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Application News

LCMS™-8045 and GCMS-TQ™8040

Method for the determination of 182 Residual Pesticides in Milk using LCMS-8045 and GCMS-TQ8040 NX

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User Benefits

- A modified QuEChERS extraction procedure has been employed for quantifying the pesticides at the desirable concentration levels using Ultra-fast technologies of LCMS-8045 and GCMS-TQ8040 NX.
- Method with shorter run time can contribute to achieving high throughputs in testing laboratories.

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 high 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 multi-residue method which is a simple, sensitive and high throughput to determinate of 182 pesticides in milk using LCMS-8045 and GCMS-TQ8040 NX. The multi residue extraction was performed with modified QuEChERS[2] method for simultaneous quantification of 113 pesticides by LC-MS/MS and 92 pesticides by GC-MS/MS. 23 out of these pesticides were common to both techniques. Out of 66 pesticides listed under FSSAI, 61 pesticides were covered in this study. These compounds are marked with # in the summary results' table. From the remaining 5, Flubendiamide and Chlorothalonil are required to be analysed at higher concentration levels than the range selected for this work. Remaining 3 pesticides i.e., Glufosinate, Paraquat and Triacontanol require single residue methods for analysis.

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
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 1 to 20 µg/L. Calibration curves were generated by external standard method with weighted regression of $1/C^2$. Fortified samples were prepared in six replicates of each 5 and 10 µg/kg. The compounds marked with asterisk (*) in summary result Tables 3 and 4 were common in both LC-MS/MS (Restek P/N: 31971) and GC-MS/MS (Restek P/N: 32562) standard mixture. So, for further calibrations and spiking calculations their concentrations were considered twice to that of other compounds e.g. 2 to 40 µg/L for above calibration range.

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

Shimadzu's LC/MS/MS Method Package for residueal pesticides Ver.3 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

In this study, single extraction procedure for LC-MS/MS and GC-MS/MS in which modified QuEChERS method was adopted. First, the sample was deproteinized with acetonitrile and divided in two parts A and B. Part A was extracted using AR grade anhydrous magnesium sulphate ($MgSO_4$) 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 1 and 2 for LC-MS/MS and GC-MS/MS, respectively.

Fig. 1 Shimadzu LCMS™-8045

Fig. 2 Shimadzu GCMS-TQ™8040 NX

2.2. Analytical Conditions

Table 1 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 , 2.2 µm) P/N: 228-41624-93

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	: B Concentration, 10% (0.0-1.0 min) → 60% (4.0 min) → 100% (14.0 -15 min) → 10% (15.2 – 20.0 min)
Run time	: 20 min
Injection volume	: 10 µL (Co-injection with 25µL water)
Column oven temp	: 40 °C

MS	
Ionization	: ESI
Nebulizing gas flow	: 3 L/min
Heating gas flow	: 8 L/min
Drying gas flow	: 8 L/min
Interface temp.	: 300 °C
DL temp.	: 150 °C
Heating block temp.	: 370 °C

Table 2 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 250kPa)
Injection volume	: 2 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	: 0.3 sec

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 3 and 4, respectively. FSSAI regulated compounds are marked with # in the table.

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 1 to 20 µg/L (2 to 40 µg/L for compounds marked with * in summary results Tables 3 and 4). All calibration standards were found within 80 to 120% accuracy as per SANTE guidelines. The linearity graphs of some representative compounds are shown in Fig. 3 and 4.

3.3. Recovery study

Recovery was evaluated by analysing fortified samples at 5 and 10 µg/kg (10 and 20 µg/kg for * marked compounds in summary result Tables 3 and 4) (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%, except 4 compounds due to poor peak shape. (Refer to Tables 3 and 4)

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 to Tables 3 and 4)

