GCMS-TQ[TM]8040 & LCMS[TM]-8050

Multiresidue pesticides analysis in Curcumin color additive powder using GCMS-TQ8040 NX and LCMS-8050

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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 the pesticides at trace levels from complex matrix like Curcumin powder by using Ultra-Fast technologies of LCMS-8050 and GCMS-TQ8040 NX.

◆ LCMS Method Package[TM] for residual pesticide Ver.3 and GCMS Smart Pesticides Database[TM] Ver.2 from Shimadzu Corporation enables ease of optimizing instrumental method.

1. Introduction

Color additives are dyes, pigments, or other substances that can impart color when added or applied to a food, drug, cosmetic, or the human body. They can be found in a range of consumer products—from cough syrup and eyeliner to contact lenses and cereal.

The food color curcumin (turmeric yellow) is obtained by solvent extraction of turmeric, i.e., the ground rhizomes of Curcuma longa L., with purification of the resultant extract by crystallization. In India, it has been used as a food preservative and as a spice in curry dishes. Hence, considering the heavy use of pesticides in their cultivation, it is important to analyze these plant-based color additives for the presence of residual pesticides.

This note shows validation data of the Multi-residue analysis method in complex matrix such as curcumin powder. The analysis was performed using modified QuEChERS[1] and triple quadrupole gas chromatography (GC-MS/MS) and liquid chromatography (LC-MS/MS) system.

Fig. 1 Curcumin color additive powder

2. Materials and Methods

The customized reference standards for 72 pesticides under study were procured from Restek:
CS-27517-1; CS-27517-2; CS-27517-3; CS-27517-4; CS-27517-5; CS-27517-6.

The curcumin powder procured from local market, was used to prepare matrix-matched calibration standards and fortified samples. The calibration standards were analyzed in the range of 0.5 to 200 µg/L and 0.1 to 20 µg/L for GC-MS/MS and LC-MS/MS, respectively. Fortified samples were prepared in six replicates of each 5, 10 and 20 µg/kg. Shimadzu GCMS-TQ8040 NX (Figure 2) and LCMS-8050 with Nexera X2 as front end (Figure 3), manufactured by Shimadzu Corporation Japan, were used as analytical tool to quantify residual pesticides in matrix.

Shimadzu's Smart Pesticides Database Ver.2 for GC-MS/MS and Method Package Ver.3 for LC-MS/MS enabled quick instrumental method optimization for higher throughput. For most of the compounds, 1 target and 2 reference MRM transitions were used in the method.

Shimadzu's data processing software 'LabSolutions Insight[TM]' 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. AR grade salts like Sodium chloride, Anhydrous magnesium sulphate ($MgSO_4$), Trisodium citrate dihydrate and Disodium hydrogen citrate sesquihydrate were used in optimised proportion to get maximum 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), Zirconium 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 diluted using Methanol and filtered through 0.22µm nylon filter.

All samples were analysed as per conditions shown in Table 1 and 2 for GC-MS/MS and LC-MS/MS, respectively.

Fig. 2 Shimadzu GCMS-TQ8040 NX

2.2. Analytical Conditions

Table 1 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.	: 250 °C
Column oven temp	: 80 °C (2 min), 20 °C/min to 180 °C, 5 °C/min to 300 °C (3 min)
Run time	: 34 min
Injection mode	: Splitless (High pressure at 250kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 40.4 cm/sec (Constant mode)

MS

Interface temp.	: 280 °C
Ion source temp.	: 230 °C
Ionization mode	: EI
Solvent cut time	: 5 min
Loop Time	: 0.3 sec
Resolution	: Unit (Q1) – Unit (Q3)

Fig. 3 Shimadzu LCMS-8050

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

System Configuration

LC-MS/MS	: LCMS-8050
Auto-sampler	: Nexera X2 SIL 30AC
Column	: Shim-pack™ Scepter, (4.6mm I.D. x 100mm, 5 µm)

LC

Flow rate	: 0.6 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	: 5-10%B (0.0 mins to 1.0 mins) → 10-55%B (1.01 min to 3.00 min) → 55-75%B (3.01 min to 5.00 min) → 75-90%B (5.01 min to 9.00 min) → 90-100%B (9.01 min to 11.00 min)
Run time	: 18 min
Injection volume	: 5 x 5 µL (Sandwich injection with 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.	: 400 °C
Resolution	: Unit (Q1) – Unit (Q3)

3. Result and Discussion

Validation parameters like linearity, recovery and precision were studied against criteria set by Standard Method Performance Requirement (SMPR) (Refer Table 3). Results obtained on GC-MS/MS and LC-MS/MS are shown in Table 4 and 5, respectively.

