98.56	78.63	10.06	8.08
185	Ipconazole	11.710	334.1>70.1	-17	0.9960	0.005	101.32	70.56	14.91	11.94
186	Triflumizole*	11.670	346>278	-6	0.9982	0.010	106.72	65.59	7.41	7.18
187	Metaflumizone	11.856	507.1>178.05	-27	0.9992	0.010	103.84	97.11	16.36	17.75
188	Fluazifop-butyl	11.956	384.2>281.9	-7	0.9977	0.010	95.00	85.53	19.19	6.09
189	Profenofos	12.075	375>304.7	-19	1.0000	0.005	97.52	76.43	6.39	10.84
190	Tolfenpyrad	11.951	384>91	-55	0.9996	0.005	95.28	69.41	14.43	6.29
191	Fluopicolide	12.109	383>108.95	-55	0.9953	0.005	100.08	79.97	14.01	12.26
192	Temephos	12.137	467>125	-38	0.9996	0.005	100.68	74.55	6.54	8.73
193	Furathiocarb	12.106	383.2>167.05	-27	0.9961	0.005	108.24	77.83	6.14	7.01
194	Tetramethrin	12.171	332.2>164.1	-24	0.9974	0.005	103.68	64.50	2.67	11.46
195	Tebufenpyrad*	12.243	334.2>147.2	-25	0.9997	0.010	105.32	75.62	8.00	8.54
196	Buprofezin	12.316	306.2>57	-19	0.9977	0.005	103.72	61.15	8.43	6.48
197	Ethion	12.460	385>198.9	-10	0.9995	0.005	100.72	64.34	8.00	6.18
198	Pirimiphos-ethyl	12.458	334.1>182.1	-18	0.9993	0.005	106.64	46.56	9.97	3.90
199	Chlorpyrifos-oxon	12.468	334>197.85	-30	0.9924	0.005	104.24	44.86	10.66	6.35
200	Piperonyl-butoxide*	12.552	356.2>177	-3	0.9998	0.010	100.52	72.14	4.07	4.28
201	Pyriproxyfen*	12.732	322.1>184.95	-24	0.9997	0.010	97.68	57.14	13.01	5.65
202	Flufenoxuron	12.756	489>158.1	-21	0.9972	0.005	98.56	68.76	6.42	7.31
203	Hexythiazox	12.774	353.1>228	-11	0.9984	0.005	96.68	60.82	9.35	6.76
204	Etoxazole	12.944	360.1>141.1	-15	0.9988	0.005	103.84	48.92	15.17	7.27
205	Propargite*	12.972	368.2>231.1	-6	0.9977	0.010	103.64	62.14	8.04	2.45
206	Quinoxifen	12.998	308>197	-31	0.9986	0.005	103.04	57.00	16.80	7.90
207	Pendimethalin	13.056	282.2>212	-11	0.9997	0.005	100.12	56.32	15.70	7.16
208	Carbophenothion	13.101	343>157.05	-13	0.9968	0.005	98.44	45.43	16.96	10.33
209	Chlorfluazuron	13.148	539.9>382.85	-20	0.9997	0.005	106.28	74.64	9.67	13.38
210	Flumetralin	13.285	422.1>107.1	-54	0.9862	0.010	100.96	91.83	13.97	10.31
211	Spirodiclofen	13.301	411.1>313.05	-14	0.9987	0.005	101.88	66.37	11.79	5.26
212	Fenpyroximate	13.283	422.1>366	-4	0.9992	0.005	101.32	61.52	13.38	8.65
213	Deltamethrin	13.373	523>281	-17	0.9899	0.005	100.84	42.70	14.10	15.92
214	Fluvalinate	13.632	503.1>180.95	-29	0.9985	0.005	94.24	69.02	14.41	17.70
215	Pyridaben*	13.764	365.2>147.1	-15	0.9994	0.010	100.60	47.12	5.85	4.18
216	Fenazaquin	14.108	307.2>161.1	-12	0.9980	0.005	103.12	48.06	10.94	5.31
217	Phenothrin (isomer)	14.317	351.2>183.05	-21	0.9992	0.010	98.72	46.92	13.87	15.72
218	Permethrin	14.476	408.1>180.15	-21	0.9994	0.010	98.60	42.81	17.45	2.50
219	Bifenthrin	14.596	440.2>183.1	-17	0.9992	0.005	99.20	55.81	15.25	11.12
220	Fludioxonil*	8.938	247>180.15	28	0.9954	0.010	97.32	80.38	18.49	19.77
221	Fipronil*	9.989	435>330	16	0.9989	0.010	96.08	77.82	4.23	4.75
222	Hexaflumuron	11.376	458.8>439	11	0.9992	0.010	100.64	120.80	17.28	6.59
223	Teflubenzuron	12.404	378.8>339	12	0.9974	0.010	97.84	119.82	8.62	11.01