Table 3 Summary results of LC-MS/MS analysis

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
1	Methamidophos#	1.97	142.20>93.95	-15	0.9973	105.75	0.005	87.63	2.24	0.81
2	Acephate#	2.63	184.00>143.00	-10	0.9989	103.85	0.005	95.42	2.36	2.18
3	Omethoate	3.42	214.10>125.00	-18	0.9978	96.34	0.010	87.74	2.05	1.41
4	Dinotefuran#	4	203.15>114.15	-13	0.9849	107.85	0.005	98.07	4.13	3.74
5	Pymetrozine	4.09	218.10>105.00	-12	0.9927	108.35	0.005	92.95	3.24	2.69
6	Methomyl#	4.29	163.00>87.90	-10	0.9834	109.60	0.005	118.92	5.98	1.93
7	Flonicamid	4.82	230.10>203.00	-16	0.9849	108.90	0.005	115.55	12.99	9.04
8	Thiamethoxam#	4.96	292.00>181.10	-22	0.9991	104.05	0.005	94.72	1.72	2.33
9	Monocrotophos#	5.09	240.90>127.10	-21	0.9975	106.75	0.005	101.54	3.57	1.85
10	Carbendazim#	4.98	192.10>160.15	-6	0.9998	100.45	0.005	99.21	2.64	1.93
11	Imidacloprid	5.45	256.10>174.95	-20	0.9978	102.90	0.005	98.41	4.86	4.01
12	Clothianidin#	5.51	250.00>132.05	-16	0.9921	110.60	0.005	96.94	5.78	2.46
13	Carbofuran-3-hydroxy#	5.7	255.00>163.15	-19	0.9898	109.60	0.005	107.09	3.57	3.51
14	Trichlorfon#	5.71	257.00>79.10	-32	0.9886	111.30	0.005	109.20	11.85	5.03
15	Dimethoate#	5.71	230.00>198.90	-4	0.9904	109.05	0.005	101.00	3.65	2.83
16	Acetamiprid#	5.74	223.10>126.10	-11	0.9938	105.10	0.005	107.78	1.21	3.43
17	Thiacloprid#	6	253.00>126.05	-11	0.9985	104.05	0.005	88.94	8.68	2.58
18	Tricyclazole*	6.18	190.10>136.00	-24	0.9943	109.80	0.010	94.97	5.07	3.02
19	Metsulfuron-methyl	6.64	382.00>166.90	-7	0.9994	99.60	0.005	99.76	7.07	5.56
20	Thiophanate-methyl#	6.65	343.00>151.15	-6	0.9969	101.22	0.010	48.87	23.49	9.65
21	Dichlorvos#	6.67	238.00>109.10	-21	0.9891	106.60	0.005	39.00	14.74	14.00
22	Carbofuran#	6.73	222.10>165.00	-6	0.9938	109.50	0.005	98.72	3.66	4.75
23	Simazine	6.76	202.10>104.00	-25	0.9924	111.00	0.005	91.92	5.69	4.31
24	Fenthion-sulfoxide#	6.81	295.00>279.90	-19	0.9896	108.62	0.010	71.46	13.16	7.78
25	Penoxsulam	6.93	484.00>195.10	-25	0.9870	112.65	0.005	96.30	7.23	4.37
26	Carbaryl (NAC)#	6.94	202.10>145.10	-12	0.9917	109.70	0.005	95.06	3.73	4.52
27	Imazalil	6.71	297.10>159.05	-23	0.9944	108.65	0.005	100.94	4.57	1.38
28	Phorate-sulfoxide#	7.21	277.00>198.90	-10	0.9963	108.25	0.005	98.15	9.58	3.46