Table3 SMPR

Analytical range	LOQ to 100 times LOQ
Recovery %	60-120
RSD_R %	≤30
RSD_r %	≤20

3.1. Linearity study

In this modified QuEChERS method, samples were diluted five times for GC-MS/MS and fifty times for LC-MS/MS analysis. Hence the matrix matched calibration standards were analyzed from much lower concentration levels i.e., 0.5 to 200 µg/L and 0.1 to 20 µg/L for GC-MS/MS and LC-MS/MS, respectively.

Accuracies of calibration curves were evaluated according to SANTE/12682/2019.[2] Representative calibration curves of compounds are shown in Figure 4 and 5. Most of the compounds showed accuracy within 80-120%. Accuracies obtained at LOQ levels, and their correlation coefficients (R^2) are displayed in Table 4 and 5.

3.2. Recovery study

Six fortified samples of each 5, 10 and 20 µg/kg were analyzed, and their mean recovery was evaluated against SMPR. All compounds showed good recovery within the range of 60 to 120% at LOQ levels. (Refer tables 4 and 5) As mentioned previously, fortified samples were diluted five times for GC-MS/MS and fifty times for LC-MS/MS, respectively.
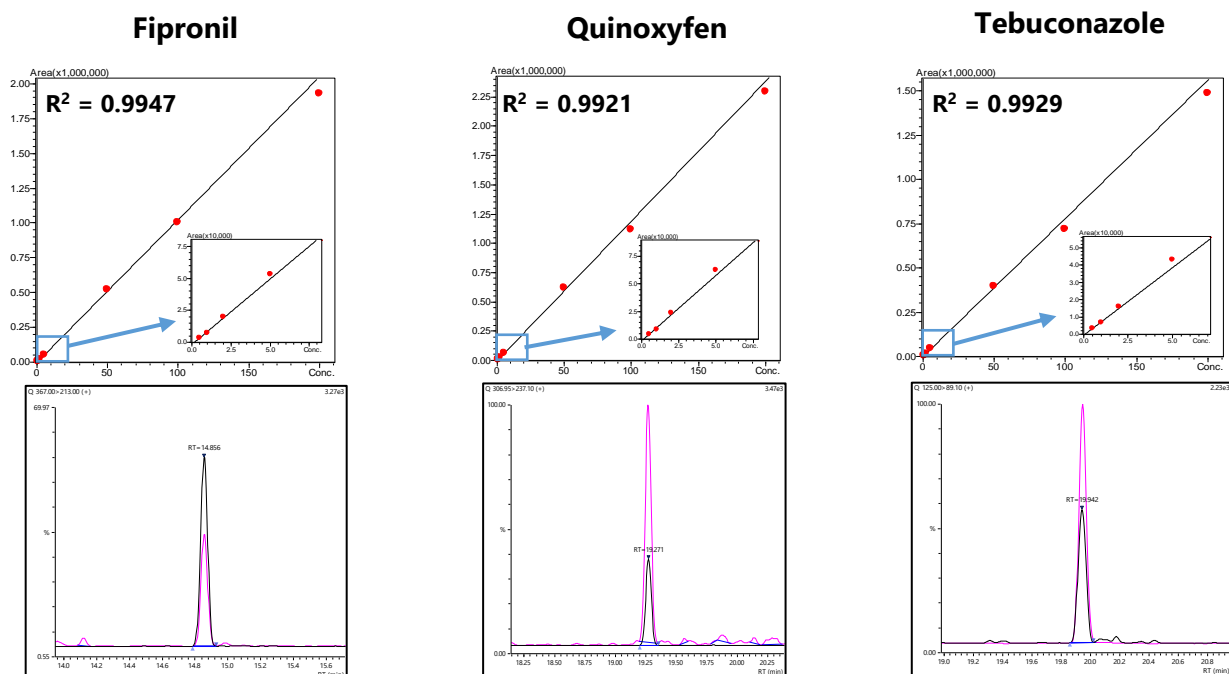
Fipronil

