

GCMS-TQ™8040 and LCMS™-8045

Analysis of residual pesticides in Carrot-oleoresin using GCMS-TQ8040 NX and LCMS-8045

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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 in complex matrix like Carrot-oleoresin using Ultra-Fast technologies of LCMS-8045 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

Carrot is often contaminated with pesticide residues, due to the application of various chemicals for the control of ectoparasites, insects and pests.

Carrot is used to prepare or extract natural colour additives in the form of oleoresin. Oleoresin is produced by solvent extraction of carrot powder or dehydrated carrot using a suitable organic solvent. Alternatively, the oleoresin can be recovered by steam distillation followed by solvent extraction. During the extraction process, the pesticide residues get concentrated in these oleoresins. The carrot oleoresins (Fig. 1) are used as colour additives in food products, cosmetics, nutraceutical and pharma drugs. Hence quantitation of residual pesticides in carrot oleoresin becomes very important. As the oleoresin is a complex matrix for extraction, it is required to develop a rugged, sensitive and easy method for residual pesticide analysis.



Fig. 1 Carrot oleoresin

Shimadzu Application Development Center (ADC), India has developed a highly sensitive method for simultaneous quantification of multiple pesticides in complex matrix of carrot oleoresin using modified QuEChERS^[1] and triple quadrupole gas chromatography (GC-MS/MS) and liquid chromatography (LC-MS/MS) system.

2. Materials and Methods

The customized reference standards for 72 pesticides under study were procured from Restek Corporation, Bellefonte, PA, USA.

CS-27517-1; CS-27517-2; CS-27517-3; CS-27517-4; CS-27517-5; CS-27517-6.

The food grade carrot oleoresin procured from market was used to prepare matrix-matched calibration standards and fortified samples. The calibration standards were analyzed in the range of 1 to 200 µg/L and 0.5 to 100 µg/L for GC-MS/MS and LC-MS/MS, respectively. Fortified samples were prepared in seven replicates of each 10 and 25 µg/kg. Shimadzu GCMS-TQ8040 NX (Fig. 2) and LCMS-8045 with Nexera™ X2 as front end (Fig. 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 for Residual Pesticide 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™' 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 (AR grade), Anhydrous magnesium sulphate (MgSO₄) (AR grade) salts 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) and Anhydrous MgSO₄ 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 (70:30 v/v) 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-TQTM8040 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 TM -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.	: 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	: 5.91 min
Loop Time	: 0.3 sec
Resolution	: Unit (Q1) – Unit (Q3)



Fig. 3 Shimadzu LCMSTM-8045

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 Scepter, (100 mm × 4.6 mm I.D., 5 μm) P/N: 227-31020-04

LC

Flow rate	: 0.6 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	: 5-10%B (0.0 min to 1.0 min) →55% (3.00 min) → 75% (5.00 min) →90% (9.00 min) →100% (11.0-14.00 min) →10% (14.25min) →5% (14.75-18.0min)
Run time	: 18 min
Injection volume	: 5 × 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 fifteen times for LC-MS/MS analysis. Hence the matrix matched calibration standards were analyzed from much lower concentration levels i.e., 1 to 200 μg/L and 0.5 to 100 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 Fig. 4 and 5. Most of the compounds showed accuracy within 80-120%. Accuracies obtained at LOQ levels, and their correlation coefficients are displayed in Table 4 and 5.

3.2. Recovery study

Seven fortified samples of each 10 and 25 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 fifteen times for LC-MS/MS, respectively.