29	Azimsulfuron	7.38	425.10>182.10	-18	0.9952	107.45	0.005	98.11	2.69	3.83
30	Thiometon	7.31	247.00>89.00	-11	0.9738	90.60	0.005	87.51	13.15	7.63
31	Orthosulfamuron	7.41	425.00>199.00	-9	0.9944	105.70	0.005	79.52	11.55	4.62
32	Atrazine	7.43	216.10>174.10	-13	0.9871	109.25	0.005	88.60	6.98	6.05
33	Sulfosulfuron	7.88	471.00>211.00	-10	0.9977	104.35	0.005	102.34	5.79	4.07
34	Chlorantraniliprole*#	7.76	483.90>452.90	-19	0.9994	102.20	0.010	109.06	4.37	2.88
35	Bensulfuron-methyl	7.8	411.10>149.05	-64	0.9965	103.50	0.005	108.70	4.28	3.21
36	Azinphos-methyl	7.82	318.00>132.05	-16	0.9940	107.40	0.005	107.01	7.54	6.07
37	Phosmet	7.82	318.00>159.90	-8	0.9904	111.10	0.005	98.31	3.82	2.85
38	Clomazone	7.93	239.90>125.00	-11	0.9968	106.25	0.005	85.56	4.21	3.81
39	Azoxystrobin#	7.97	404.00>371.95	-5	0.9927	102.10	0.005	112.90	4.72	5.31
40	Phorate-sulfone#	7.19	293.00>96.90	-34	0.9996	101.75	0.005	103.52	18.20	12.90
41	Fenobucarb	8.09	208.10>95.00	-10	0.9964	103.40	0.005	81.96	9.53	5.53
42	Bispyribac-sodium	8.41	431.10>275.10	-7	0.9982	103.65	0.005	77.81	14.46	6.64
43	Chlorimuron-ethyl	8.89	415.10>82.90	-46	0.9945	109.40	0.005	101.84	7.17	4.84
44	Ethoxysulfuron	8.61	399.10>217.90	-25	0.9817	117.80	0.005	88.55	7.14	5.73
45	Triadimenol (isomer)*	8.94	296.10>70.05	-22	0.9846	115.90	0.010	110.97	9.06	1.20
46	Malathion*	8.53	348.10>127.05	-17	0.9972	105.45	0.010	63.23	14.24	3.51
47	Fluxapyroxad	8.61	382.00>362.05	-14	0.9895	112.30	0.005	80.64	10.69	3.58
48	Triadimefon*#	8.69	294.10>69.00	-22	0.9924	108.90	0.010	98.85	5.73	1.37
49	Pyrazosulfuron-ethyl	8.9	415.10>182.00	-10	0.9989	104.30	0.005	107.39	5.88	4.17
50	Triazophos	8.83	314.10>96.90	-34	0.9907	108.75	0.005	89.30	5.00	3.19
51	Chromafenozide	8.93	395.20>174.85	-32	0.9904	110.10	0.005	85.05	12.76	4.43
52	Isoprothiolane	8.54	290.80>231.00	-7	0.9934	109.40	0.005	74.06	8.92	2.74
53	Azinphos-ethyl	9.07	346.00>77.05	-45	0.9946	105.45	0.005	87.97	12.50	5.61
54	Flufenacet	9.13	364.10>194.00	-7	0.9946	108.00	0.005	96.36	5.83	5.63
55	Epoxiconazole	9.29	330.00>101.10	-44	0.9890	113.60	0.005	116.49	6.78	5.72
56	Metolachlor#	9.34	284.10>176.10	-26	0.9904	111.30	0.005	94.33	6.66	3.94