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)	% RSD _r (n=6)
1	Allidochlor	4.749	132.10>56.00	8	0.9965	0.005	101.60	65.23	10.07	8.04
2	Dichlobenil	5.081	170.90>136.00	14	0.9991	0.005	98.00	55.30	9.75	6.03
3	Biphenyl	5.257	154.10>115.10	24	0.9993	0.005	96.00	38.83	17.73	6.80
4	Mevinphos-1*	5.451	127.00>95.00	18	0.9959	0.010	100.80	51.73	19.31	17.51
5	Mevinphos-2*	5.452	127.00>95.00	18	0.9978	0.010	111.20	72.37	18.22	6.67
6	Etridiazole	5.570	210.90>139.90	22	0.9994	0.005	97.60	65.27	16.69	13.85
7	3,4-Dichloroaniline	5.586	161.00>99.00	22	0.9967	0.005	113.20	46.30	12.43	8.89
8	Methacrifos	5.774	240.00>208.00	4	0.9964	0.010	100.80	59.10	13.53	11.84
9	Chloroneb	5.886	193.00>113.00	18	0.9919	0.005	102.00	68.03	15.34	10.46
10	Pentachlorobenzene	6.077	251.90>214.90	22	0.9937	0.005	99.60	56.80	7.01	3.12
11	2-Phenylphenol	6.077	170.10>141.10	24	0.9981	0.010	88.80	63.88	13.55	17.19
12	Tecnazene	6.535	260.90>202.90	14	0.9987	0.010	102.40	61.55	14.26	18.13
13	Propachlor	6.597	120.00>77.00	20	0.9980	0.005	100.00	82.60	15.34	12.59
14	Ethalfuralin	6.746	276.00>202.00	18	0.9977	0.005	115.60	83.70	8.64	6.77
15	Cycloate	6.784	154.20>83.10	8	0.9963	0.005	102.40	65.93	16.33	15.54
16	Trifluralin	6.846	264.10>206.10	8	0.9998	0.010	102.00	65.40	14.93	15.46
17	Diphenylamine	6.777	169.10>66.00	24	0.9983	0.005	100.80	73.57	18.67	6.99
18	Benfluralin	6.885	292.10>160.00	22	0.9987	0.005	102.00	70.00	15.60	12.26
19	2,3,5,6-Tetrachloroaniline	6.818	230.90>158.00	22	0.9970	0.005	96.40	64.97	15.32	6.87
20	Sulfotep	6.958	266.00>146.00	18	0.9995	0.005	104.40	65.87	19.20	15.83
21	Chlorpropham	6.937	213.10>127.10	14	0.9965	0.005	96.80	80.77	15.20	13.03
22	Di-allate-1	7.172	234.10>192.10	14	0.9994	0.005	102.40	63.57	12.67	14.11
23	alpha-BHC	7.346	180.90>144.90	16	0.9980	0.005	99.60	75.47	12.54	8.77
24	Hexachlorobenzene	7.430	283.80>213.80	28	0.9969	0.005	100.00	60.23	10.81	9.75
25	Pentachloroanisole	7.484	264.80>236.80	16	0.9996	0.005	97.60	74.87	17.12	11.09
26	Atrazine	7.643	200.10>104.10	18	0.9910	0.010	100.00	70.13	15.69	9.06
27	Clomazone	7.716	204.10>107.00	20	0.9998	0.005	99.60	80.33	13.37	12.23
28	Dicloran	7.635	206.00>176.00	10	0.9913	0.005	118.40	70.40	11.95	19.90
29	Terbufos	7.822	231.00>128.90	26	0.9982	0.005	95.60	76.73	16.67	9.25
30	Diazinon	7.860	179.10>137.10	18	0.9978	0.010	82.40	70.20	18.52	7.59
31	Quintozene	7.792	294.80>236.80	16	0.9771	0.005	101.60	81.43	13.81	14.72
32	Terbutylazine	7.841	229.10>173.10	6	0.9991	0.005	88.00	77.07	18.29	14.04
33	beta-BHC	7.783	180.90>144.90	16	0.9997	0.005	100.40	85.87	8.31	12.71
34	Fluchloralin	7.886	326.00>63.00	16	0.9982	0.005	99.20	80.73	17.85	17.18
35	Fonofos	7.939	137.10>109.10	8	0.9979	0.005	98.00	74.40	9.80	8.47
36	Pentachlorobenzonitrile	7.894	274.80>239.80	18	0.9993	0.005	100.00	72.87	11.66	9.43
37	Phorate	7.822	231.00>129.00	24	0.9979	0.005	94.40	80.10	10.40	6.17
38	Tefluthrin	8.046	177.00>137.10	16	0.9994	0.005	98.00	70.80	4.91	7.52
39	Propyzamide	7.909	172.90>144.90	16	0.9949	0.010	103.60	73.67	9.40	9.71
40	gamma-BHC (Lindane)	7.856	180.90>144.90	16	0.9984	0.005	92.80	71.23	13.43	13.09
41	Isazofos	8.087	257.00>119.00	18	0.9928	0.005	110.40	89.17	11.56	17.24
42	Disulfoton	7.824	186.00>153.00	6	0.9922	0.010	114.00	67.00	9.34	17.38
43	Pyrimethanil*	8.063	198.10>158.10	18	0.9993	0.010	98.00	78.80	13.81	15.63
44	Tri-allate	8.225	268.10>184.00	20	0.9868	0.005	98.40	62.50	11.32	11.48
45	Chlorothalonil	8.244	263.90>168.00	24	0.9989	0.005	114.80	64.90	13.06	17.29
46	Terbacil	8.243	161.00>144.00	14	0.9987	0.010	87.60	78.62	13.34	16.12
47	delta-BHC	8.349	180.90>144.90	16	0.9994	0.005	104.00	77.63	19.13	7.07
48	Dimethachlor	8.640	197.10>148.10	10	0.9988	0.005	102.40	59.13	13.54	7.70
49	Acetochlor	8.683	223.10>132.10	22	0.9966	0.005	80.40	85.33	19.11	11.82
50	Pentachloroaniline	8.612	262.90>191.90	22	0.9988	0.005	96.40	70.37	16.61	12.51
51	Chlorpyrifos-methyl	8.734	285.90>93.00	22	0.9997	0.005	101.20	88.70	18.76	10.40
52	Transfluthrin	8.810	163.10>143.10	16	0.9988	0.005	90.00	73.27	18.83	11.08
53	Vinclozolin	8.779	285.00>212.00	12	0.9960	0.010	108.00	81.48	11.46	11.78
54	Tolclofos-methyl	8.859	264.90>93.00	24	0.9998	0.010	96.40	70.03	8.73	13.75
55	Propisochlor	8.896	162.10>144.10	12	0.9993	0.005	99.20	80.37	15.93	16.15
56	Parathion-methyl	8.863	125.00>47.00	12	0.9999	0.005	97.60	82.50	9.29	6.92