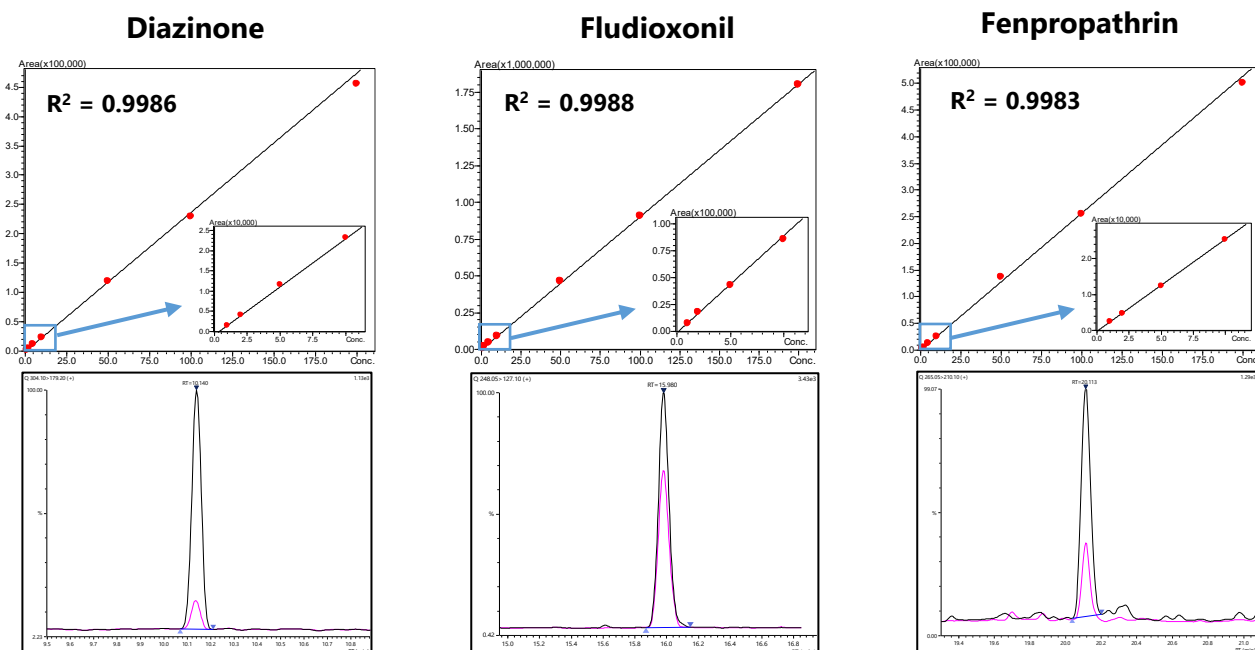


Fig. 4 Representative linearity graphs and chromatograms at LOQ level for GC-MS/MS compounds

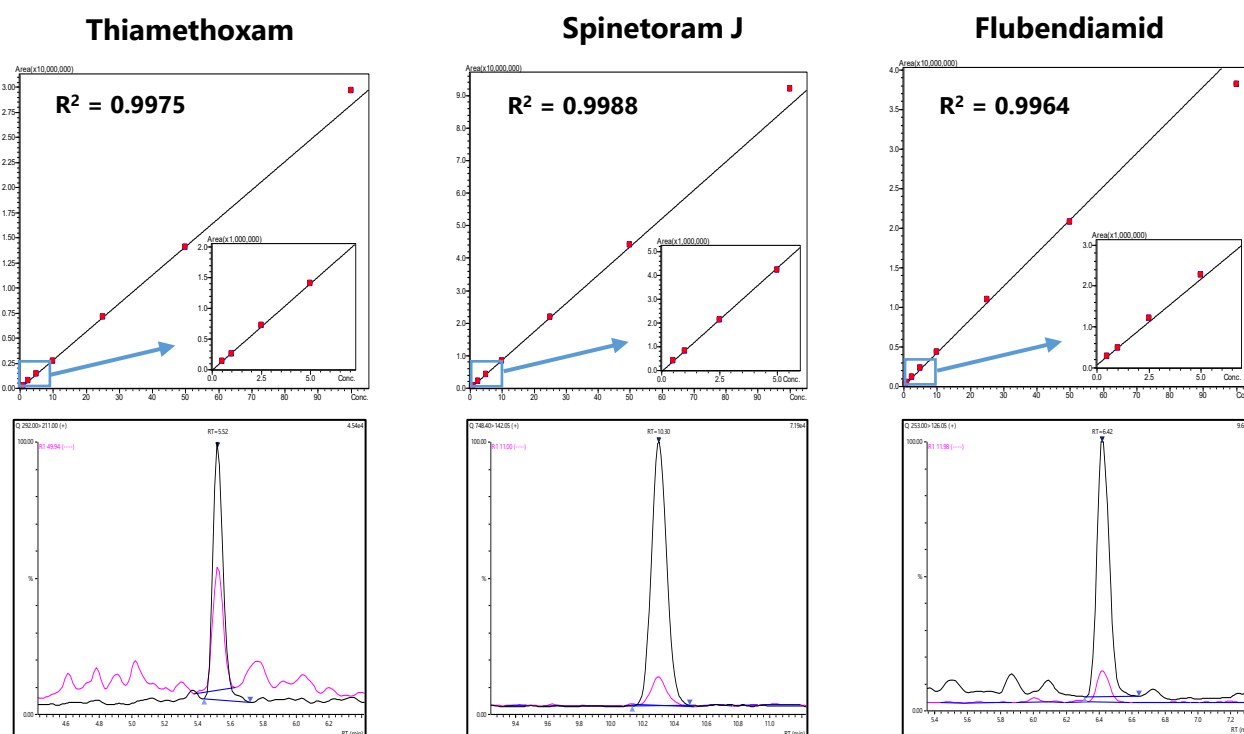


Fig. 5 Representative linearity graphs and chromatograms at LOQ level for LC-MS/MS compounds