Table 3 Summary results of LC-MS/MS analysis (Contd.)

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
57	Thifluzamide	9.39	528.60>148.05	-42	0.9891	93.45	0.005	109.74	8.77	8.42
58	Picoxystrobin	9.57	368.00>145.10	-12	0.9946	109.60	0.005	100.49	3.10	5.95
59	Flusilazole*#	9.58	316.10>247.00	-13	0.9963	107.30	0.010	100.87	4.61	2.99
60	Iprobenfos	9.7	289.10>204.95	-6	0.9976	105.05	0.005	94.13	3.63	4.95
61	Phenthoate#	9.69	321.00>246.85	-12	0.9702	123.40	0.005	100.71	4.45	3.60
62	Quinalphos	9.74	299.10>163.00	-22	0.9816	116.30	0.005	95.31	4.80	3.22
63	Fenoxaprop	10.01	334.10>287.85	-18	0.9935	103.05	0.005	95.33	13.56	18.59
64	Kresoxim-methyl#	9.81	314.10>267.00	-8	0.9874	114.95	0.005	108.83	3.23	2.39
65	Carfentrazone-ethyl*	9.8	412.00>345.90	-24	0.9903	104.05	0.010	122.55	16.88	9.37
66	Edifenphos#	9.93	311.00>282.95	-9	0.9925	110.45	0.005	99.81	3.85	7.22
67	Penconazole*#	9.97	284.10>70.05	-17	0.9923	110.75	0.010	93.55	6.29	2.94
68	Anilofos	9.99	368.00>170.90	-16	0.9930	111.35	0.005	98.66	6.32	6.52
69	Fenthion#	10.03	278.90>247.00	-14	0.9850	115.75	0.005	81.86	6.85	5.63
70	Carpropamid	10.03	334.10>139.10	-21	0.9880	109.45	0.005	94.83	5.70	2.08
71	Tebuconazole*#	10.05	308.20>70.05	-22	0.9907	105.20	0.010	98.31	4.87	2.46
72	Oxydemeton-methyl#	10.13	247.00>109.05	-16	0.9943	110.05	0.005	76.42	6.11	4.76
73	Diazinon	10.14	305.10>169.10	-12	0.9974	104.15	0.005	83.11	5.01	2.86
74	Propiconazole (stereo isomer)#	10.18	342.00>158.90	-28	0.9938	110.45	0.005	100.95	5.21	7.58
75	Pyraclostrobin#	10.37	388.00>194.10	-8	0.9950	109.20	0.005	88.99	6.47	2.76
76	Hexaconazole	10.42	314.10>70.00	-22	0.9931	109.15	0.005	103.91	3.00	6.33
77	Oxadiargyl	10.41	358.10>340.90	-11	0.9871	101.80	0.005	94.14	9.13	17.77
78	Phorate#	10.5	261.00>75.15	-11	0.9754	119.40	0.005	61.88	14.79	10.17
79	Phosalone	10.51	368.00>182.00	-17	0.9883	111.20	0.005	95.10	5.93	4.69
80	Pirimiphos-methyl#	10.5	306.10>164.10	-18	0.9927	109.85	0.005	83.33	5.76	4.54
81	Bitertanol#	10.54	338.00>99.15	-15	0.9968	106.65	0.005	107.73	5.10	1.99
82	Pencycuron	10.71	329.10>125.00	-10	0.9927	109.05	0.005	95.89	3.94	6.43
83	Chlorpyrifos-methyl#	10.89	324.00>125.10	-21	0.9874	114.05	0.005	82.83	10.86	6.64
84	Difenoconazole (isomer)#	10.95	406.10>250.90	-20	0.9953	106.75	0.005	88.42	7.06	3.04