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)	% RSD _r (n=6)
57	Metalaxyl (Mefenoxam)*	8.961	249.20>146.10	22	0.9972	0.010	118.40	71.03	10.36	11.32
58	Fenchlorphos	9.017	284.90>239.90	26	0.9983	0.005	83.20	58.60	17.67	18.90
59	Pirimiphos-methyl	9.149	305.10>290.10	12	0.9976	0.010	110.00	75.88	18.50	10.63
60	Alachlor	8.838	188.10>160.10	10	0.9987	0.005	80.00	68.63	10.87	15.58
61	Prodiamine	9.162	321.10>279.10	6	0.9895	0.005	102.00	87.37	10.42	9.48
62	Fenitrothion	9.266	277.00>109.10	14	0.9972	0.010	80.40	66.52	17.22	9.12
63	Malathion	9.348	158.10>125.00	10	0.9988	0.005	100.80	88.03	16.88	7.63
64	Dichlofluanid	9.389	223.90>123.10	8	0.9992	0.010	85.60	45.32	19.18	8.88
65	Metolachlor (S-Metolachlor)	9.491	238.10>162.10	12	0.9985	0.005	100.00	81.00	12.03	12.70
66	Chlorpyrifos	9.506	196.90>168.90	14	0.9998	0.005	92.00	77.93	16.30	18.16
67	Fenthion	9.591	278.00>109.00	20	0.9993	0.005	83.20	66.87	17.51	16.62
68	Chlorthal-dimethyl	9.612	298.90>220.90	24	0.9869	0.010	102.00	75.05	13.17	7.32
69	Triadimefon*	9.710	181.00>127.00	8	0.9993	0.010	99.20	78.83	7.45	20.29
70	Pirimiphos ethyl	9.825	304.10>168.10	12	0.9999	0.005	99.20	105.67	9.19	16.53
71	Isopropalin	9.936	280.10>238.10	8	0.9997	0.005	88.00	75.67	17.92	18.78
72	4,4'-Dichlorobenzophenone	9.864	249.90>139.00	16	0.9970	0.005	98.80	59.23	18.94	16.96
73	Bromophos	9.925	328.90>313.90	18	0.9909	0.010	85.60	67.22	11.72	7.89
74	Fenson	9.960	141.00>77.00	16	0.9953	0.005	97.20	85.63	10.58	4.98
75	Pendimethalin	10.097	252.10>162.10	10	0.9995	0.010	85.60	67.03	15.11	10.46
76	Cyprodinil*	10.150	224.10>197.10	22	0.9949	0.010	108.80	88.53	17.66	13.43
77	Metazachlor	10.172	209.10>132.10	18	0.9982	0.005	83.20	63.57	13.13	13.88
78	Tolyfluanid	10.275	238.00>137.10	14	0.9950	0.005	82.80	45.87	9.97	18.25
79	(E)-Chlorfenvinphos	10.278	267.00>159.00	18	0.9998	0.005	107.60	87.27	19.21	14.70
80	Heptachlor-exo-epoxide	10.326	352.80>262.90	14	0.9964	0.010	112.40	73.23	9.78	14.75
81	Penconazole*	10.250	248.10>157.10	26	0.9996	0.010	86.00	71.63	18.49	10.00
82	Bromfenvinfos-methyl	10.328	294.90>109.00	16	0.9951	0.005	107.60	58.60	13.40	13.08
83	Quinalphos	10.400	146.10>118.00	10	0.9988	0.005	100.00	92.00	19.76	6.86