$R^2 = 0.9947$

Quinoxyfen

$R^2 = 0.9921$

Tebuconazole

$R^2 = 0.9929$

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

Omethoate

$R^2 = 0.9970$

Acetamipirid

$R^2 = 0.9993$
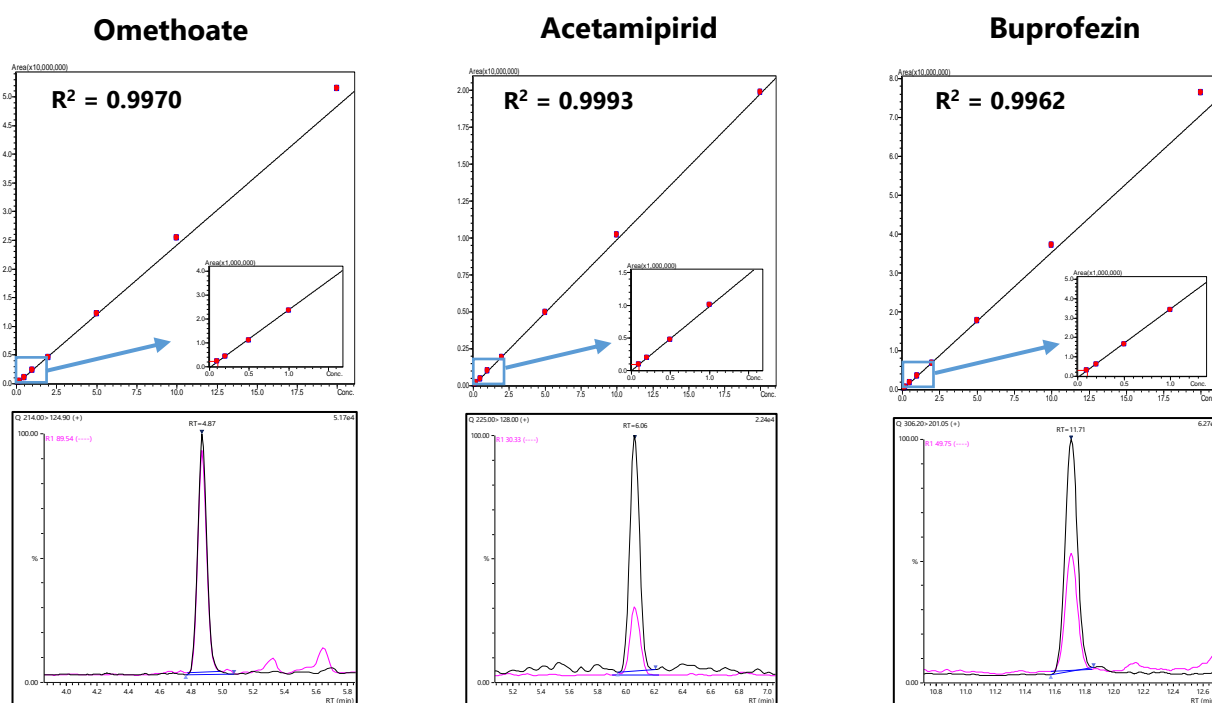
Buprofezin

$R^2 = 0.9962$

Fig. 5 Representative linearity graphs and chromatograms at LOQ level for LC-MS/MS compounds

3.3. Precision study

For precision, repeatability and within-laboratory reproducibility studies were carried out.

Repeatability (RSD$_r$): Repeatability experiment was performed by injecting six replicates at 5, 10 and 20µg/L 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 spiked samples at 5, 10 and 20 µg/L concentration levels. The % RSD for recovery of six spiked samples at their respective LOQ levels were found to be less than 30%. (Refer tables 4 and 5)

Trend graphs for recovery and precision data obtained on GC-MS/MS and LC-MS/MS are shown in Figure 6 and 7, respectively.

Out of 72 compounds analyzed, LOQs of five compounds were found to be higher than the recovery levels analyzed in this study. Among these, Cyfluthrin, Cypermethrin and Spinetoram were having less than 60% recoveries in the spiked samples whereas Linuron and Methoxyfenozide showed poor response.