85	Emamectin B1b#	10.58	872.20>158.20	-37	0.9896	103.40	0.005	104.07	14.21	12.48
86	Indoxacarb#	11.03	528.10>218.00	-24	0.9852	112.20	0.005	101.96	7.99	4.41
87	Trifloxystrobin	11.05	409.10>145.10	-29	0.9992	100.75	0.005	102.78	7.46	2.54
88	Pretilachlor	11.19	312.20>252.05	-7	0.9954	108.15	0.005	100.20	10.44	5.95
89	Cyflumetofen	11.43	465.20>172.80	-24	0.9968	107.20	0.005	101.54	5.66	3.15
90	Emamectin B1a#	11.06	886.40>158.20	-31	0.9891	106.00	0.005	119.75	4.03	4.75
91	Fenoxaprop-P-ethyl	11.55	362.10>121.00	-28	0.9972	103.20	0.005	98.57	7.69	9.70
92	Fenoxaprop-ethyl	11.55	362.10>287.90	-13	0.9972	107.10	0.005	94.87	7.41	3.09
93	Buprofezin#	11.83	306.20>57.00	-19	0.9961	106.55	0.005	91.77	5.81	5.40
94	Chlorpyrifos-oxon	11.86	334.00>197.85	-30	0.9937	110.55	0.005	85.56	6.93	2.21
95	Pirimiphos-ethyl	11.87	334.10>198.15	-12	0.9913	109.75	0.005	83.96	9.38	5.30
96	Butachlor	11.95	312.20>238.00	-12	0.9933	103.10	0.005	112.07	4.64	4.79
97	Oxadiazon	12.02	345.10>219.90	-20	0.9900	102.80	0.005	81.18	11.07	18.29
98	Ethion#	12.08	385.00>198.90	-10	0.9975	106.40	0.005	88.51	3.52	3.91
99	Pyriproxyfen*	12.19	322.10>184.95	-24	0.9992	102.55	0.010	81.67	1.56	2.64
100	Chlorpyrifos#	12.36	350.00>197.95	-21	0.9975	106.05	0.005	69.66	13.98	7.55
101	Hexythiazox	12.37	353.10>228.00	-11	0.9892	113.35	0.005	81.98	9.83	4.88
102	Pendimethalin	12.46	282.20>212.00	-11	0.9990	103.70	0.005	74.99	7.50	6.03
103	Fenpropathrin#	12.88	367.00>125.10	-17	0.9852	108.65	0.005	79.15	4.77	7.00
104	Cypermethrin#	13.4	432.90>191.00	-15	0.9924	90.60	0.005	72.38	14.40	9.87
105	Deltamethrin#	13.37	523.00>281.00	-17	0.9904	109.00	0.005	57.55	9.48	10.37
106	Avermectin B1a	14.09	890.50>305.20	-29	0.9839	112.50	0.005	110.16	10.56	5.28
107	Etofenprox#	14.55	394.20>177.05	-6	0.9958	107.10	0.005	49.04	5.26	3.51
108	Bifenthrin#	14.7	440.20>181.15	-17	0.9958	99.05	0.005	39.33	8.69	11.87
109	Bentazone	6.6	239.00>175.10	19	0.9839	101.45	0.005	78.96	17.04	5.72
110	2,4-D (2,4-PA)#	7.72	218.90>161.15	15	0.9962	104.15	0.005	78.85	16.55	21.07
111	MCPA (MCP)#	7.85	199.00>141.15	14	0.9968	93.95	0.005	84.10	9.43	14.29
112	Fipronil*#	9.63	435.00>330.00	16	0.9954	108.20	0.010	106.71	4.53	3.65
113	Novaluron	11.42	491.00>470.90	13	0.9938	102.10	0.005	95.94	7.92	1.65