84	Fipronil*	10.174	366.90>212.90	30	0.9998	0.010	101.60	87.47	12.41	13.42
85	Procydione	10.476	285.00>68.00	24	0.9965	0.010	104.00	75.62	8.93	12.61
86	Triadimenol-1*	10.479	168.10>70.00	10	0.9996	0.010	116.40	95.53	9.08	9.09
87	o,p'-DDE	10.754	246.00>176.00	30	0.9998	0.005	98.40	75.80	13.64	9.69
88	Chlorbenside	10.757	125.00>89.00	16	0.9992	0.005	95.20	70.30	14.57	11.38
89	Paclobutrazol*	10.838	236.10>125.00	14	0.9997	0.010	83.20	76.30	13.24	9.20
90	Bromfenvinphos	10.996	266.90>159.00	14	0.9956	0.005	105.20	90.73	17.82	4.63
91	Prothiofos	11.155	266.90>238.90	10	0.9988	0.005	81.20	72.23	14.41	10.60
92	Pretilachlor	11.165	262.10>202.10	10	0.9938	0.005	106.80	67.33	19.37	19.49
93	Flutriafol*	11.048	219.10>123.10	14	0.9987	0.010	100.40	89.87	10.49	3.17
94	Flutolanil*	11.083	281.10>173.00	12	0.9991	0.010	100.00	89.17	10.25	4.53
95	Oxadiazon	11.269	258.00>175.00	8	0.9997	0.005	86.80	71.63	18.56	7.43
96	Chlorfenson	11.175	175.00>111.00	12	0.9993	0.005	105.20	81.83	7.87	11.84
97	Profenofos	11.249	336.90>266.90	14	0.9992	0.005	94.80	93.00	18.43	12.62
98	p,p'-DDE	10.753	246.00>176.00	30	0.9998	0.005	98.40	76.00	13.61	9.75
99	Bupirimate*	11.401	273.10>108.10	16	0.9989	0.010	86.00	89.57	10.22	6.77
100	o,p'-DDD	11.467	235.00>165.00	24	0.9979	0.005	80.80	76.13	13.06	5.20
101	Flusilazole*	11.430	206.10>151.10	16	0.9999	0.010	95.20	83.70	8.57	11.75
102	Myclobutanil*	11.424	179.10>125.00	14	0.9993	0.010	84.40	90.70	8.65	9.22
103	Fluazifop-P-butyl	11.697	282.10>238.10	18	0.9952	0.005	96.40	83.70	16.68	15.75
104	1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane	11.764	223.20>167.10	14	0.9997	0.010	99.20	70.17	11.86	7.79
105	Chlorobenzilate	11.911	251.00>139.00	14	0.9976	0.005	86.80	93.13	9.54	7.80
106	Nitrofen	11.834	282.90>202.00	14	0.9988	0.010	98.40	71.62	7.33	17.83
107	Ethion	12.038	230.90>129.00	24	0.9967	0.005	80.80	80.30	10.48	6.32
108	Chlorthiophos-2	12.097	324.90>268.90	14	0.9955	0.005	83.60	63.30	14.85	16.11
109	Chlorthiophos-3	12.097	324.90>268.90	14	0.9942	0.005	84.40	63.47	15.04	16.29
110	p,p'-DDD	12.120	235.00>165.00	24	0.9986	0.005	84.00	71.50	6.89	4.37
111	o,p'-DDT	12.120	235.00>165.00	24	0.9986	0.005	84.00	71.50	6.89	4.37
112	Sulprofos	12.358	322.00>139.00	14	0.9996	0.010	87.60	80.55	11.08	13.47