This method successfully achieved 5µg/kg LOQs on GC-MS/MS and LC-MS/MS for 61 compounds. Whereas 3 compounds showed 20µg/kg LOQ on GC-MS/MS and other 3 showed LOQ at 10µg/kg on LC-MS/MS. Refer summary Tables 4 and 5. Representative chromatograms of few compounds at their LOQ levels are shown in Figure 4 and 5.

Table 4 Summary results of GC-MS/MS analysis

ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Matrix match linearity (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision % RSD$_R$ (n=6)	% RSD$_r$ (n=6)
1	Tetrahydrophthalimide (THPI) as Captan deg.	8.150	151.10>79.00	18	0.9752	105.25	0.02	66.65	18.42	6.22
2	Diazinone	11.152	304.10>179.20	19	0.9919	90.65	0.005	87.07	19.04	12.05
3	Pyrimethanil	11.313	198.10>118.10	30	0.9855	93.18	0.005	73.45	11.59	9.15
4	Metalaxyl	12.787	234.10>146.20	20	0.9884	87.67	0.005	70.94	15.52	13.99
5	Malathion	13.481	157.95>125.00	9	0.9901	87.80	0.005	68.29	22.44	9.85
6	Chlorpyrifos	13.703	313.95>257.90	17	0.9828	77.30	0.005	63.30	30.85	20.37
7	Cyprodinil	14.717	224.15>222.10	24	0.9895	103.57	0.005	85.01	10.94	7.80
8	Fipronil	14.840	367.00>213.00	29	0.9947	90.09	0.005	81.33	6.83	12.28
9	Triflumizole	15.388	278.05>73.10	8	0.9961	101.48	0.005	70.96	12.44	9.78
10	Flutriafol	16.253	219.10>123.10	21	0.9875	89.16	0.005	78.96	18.01	13.11
11	Fludioxonil	16.518	248.05>127.10	27	0.9889	100.16	0.005	76.47	11.55	4.15
12	Myclobutanil	16.951	179.05>125.00	18	0.9869	89.74	0.005	72.99	14.50	8.23
13	Buprofezin	17.074	172.10>57.10	21	0.9843	110.61	0.005	83.96	11.51	10.07
14	Chlorfenapyr	17.373	247.00>227.00	14	0.9591	76.81	0.005	73.97	11.80	16.00
15	Propiconazole-1	19.239	172.95>109.00	25	0.9905	105.33	0.005	68.88	25.18	15.40
16	Trifloxystrobin	19.242	222.05>190.10	5	0.9841	105.57	0.005	90.11	10.36	2.84
17	Quinoxyfen	19.257	306.95>237.10	24	0.9921	87.77	0.005	77.89	9.82	13.49
18	Fenhexamid	19.444	177.00>113.00	17	0.9824	89.93	0.005	68.68	11.65	18.51
19	Propiconazole-2	19.458	172.95>109.00	25	0.9948	97.86	0.005	73.89	9.86	13.31
20	Fluopicolide	19.555	209.00>182.00	19	0.9788	99.78	0.005	84.60	18.63	6.83
21	Tebuconazole	19.936	125.00>89.10	21	0.9929	92.40	0.005	65.68	23.45	14.03
22	Piperonyl butoxide	20.285	176.05>131.10	13	0.9898	97.47	0.005	73.64	14.91	7.87
23	Fluxapyroxad	21.104	381.10>159.10	16	0.9813	94.57	0.005	90.86	12.88	7.23
24	Iprodione	20.864	187.00>124.00	24	0.9946	100.17	0.02	73.73	10.52	12.14
25	Chlorantraniliprole	21.327	278.00>249.00	20	0.9699	86.69	0.005	67.99	22.17	5.94
26	Bifenthrin	21.217	181.05>165.10	22	0.9735	96.84	0.005	74.80	12.05	5.60
27	Bifenazate	21.358	300.10>258.10	9	0.9701	89.79	0.005	68.16	14.04	11.50
28	Etoxazole	21.490	359.15>187.20	21	0.9848	99.68	0.02	60.16	4.94	12.22
29	Fenpropathrin	21.530	265.05>210.10	12	0.9895	100.05	0.005	72.25	16.15	7.18
30	Lambda-Cyhalothrin	23.107	208.05>181.10	9	0.9642	103.38	0.005	74.67	13.23	12.53
31	Pyridaben	24.826	147.15>117.10	24	0.9897	100.77	0.005	65.76	10.91	9.90
32	Boscalid	26.285	140.10>112.10	12	0.9635	94.48	0.005	66.44	11.98	14.77