Table 4 Summary results of GC-MS/MS analysis

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision	
									% RSD_R (n=6)	% RSD_r (n=6)
1	Fenobucarb	6.398	121.10>77.00	20	0.9979	102.00	0.005	71.63	7.23	3.41
2	alpha-BHC#	7.191	218.90>144.90	20	0.9884	106.40	0.005	60.51	10.48	6.50
3	Dimethoate#	7.232	125.00>47.00	14	0.9964	103.60	0.005	56.00	11.71	4.66
4	THPI (Tetrahydrophthalimide)	7.282	151.10>79.00	18	0.9786	101.70	0.010	71.37	7.02	5.80
5	Thiometon	7.232	88.00>60.00	8	0.9965	105.80	0.005	60.86	11.39	1.85
6	Clomazone	7.552	204.10>78.00	26	0.9899	105.80	0.005	74.43	9.62	4.83
7	Phorate#	7.638	231.00>129.00	24	0.9996	99.80	0.005	69.06	8.68	2.62
8	Diazinon	7.68	179.10>137.10	18	0.9911	93.40	0.005	78.37	8.78	11.58
9	beta-BHC#	7.709	180.90>144.90	16	0.9985	104.60	0.005	78.66	6.67	4.26
10	gamma-BHC (Lindane)#	7.709	180.90>144.90	16	0.9985	104.60	0.005	78.66	6.67	4.26
11	Iprobenfos	8.182	204.00>91.00	8	0.9974	97.40	0.005	86.20	8.05	2.50
12	delta-BHC#	8.225	218.90>182.90	8	0.9885	108.80	0.005	82.89	16.20	6.48
13	Chlorpyrifos-methyl#	8.552	285.90>93.00	22	0.9945	108.60	0.005	71.77	10.88	7.02
14	Parathion-methyl	8.711	263.00>109.00	14	0.9936	110.40	0.005	82.11	10.37	5.60
15	Metalaxyl* (Mefenoxam)	8.776	249.20>190.10	8	0.9995	97.60	0.010	80.63	10.73	3.94
16	Heptachlor#	8.821	271.80>236.90	20	0.9942	105.20	0.005	62.40	10.41	8.67
17	Pirimiphos-methyl#	8.953	290.10>125.00	22	0.9937	110.20	0.005	70.97	4.62	10.43
18	Fenitrothion#	9.095	277.00>260.00	6	0.9871	99.40	0.005	85.89	4.50	6.95
19	Malathion	9.155	173.10>99.00	14	0.9857	102.00	0.005	97.00	13.58	8.51
20	Metolachlor (S-Metolachlor)#	9.295	162.10>133.10	16	0.9961	101.60	0.005	85.60	6.05	1.96
21	Chlorpyrifos#	9.312	313.90>285.90	8	0.9921	92.20	0.010	72.80	13.68	14.15
22	Aldrin#	9.43	262.90>191.00	34	0.9802	91.70	0.010	47.00	16.86	21.80
23	Fenthion#	9.412	278.00>109.00	20	0.9956	92.60	0.005	89.29	7.30	4.55
24	Flufenacet	9.448	151.00>95.00	26	0.9828	101.50	0.010	86.81	11.49	8.02
25	Triadimefon*#	9.529	208.10>111.00	22	0.9891	103.80	0.010	89.31	13.37	8.03
26	Pirimiphos ethyl	9.619	304.10>168.10	12	0.9843	111.60	0.005	81.06	11.86	5.15
27	Pendimethalin	9.902	252.10>162.10	10	0.9847	96.20	0.005	80.83	12.99	8.26
28	Fipronil*#	10.042	366.90>212.90	30	0.9957	105.20	0.010	86.49	7.13	5.43
29	Penconazole*#	10.088	248.10>157.10	26	0.9997	102.00	0.010	80.20	6.09	3.85
30	(E)-Chlorfenvinphos#	10.09	323.00>267.00	16	0.9895	110.40	0.005	85.66	11.56	6.92
31	Heptachlor-exo-epoxide#	10.114	352.80>262.90	14	0.9990	103.40	0.005	74.06	17.82	22.57
32	Phenthoate#	10.162	273.90>125.00	20	0.9941	97.00	0.005	86.83	7.40	8.89
33	Quinalphos	10.219	146.10>118.00	10	0.9950	106.60	0.005	88.57	8.48	6.08
34	trans-Chlordane#	10.55	372.80>263.90	28	0.9950	105.80	0.005	66.80	16.79	15.57
35	p,p'-DDE#	10.55	246.00>211.00	22	0.9962	101.80	0.005	77.40	10.69	19.46
36	Butachlor	10.571	176.10>147.10	14	0.9925	110.80	0.005	97.66	7.81	8.76
37	cis-Chlordane#	10.765	374.80>265.90	26	0.9983	102.20	0.005	77.54	10.32	12.00
38	(Z)-Chlorfenvinphos#	10.81	267.00>159.00	18	0.9913	94.40	0.005	87.11	9.64	7.35
39	Pretilachlor	10.962	262.10>202.10	10	0.9987	99.80	0.005	86.97	11.22	9.42
40	Hexaconazole	11.01	214.00>172.00	20	0.9816	95.20	0.010	73.13	14.92	14.19
41	Isoprothiolane	11.011	231.10>189.00	10	0.9884	103.80	0.005	87.91	14.53	7.97
42	Oxadiazon	11.066	258.00>175.00	8	0.9995	100.20	0.005	92.51	6.27	9.74
43	o,p'-DDE#	11.137	246.00>176.00	30	0.9984	95.60	0.005	64.09	6.87	6.98
44	Thifluzamide	11.169	448.90>428.90	20	0.9982	103.20	0.005	82.66	12.43	11.77
45	Kresoxim-methyl#	11.211	206.10>116.10	6	0.9840	105.60	0.005	93.43	11.41	10.44
46	Oxyfluorfen	11.204	252.00>196.00	22	0.9916	96.00	0.010	77.61	10.25	10.37