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)	% RSD _r (n=6)
113	Triazophos	12.354	257.00>162.00	8	0.9961	0.005	94.80	84.47	7.92	13.38
114	Carfentrazone-ethyl*	12.446	340.10>312.10	14	0.9995	0.010	97.60	88.83	9.34	10.48
115	4,4'-methoxychlor olefin	12.539	308.00>238.10	16	0.9999	0.005	99.20	76.83	16.10	15.58
116	Carbophenothion	12.565	341.90>157.00	14	0.9979	0.005	98.80	78.13	13.21	15.85
117	p,p'-DDT	12.120	235.00>165.00	24	0.9986	0.005	84.00	71.53	6.88	4.37
118	2,4'-Methoxychlor	12.862	227.10>121.10	16	0.9988	0.005	90.80	78.43	13.64	3.66
119	Lenacil	12.810	153.10>136.10	14	0.9989	0.005	89.20	84.33	11.67	17.63
120	Piperonyl butoxide*	13.106	176.10>131.10	12	0.9993	0.010	99.60	91.87	7.79	8.04
121	Resmethrin-2 (Bioresmethrin)	13.143	143.10>128.10	10	0.9962	0.005	86.80	94.23	5.91	10.41
122	Dicofol	13.012	139.00>111.00	12	0.9989	0.010	117.60	76.65	14.48	9.14
123	Tebuconazole*	13.056	250.10>125.10	22	0.9990	0.010	88.40	76.07	9.68	8.40
124	Nitralin	13.141	316.10>274.00	8	0.9982	0.005	96.00	103.80	10.12	8.21
125	Tetramethrin-1	13.654	164.10>77.00	22	0.9964	0.005	100.00	92.00	16.64	14.97
126	Bifenthrin	13.568	181.10>166.10	12	0.9990	0.005	100.00	84.83	7.64	1.69
127	Pyridaphenthion	13.482	340.00>199.10	8	0.9996	0.005	85.20	85.40	8.55	17.26
128	Bromopropylate	13.681	340.90>182.90	18	0.9995	0.005	82.00	91.53	5.53	11.18
129	EPN	13.674	156.90>77.00	24	0.9999	0.005	90.40	95.07	15.16	9.88
130	Fenpropathrin	13.774	265.10>210.10	12	0.9944	0.005	86.80	83.87	11.71	8.92
131	Methoxychlor	13.769	227.10>169.10	24	0.9978	0.005	91.20	77.10	9.68	4.66
132	Phosmet	13.681	160.00>77.00	24	0.9989	0.005	100.80	88.10	15.13	11.73
133	Tebufenpyrad*	13.881	333.10>171.10	20	0.9992	0.010	96.80	82.77	9.89	8.02
134	Tetradifon	14.204	226.90>199.00	16	0.9912	0.005	94.80	81.80	13.98	8.51
135	Leptophos	14.304	376.90>361.90	24	0.9993	0.005	86.00	55.03	11.78	18.82
136	Pyriproxyfen*	14.418	136.10>96.00	14	0.9993	0.010	84.80	79.40	8.09	9.11
137	lambda-Cyhalothrin	14.547	197.00>141.00	12	0.9997	0.005	96.40	81.40	12.17	6.01
138	Azinphos-methyl	14.422	160.10>132.10	6	0.9875	0.010	104.40	82.37	19.77	10.46
139	Acrinathrin-2	14.679	289.10>93.00	14	0.9974	0.005	91.20	83.20	13.00	11.65