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 10 µg/L and 25 µg/L 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 seven different spiked samples at 10 µg/L and 25 µg/L concentration levels. The % RSD for recovery of seven spiked samples at their respective LOQ levels were found to be less than 30%. (Refer to Tables 4 and 5)

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

Out of 72 compounds analyzed, Etoazole and Chlorfenapyr showed lower recovery than SMPR requirement, whereas neither Captan nor its degradant Tetrahydrophthalimide (THPI) could be detected due to matrix interference. Boscalid and Azoxystrobin were present in large concentrations in sample matrix, hence their LOQs could not be studied.

This method successfully achieved 10 µg/kg LOQs on GC-MS/MS and LC-MS/MS for 66 compounds. LOQ of Fonicamid was found to be 25 µg/kg on LC-MS/MS. Refer to summary Tables 4 and 5. Representative chromatograms of compounds at their LOQ levels are shown in Fig. 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=7)	% RSD _r (n=6)
1	Diazinone	10.116	304.10>179.20	19	0.9986	103.30	0.010	85.71	12.60	6.81
2	Pyrimethanil	10.352	198.10>118.10	30	0.9987	95.15	0.010	88.09	6.25	3.78
4	Malathion	12.269	157.95>125.00	9	0.9981	103.87	0.010	97.88	6.05	5.00
4	Chlorpyrifos	12.445	313.95>257.90	17	0.9981	99.91	0.010	73.58	20.46	18.94
5	Cyprodinil	13.695	224.15>222.10	24	0.9947	89.81	0.010	87.33	14.09	9.33
6	Fipronil	13.833	367.00>213.00	29	0.9932	91.08	0.010	101.87	9.31	6.47
7	Triflumizole	14.215	278.05>73.10	8	0.9883	83.92	0.010	64.53	11.68	14.13
8	Profenofos	15.378	337.00>266.90	15	0.9860	82.71	0.010	82.31	10.85	9.61
9	Buprofezin	15.731	172.10>57.10	21	0.9984	96.99	0.010	96.50	6.15	4.54
10	Myclobutanil	16.225	179.05>125.00	18	0.9988	102.38	0.010	103.73	6.44	4.24
11	Fludioxonil	15.897	248.05>127.10	27	0.9988	105.02	0.010	102.57	3.76	4.03
12	Trifloxystrobin	17.862	222.05>190.10	5	0.9887	82.65	0.010	96.50	4.44	10.19
13	Propiconazole-1	17.942	172.95>109.00	25	0.9893	81.37	0.010	93.84	12.46	8.18
14	Quinoxifen	17.961	306.95>237.10	24	0.9963	92.96	0.010	80.93	7.90	8.46
15	Propiconazole-2	18.138	172.95>109.00	25	0.9971	104.74	0.010	87.44	10.05	5.10
16	Fenhexamid	18.172	177.00>113.00	17	0.9955	88.60	0.010	94.97	10.28	6.48
17	Fluopicolide	18.286	209.00>182.00	19	0.9976	105.01	0.010	103.01	7.90	4.80
18	Tebuconazole	18.747	125.00>89.10	21	0.9975	99.68	0.010	90.12	4.89	11.01
19	Piperonyl-butoxide	18.890	176.05>131.10	13	0.9970	102.22	0.010	86.96	11.76	6.41
20	Iprodione	19.687	187.00>124.00	24	0.9830	98.94	0.010	63.51	24.57	12.25
21	Bifenthrin	19.723	181.05>165.10	22	0.9932	85.75	0.010	72.94	20.83	17.93
22	Fluxapyroxad	19.953	381.10>159.10	16	0.9984	94.58	0.010	99.28	6.07	5.62
23	Fenpropathrin	20.070	265.05>210.10	12	0.9983	96.61	0.010	86.29	11.85	4.68
24	Bifenazate	20.147	300.10>258.10	9	0.9988	98.84	0.010	88.84	13.07	6.81
25	Pyriproxyfen	21.294	136.10>78.00	24	0.9976	98.35	0.010	110.65	12.61	5.96
26	Lambda-cyhalothrin	21.665	208.05>181.10	9	0.9975	92.16	0.010	109.33	13.01	9.98
27	Fenbuconazole	24.249	198.10>129.10	12	0.9983	99.34	0.010	97.04	4.08	2.68
28	Cyfluthrin-1	24.266	226.05>206.10	15	0.9812	79.30	0.010	88.29	6.73	6.15
29	Cyfluthrin- 2	24.470	226.05>206.10	15	0.9877	88.99	0.010	75.49	13.39	16.17
30	Cyfluthrin- 3	24.557	226.05>206.10	15	0.9739	75.82	0.010	79.82	10.36	16.67
31	Cyfluthrin- 4	24.663	226.05>206.10	15	0.9769	84.01	0.010	84.19	19.13	7.97
32	Cypermethrin-1	24.844	162.95>127.00	9	0.9939	113.23	0.010	82.48	17.54	4.06
33	Cypermethrin-2	25.055	162.95>127.00	9	0.9967	96.17	0.010	81.64	13.85	14.83
34	Cypermethrin-3	25.141	162.95>127.00	9	0.9926	85.01	0.010	87.75	25.16	15.90
35	Cypermethrin-4	25.236	162.95>127.00	9	0.9956	107.29	0.010	82.25	16.17	6.14
36	Pyraclostrobin	26.709	164.05>132.10	12	0.9986	94.10	0.010	79.77	6.89	3.73
37	Difenoconazole-1	27.382	323.05>264.90	18	0.9977	101.88	0.010	94.65	3.27	3.51
38	Difenoconazole-2	27.500	323.05>264.90	18	0.9979	99.13	0.010	88.75	5.83	5.39
39	Indoxacarb	27.725	264.05>148.10	28	0.9851	103.50	0.010	98.66	10.85	12.12
40	Dimethomorph-1	28.546	301.05>165.10	15	0.9989	100.43	0.010	94.98	3.49	3.32
41	Dimethomorph- 2	29.134	301.05>165.10	15	0.9979	100.02	0.010	93.05	3.93	4.21