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

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Determination Coefficient (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision % RSD_R (n=6)	Precision % RSD_r (n=6)
47	Dieldrin#	11.263	276.90>241.00	8	0.9738	117.00	0.010	80.24	17.93	21.06
48	Flusilazole*#	11.264	233.10>165.10	14	0.9957	106.80	0.010	79.94	8.27	6.50
49	o,p'-DDD#	11.279	235.00>165.00	24	0.9972	102.00	0.005	79.46	7.29	6.50
50	Myclobutanil*	11.288	179.10>125.00	14	0.9933	100.80	0.010	87.89	4.85	4.13
51	Ethion#	11.835	230.90>129.00	24	0.9980	105.00	0.005	90.60	7.86	5.44
52	beta-Endosulfan	11.906	194.90>160.00	8	0.9963	98.80	0.010	77.10	7.09	15.07
53	p,p'-DDD#	11.94	235.00>165.00	24	0.9951	107.60	0.005	76.34	4.42	3.83
54	o,p'-DDT#	11.941	235.00>165.00	24	0.9981	100.00	0.005	75.89	4.43	4.14
55	Triazophos	12.203	257.00>162.00	8	0.9822	101.80	0.005	96.83	7.74	10.29
56	Carfentrazone-ethyl*	12.256	340.10>312.10	14	0.9971	106.60	0.010	86.06	4.78	4.76
57	Trifloxystrobin	12.303	222.10>130.10	12	0.9697	89.00	0.005	95.69	11.14	13.64
58	Edifenphos#	12.478	173.00>109.00	10	0.9981	100.00	0.005	90.29	6.97	2.68
59	p,p'-DDT#	12.596	235.00>165.00	24	0.9998	99.60	0.005	72.43	4.87	3.76
60	Propiconazole-1#	12.634	173.00>145.00	16	0.9965	106.40	0.005	92.57	7.35	4.66
61	Propargite-1*	12.829	135.10>95.00	14	0.9946	99.20	0.010	68.54	19.56	11.97
62	Dicofol	12.839	139.00>111.00	12	0.9845	109.80	0.005	88.09	6.03	8.47
63	Tebuconazole*#	12.902	250.10>125.10	22	0.9974	104.40	0.010	83.77	6.63	2.88
64	Bifenthrin#	13.358	181.10>166.10	12	0.9986	103.00	0.005	75.03	6.61	4.06
65	Iprodione	13.363	316.00>247.00	12	0.9702	94.30	0.010	87.53	15.08	15.75
66	Etoxazole	13.54	330.10>300.10	26	0.9907	109.40	0.005	81.86	12.65	18.37
67	Fenpropathrin#	13.577	265.10>210.10	12	0.9864	110.80	0.005	85.37	7.51	8.51
68	Anilofos	13.757	226.10>157.00	14	0.9851	106.60	0.005	91.43	2.28	3.43
69	Fenazaquin	13.814	160.20>145.10	8	0.9789	118.40	0.005	66.17	7.19	5.63
70	Chlorantraniliprole*#	13.902	280.00>251.00	20	0.9828	94.40	0.010	77.60	11.51	5.87
71	Pyriproxyfen*	14.238	136.10>78.00	20	0.9950	107.40	0.010	86.00	9.19	19.75
72	Cyhalofop-butyl	14.279	256.10>120.10	12	0.9993	101.20	0.005	90.71	8.03	3.61
73	lambda-Cyhalothrin	14.352	208.00>181.00	8	0.9993	103.20	0.005	86.09	10.33	7.69
74	Cyhalothrin-2	14.352	208.00>181.00	8	0.9877	98.60	0.005	82.71	7.61	3.10
75	Phosalone	14.158	182.00>102.00	14	0.9987	100.60	0.005	91.37	7.11	8.00
76	Fenoxaprop-ethyl (Fenoxaprop-P-ethyl)	14.967	361.10>288.10	12	0.9966	101.40	0.005	84.57	7.44	9.18
77	cis-Permethrine	15.169	183.10>153.10	14	0.9928	107.20	0.005	72.83	5.95	4.16
78	Bitertanol-1#	15.188	170.10>141.10	22	0.9932	106.80	0.005	86.63	4.99	2.02
79	trans-Permethrine	15.301	163.10>127.10	6	0.9988	104.00	0.005	78.20	5.36	5.19
80	Bitertanol-2#	15.312	170.10>141.00	22	0.9954	103.20	0.005	95.91	7.10	5.84
81	Cyfluthrin	15.731	226.10>206.10	14	0.9873	107.20	0.005	89.83	17.71	14.51
82	Cypermethrin#	16.043	163.10>127.10	6	0.9951	98.20	0.005	81.03	13.66	8.79
83	Etofenprox#	16.359	163.10>135.10	10	0.9966	105.40	0.005	80.40	7.14	4.98
84	Pyridalyl	16.386	204.10>148.10	20	0.9939	97.40	0.005	71.37	10.32	6.89
85	Fenvalerate-1#	16.949	225.10>119.10	20	0.9975	106.40	0.005	79.06	8.22	8.50
86	tau-Fluvalinate-1	17.04	250.10>200.10	16	0.9964	98.80	0.005	91.40	16.23	10.29
87	tau-Fluvalinate-2	17.095	250.10>200.10	16	0.9947	105.80	0.005	86.60	10.80	15.09
88	Fenvalerate-2# (Esfenvalerate)	17.153	225.10>119.10	20	0.9937	90.20	0.005	79.54	16.61	15.06
89	Difenoconazole-1#	17.5	323.00>265.00	14	0.9980	96.00	0.005	80.09	4.18	6.74
90	Indoxacarb#	17.506	218.00>203.00	10	0.9873	108.60	0.005	97.03	5.56	6.82
91	Deltamethrin-1# (Tralomethrin deg.-1)	17.688	252.90>93.00	20	0.9944	91.80	0.005	85.06	17.31	9.09
92	Azoxystrobin	17.88	344.10>329.10	16	0.9876	109.20	0.005	83.06	7.55	5.71