140	Mirex	14.760	271.80>236.80	18	0.9991	0.005	94.40	66.60	10.40	4.72
141	Pyrazophos	14.765	221.10>193.10	12	0.9992	0.005	96.80	85.17	9.10	6.73
142	Fenarimol*	14.851	251.00>111.00	26	0.9993	0.010	93.20	91.80	7.50	11.48
143	Azinphos-ethyl	14.971	160.10>132.10	4	0.9983	0.005	118.40	94.47	11.10	9.47
144	Pyraclufos	15.097	360.10>194.00	14	0.9951	0.005	102.80	77.27	11.51	13.18
145	cis-Permethrine	15.367	183.10>153.10	14	0.9994	0.005	94.80	79.60	6.08	11.26
146	trans-Permethrine	15.493	183.10>153.10	14	0.9971	0.005	82.40	61.83	15.55	12.49
147	Pyridaben*	15.560	364.10>147.10	22	0.9996	0.010	86.40	92.83	7.27	6.40
148	Fluquinconazole*	15.570	340.00>298.00	20	0.9960	0.010	92.40	93.50	4.73	4.56
149	Coumaphos	15.567	362.00>109.00	16	0.9947	0.010	96.80	77.18	7.12	6.35
150	Prochloraz*	15.637	308.10>70.00	20	0.9999	0.010	99.60	59.07	12.22	19.99
151	Cyfluthrin-1	15.902	163.10>127.10	6	0.9913	0.005	81.20	79.40	9.60	17.29
152	Cyfluthrin-2	15.998	163.10>127.10	6	0.9969	0.005	80.00	97.27	15.82	10.35
153	Cyfluthrin-3	16.087	163.10>127.10	6	0.9999	0.005	92.40	92.17	14.46	12.56
154	Cyfluthrin-4	16.087	163.10>127.10	6	0.9999	0.005	92.40	92.17	14.46	12.56
155	Cypermethrin-1	16.228	163.10>127.10	6	0.9988	0.005	102.80	85.17	6.22	9.80
156	Cypermethrin-2	16.334	163.10>127.10	6	0.9977	0.005	94.00	98.93	12.71	8.60
157	Cypermethrin-3	16.402	163.10>127.10	6	0.9969	0.005	81.60	78.07	8.56	7.07
158	Flucythrinate-1	16.389	157.10>107.10	12	0.9988	0.005	80.00	87.47	6.63	5.49
159	Cypermethrin-4	16.402	163.10>127.10	6	0.9969	0.005	81.60	78.07	8.56	7.07
160	Etofenprox	16.549	163.10>135.10	10	0.9994	0.005	84.40	74.37	4.77	7.45
161	Flucythrinate-2	16.583	157.10>107.10	12	0.9996	0.005	88.00	84.20	2.45	4.79
162	Fenvalerate-1	17.133	225.10>147.10	10	0.9930	0.005	107.60	79.63	7.23	9.79
163	tau-Fluvalinate-1	17.222	250.10>55.00	18	0.9998	0.005	89.60	88.90	8.12	2.53
164	Fluridone	16.941	328.10>259.00	24	0.9976	0.005	107.20	76.13	12.68	17.95
165	tau-Fluvalinate-2	17.281	250.10>55.00	18	0.9995	0.005	84.80	60.07	14.65	3.03
166	Fenvalerate-2 (Esfenvalerate)	17.330	225.10>119.10	20	0.9988	0.005	107.20	80.63	15.24	11.54
167	Deltamethrin-2 (Tralomethrin deg.-2)	17.862	252.90>171.90	8	0.9987	0.005	102.40	81.17	7.89	8.31