Table 5 Summary results of LC-MS/MS analysis

| ID	Compound Name	Ret. Time (min)	Target MRM (m/z)	CE	Matrix match linearity (R^2)	% Accuracy at LOQ	LOQ mg/kg	Recovery at LOQ (%)	Precision % RSD$_R$ (n=7)	% RSD$_r$ (n=6)
1	Methamidophos	4.416	142.00>94.05	-15	0.9971	103.8	0.005	89.87	2.78	1.16
2	Acephate	4.706	183.90>143.00	-10	0.9983	102.6	0.005	99.99	3.92	4.5
3	Propamocarb	4.833	189.10>102.15	-17	0.9984	103.7	0.005	64.35	3.02	3.99
4	Omethoate	4.882	214.00>124.90	-22	0.9970	103.3	0.005	101.5	4.98	1.87
5	Dinotefuran	4.993	203.05>87.00	-15	0.9990	101	0.005	106.83	3.63	3.9
6	Methomyl	5.465	163.00>88.00	-9	0.9966	103.2	0.005	107.76	5.55	2.69
7	Thiamethoxam	5.426	292.00>211.00	-12	0.9948	106.7	0.005	106.24	3.8	2.82
8	Imidacloprid	5.793	256.00>175.05	-19	0.9980	102	0.005	110.32	17.39	4.72
9	Clothianidin	5.908	250.00>169.00	-13	0.9955	105.7	0.005	103.54	6.5	7.62
10	Flupyradifurone	6.015	288.95>125.95	-20	0.9991	101.8	0.005	115.31	3.81	2.45
11	Acetamiprid	6.082	225.00>128.00	-20	0.9993	99.9	0.005	100.87	6.54	5.27
12	Carbendazim	6.102	192.00>160.05	-18	0.9962	103.6	0.005	104.71	7.51	2.74
13	Sulfoxaflor	6.21	277.95>174.10	-8	0.9964	104.2	0.005	103.79	9.44	4.56
14	Dimethoate	6.196	230.00>198.90	-10	0.9972	104.5	0.005	107.07	2.88	2.79
15	Thiacloprid	6.342	253.00>126.05	-20	0.9948	104.6	0.005	112.16	2.01	2.96
16	Thiabendazole	6.673	202.00>175.00	-25	0.9964	104.2	0.005	91.54	3.71	1.57
17	Carbaryl (NAC)	7.533	202.00>145.00	-11	0.9982	100.1	0.005	104.4	15.24	6.58
18	Imazalil	7.683	297.00>158.95	-21	0.9984	101.2	0.005	80.08	5.49	7.89