Out of total compounds analyzed, mean recoveries for 186 were found to be within 70-120 %, and for 18 within 35-70 %. Only 1 compound 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 to Tables 3 and 4)

The method successfully achieved 5 µg/kg LOQ on LC-MS/MS for 101 compounds and on GC-MS/MS for 73 compounds. Remaining 31 compounds showed LOQ of 10 µg/kg. Out of these, 12 were analyzed on LC-MS/MS and 19 on GC-MS/MS (Refer Tables 3 and 4). Representative chromatograms of few compounds at their LOQ levels are shown in Fig. 3 and 4.

Pirimiphos-methyl

R^2 = 0.9937

Heptachlor

R^2 = 0.9942

o,p'-DDE

R^2 = 0.9984

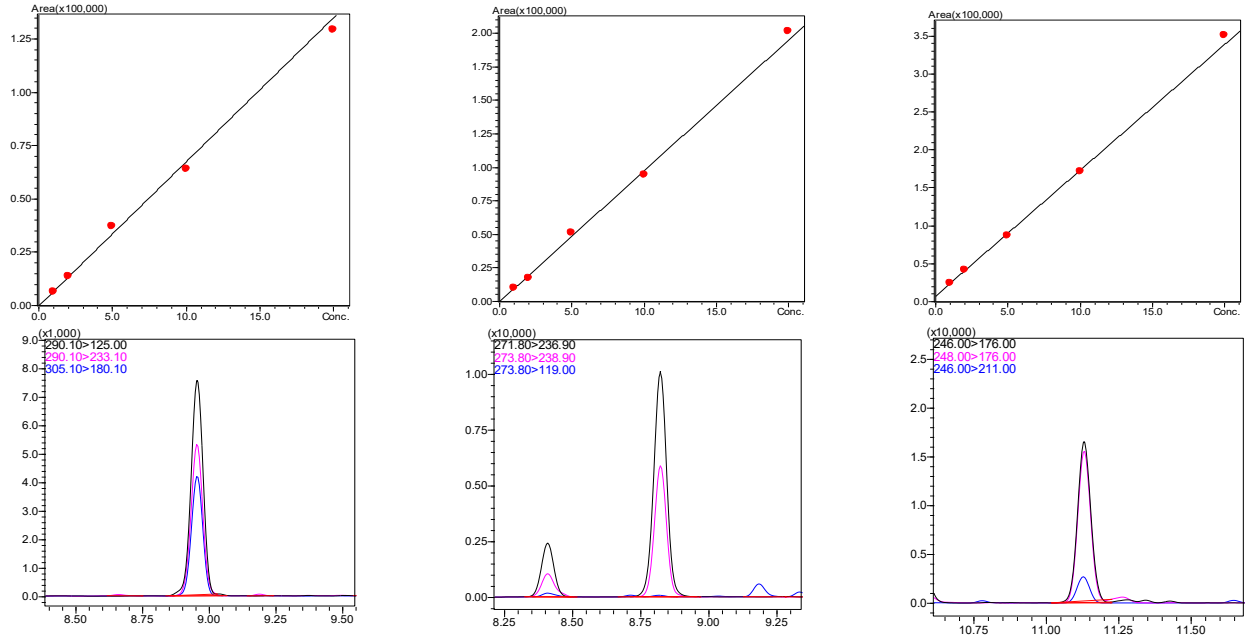
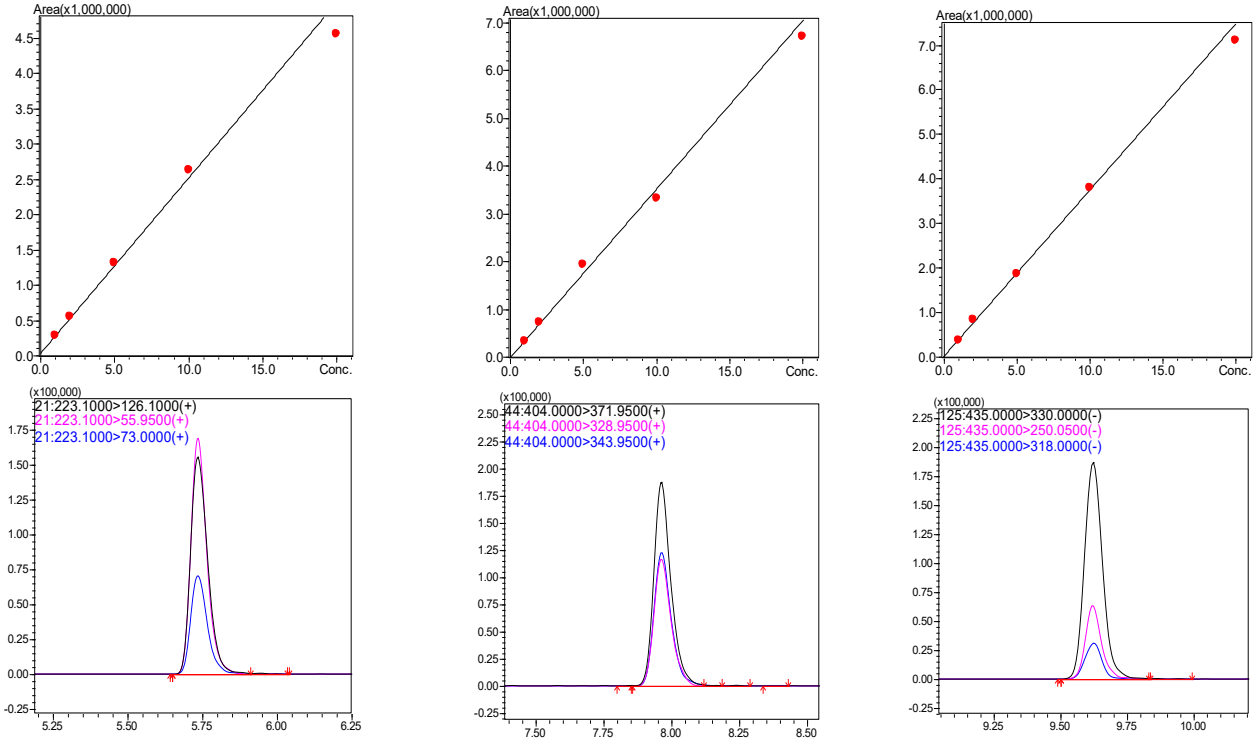


Fig. 3 Representative linearity graphs and chromatograms at LOQ level for LC-MS/MS compounds



Acetamipirid

R^2 = 0.9938

Azoxystrobin

R^2 = 0.9927

Fipronil

R^2 = 0.9954

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 182 pesticides in milk sample by LC-MS/MS and GC-MS/MS. 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 for multi-residue analysis of milk samples in testing laboratories as per FSSAI regulations.

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

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