Out of total compounds, mean recoveries for 294 were found to be within 70-120 %, and 93 within 30-70 %. Only three compounds showed higher recovery 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 tables 4 and 5)

The method successfully achieved 5 µg/kg LOQ on LC-MS/MS for 165 compounds and on GC-MS/MS for 118 compounds. Remaining 107 compounds showed LOQ of 10 µg/kg. Out of these, 58 were analyzed on LC-MS/MS and 49 on GC-MS/MS (Refer tables 4 and 5). Representative chromatograms of few compounds at their LOQ levels are shown in figure 3 and 4.

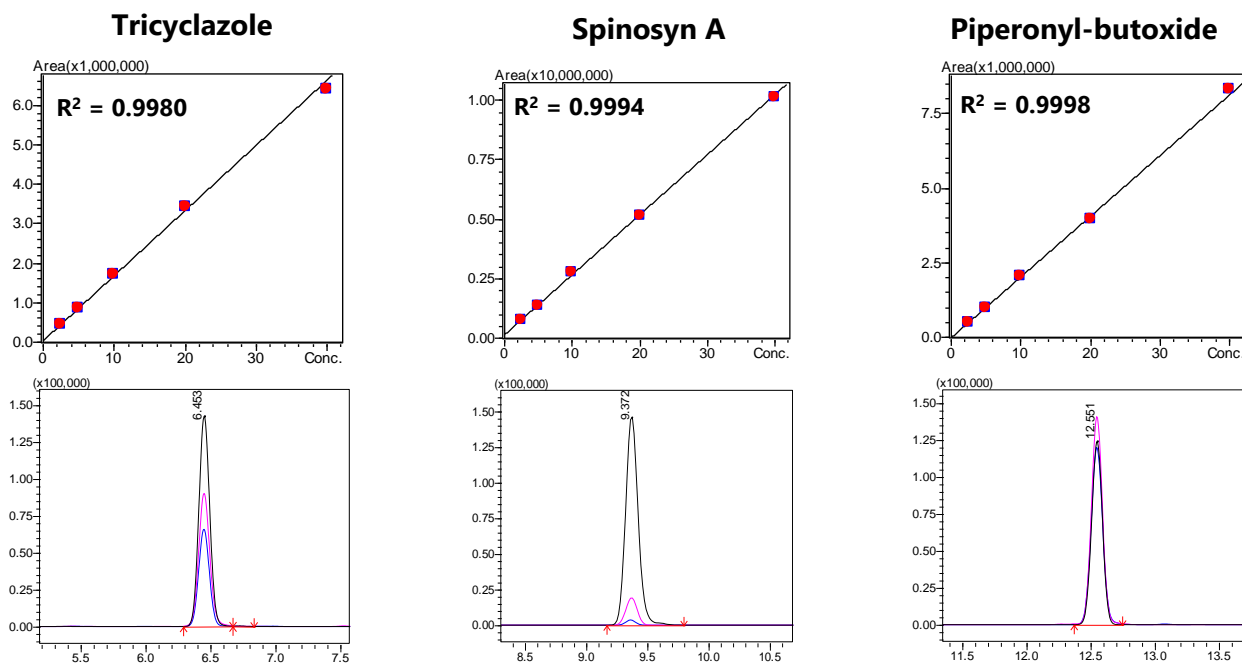


Fig. 3 Representative linearity graphs and chromatograms at LOQ level for LC-MS/MS compounds