19	Flutriafol	7.75	302.10>70.05	-17	0.9980	103.2	0.005	118.62	6.32	5.18
20	Metalaxyl	8.03	280.10>220.10	-14	0.9959	104.4	0.005	116.74	6.41	2.22
21	Azoxystrobin	8.333	404.00>371.95	-15	0.9955	103.6	0.005	114.99	3.67	4.8
22	Mandipropamid	8.568	412.00>328.00	-15	0.9914	108.6	0.005	112.51	9.27	6.29
23	Dimethomorph	8.831	388.00>301.00	-21	0.9974	96.95	0.01	111.76	7.19	5.12
24	Bifenazate	9.109	301.10>198.10	-10	0.9982	103.1	0.005	94.62	9.51	7.18
25	Fluopyram	9.097	396.90>207.90	-21	0.9983	101.5	0.005	97.33	8.42	13.99
26	Pyrimethanil	9.188	200.10>107.10	-25	0.9932	99.5	0.005	99.59	11.42	9.86
27	Spirotetramat	9.152	374.10>216.00	-33	0.9975	103.8	0.005	112.96	5.37	7.35
28	Pyriproxyfen	9.393	338.95>69.95	-22	0.9899	109	0.01	87.25	16.87	12.45
29	Fenbuconazole	9.376	337.00>124.95	-28	0.9953	95.4	0.005	112.44	19.5	10.04
30	Cyazofamid	9.466	325.00>107.90	-16	0.9988	101.3	0.005	102.44	10.7	17.23
31	Diflubenzuron	9.714	311.00>158.10	-14	0.9936	100.7	0.005	97.9	11.21	11.09
32	Tebuconazole	10.009	308.10>69.95	-24	0.9965	103.8	0.005	103.75	7.36	5.29
33	Propiconazole	10.256	342.00>158.90	-27	0.9923	108.4	0.005	102.58	14.09	19.18
34	Pyraclostrobin	10.475	388.00>194.00	-13	0.9934	103.1	0.005	112.16	7.06	3.15
35	Diazinone	10.472	305.00>169.10	-21	0.9979	103	0.005	111.97	5.22	3.69
36	Cyprodinil	10.582	226.10>93.10	-37	0.9988	99.4	0.005	103.42	11.75	8.62
37	Indoxacarb	10.611	528.00>202.90	-40	0.9977	108.65	0.01	112.9	13.35	13.95
38	Difenoconazole	10.695	406.00>250.90	-26	0.9962	104.7	0.005	115.8	8.06	4.07
39	Trifloxystrobin	10.904	409.00>186.00	-20	0.9966	103	0.005	113.88	3.81	0.94
40	Triflumizole	11.013	346.10>278.00	-10	0.9965	104.5	0.005	110.02	4.76	2.65
41	Profenofos	11.482	372.80>302.80	-19	0.9965	104.5	0.005	76.64	11.81	12.69
42	Buprofezin	11.694	306.20>201.05	-13	0.9962	104.7	0.005	109.5	4.34	2.63
43	Piperonyl-butoxide	11.969	356.10>177.00	-20	0.9965	103.5	0.005	114.68	4.17	1.82
44	Spirodiclofen	12.542	411.10>313.05	-14	0.9982	103	0.005	118.38	11.78	13.65
45	Pyridaben	12.989	365.20>147.20	-25	0.9971	103.8	0.005	101.67	9.11	11.94
46	Flonicamid	5.466	227.95>81.00	8	0.9962	102.3	0.005	111.04	5.55	7.32
47	Fludioxonil	8.796	247.10>180.15	28	0.9863	102	0.005	106.77	2.98	2.8
48	Fipronil	9.42	434.90>330.00	16	0.9970	104.5	0.005	100.93	6.98	2.8
49	Flubendiamide	9.46	680.90>254.10	27	0.9947	106.7	0.005	105.94	5.4	3.02
50	Novaluron	10.797	491.00>470.90	13	0.9997	101.1	0.005	114.12	9.02	10.05
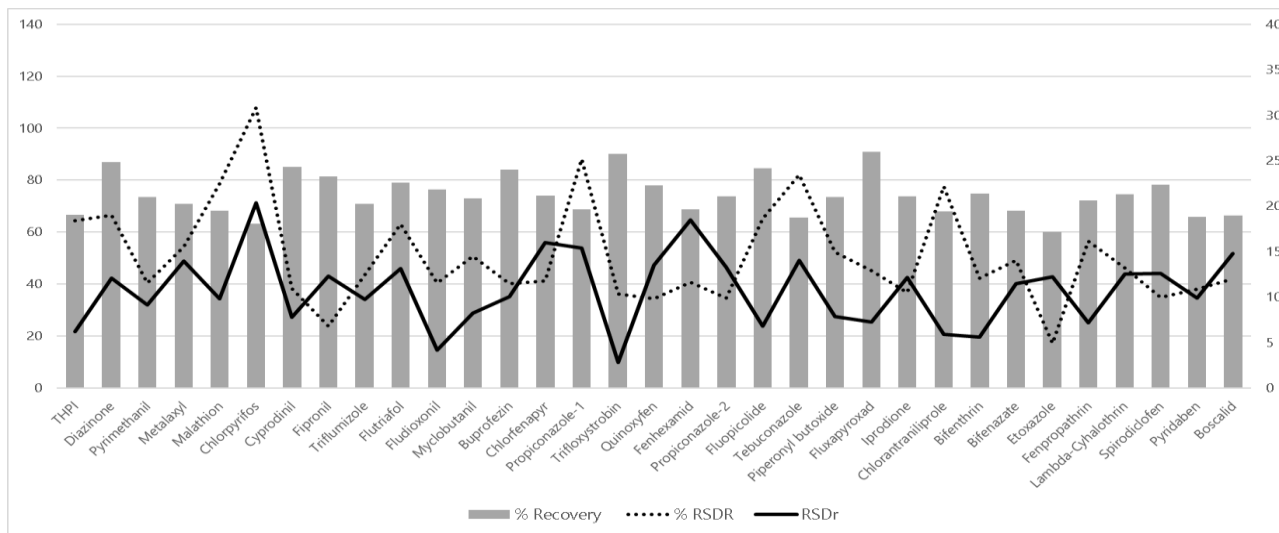
Fig. 6 Trend graph of summary results on GC-MS/MS
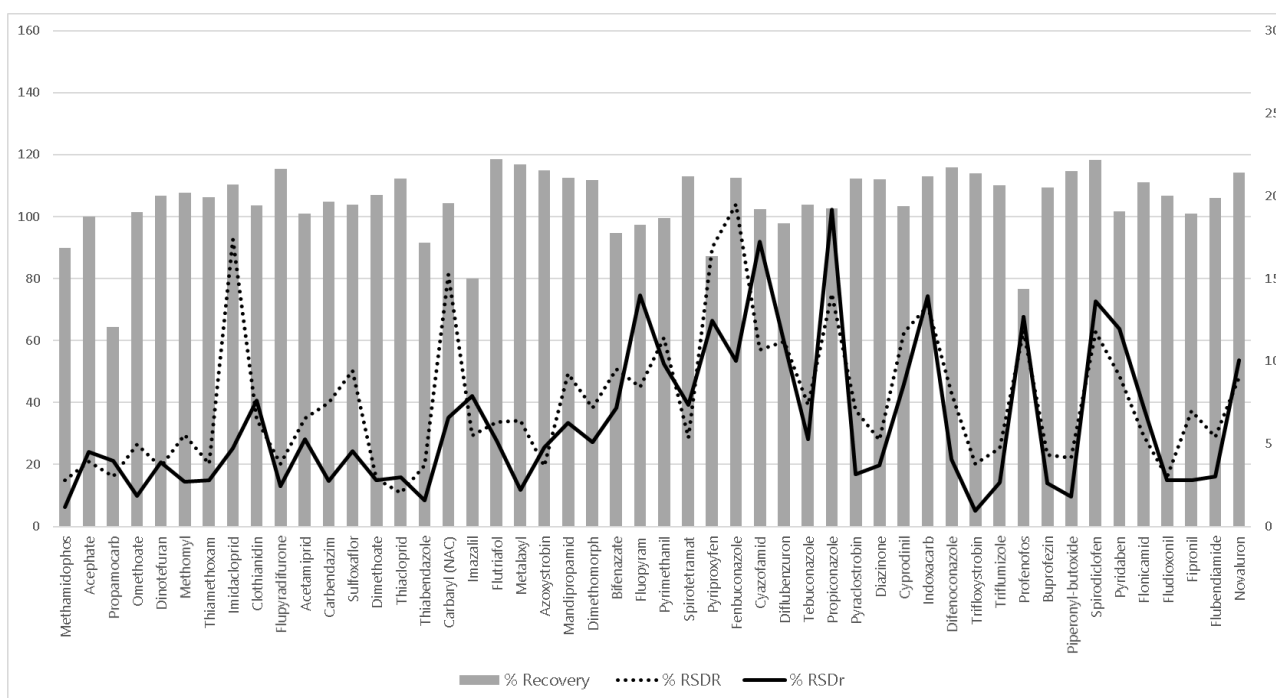

Fig. 7 Trend graph of summary results on LC-MS/MS

4. Conclusion

This study shows that the modified QuEChERS method combined with GC-MS/MS and LC-MS/MS achieved consistent pesticides monitoring in curcumin color additive sample.

Although it is a complex and difficult matrix, the modified QuEChERS method, suppressed interference from matrix.

The GC-MS/MS and LC-MS/MS detected trace levels of pesticides even though the sample was diluted.

As this method involves both the techniques, based on LOQ requirement, best suitable analytical tool can be selected.

5. References

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