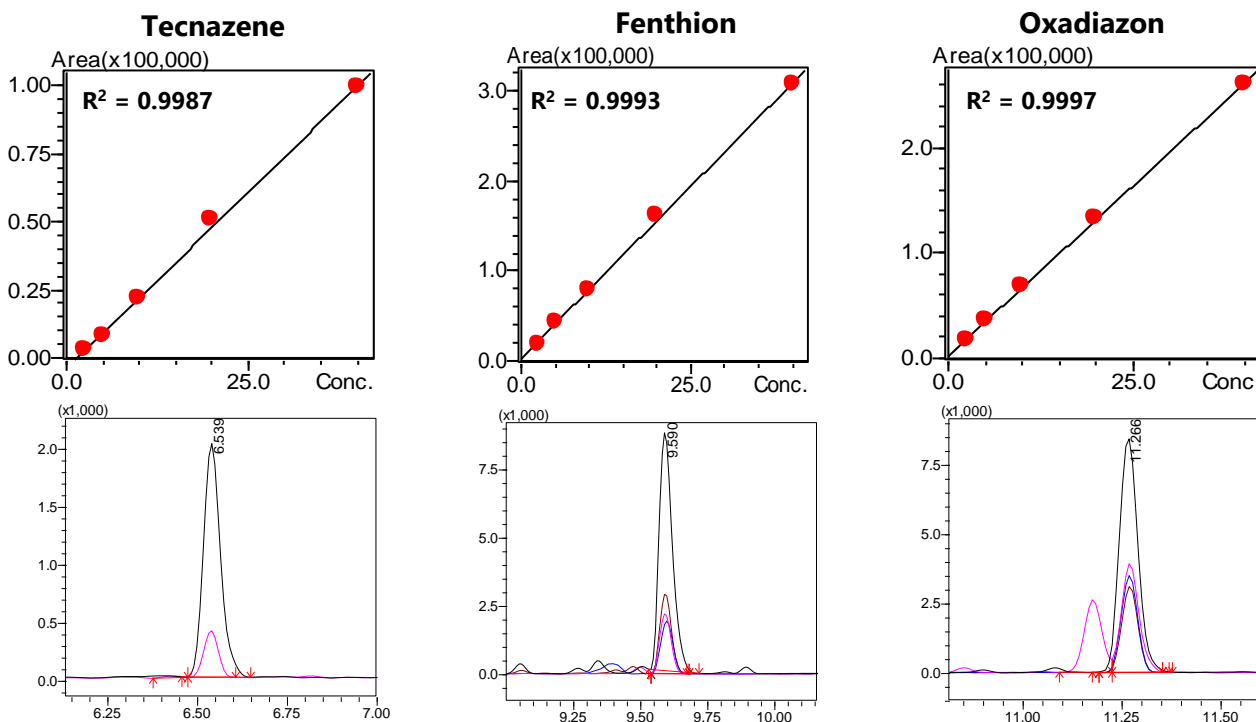


Fig. 4 Representative linearity graphs and chromatograms at LOQ level for GC-MS/MS compounds

4. Conclusion

A simple, sensitive and rapid method has been developed to quantify 346 pesticides by LC-MS/MS and GC-MS/MS in milk sample. Quantification of pesticides in milk is challenging due to complexity of matrix. 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 milk samples.

5. References

1. GB 2763-2019 National Food Safety Standard—Maximum Residue Limits for Pesticides in Food: <https://www.codeofchina.com/standard/GB2763-2019.html>.
2. M. Anastassiades, S. J. Lehotay, D. Štajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, *J. AOAC Int.*, 86: 412–431 (2003)
3. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/12682/2019

GCMS-TQ, Smart Pesticides Database, SH-Rxi and AOC are trademarks of Shimadzu Corporation in Japan and/or other countries.
LCMS, Method package, Shim-pack and LabSolutions Insight are trademarks of Shimadzu Corporation in Japan and/or other countries.



Shimadzu Corporation

www.shimadzu.com/an/

Shimadzu Analytical (India) Pvt.Ltd.

www.shimadzu.in

For Research Use Only. Not for use in diagnostic procedure.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.

See <http://www.shimadzu.com/about/trademarks/index.html> for details.

Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

01-00000-EN

First Edition: Aug. 2021