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	0.9994	101.00	0.010	89.65	8.03	4.63
2	Acephate	4.706	183.90>143.00	-10	0.9994	101.20	0.010	86.41	10.13	2.47
3	Propamocarb	4.833	189.10>102.15	-17	0.9758	98.40	0.010	64.24	3.08	3.25
4	Omethoate	4.882	214.00>124.90	-22	0.9985	100.20	0.010	101.64	6.72	2.64
5	Dinotefuran	4.993	203.05>87.00	-15	0.9987	101.80	0.010	113.17	9.02	9.36
6	Thiamethoxam	5.426	292.00>211.00	-12	0.9975	103.60	0.010	115.91	11.43	7.25
7	Methomyl	5.466	163.00>88.00	-9	0.9923	103.40	0.010	111.90	8.95	8.17
8	Fonicamid	5.466	227.95>81.00	10	0.9862	80.90	0.025	109.89	17.26	2.42
9	Imidacloprid	5.793	256.00>175.05	-19	0.9886	92.20	0.010	118.65	14.13	15.15
10	Clothianidin	5.908	250.00>169.00	-13	0.9939	104.20	0.010	106.44	12.64	14.42
11	Flupyradifurone	6.015	288.95>72.90	-20	0.9952	100.40	0.010	142.75	13.36	10.14
12	Acetamiprid	6.082	225.00>56.05	-20	0.9932	101.80	0.010	120.58	8.54	6.11
13	Carbendazim	6.102	192.00>160.05	-18	0.9944	104.80	0.010	113.75	8.80	6.38
14	Dimethoate	6.196	230.00>198.90	-10	0.9970	103.60	0.010	115.41	7.32	3.24
15	Sulfoxaflor	6.210	277.95>174.10	-8	0.9963	98.20	0.010	120.30	7.02	4.69
16	Thiacloprid	6.342	253.00>126.05	-20	0.9964	103.80	0.010	104.88	9.39	3.52
17	Thiabendazole	6.673	202.00>175.00	-25	0.9972	102.80	0.010	101.53	8.39	2.00
18	Carbaryl (NAC)	7.533	202.00>145.00	-11	0.9963	100.80	0.010	112.51	12.20	6.64
19	Imazalil	7.683	297.00>158.95	-21	0.9975	103.40	0.010	102.37	6.04	7.75
20	Flutriafol	7.750	302.10>70.05	-17	0.9970	103.00	0.010	114.90	6.54	2.43
21	Metalaxyl	8.030	280.10>220.10	-14	0.9896	98.70	0.010	115.54	7.71	2.73
22	Chlorantraniliprole	8.189	483.80>285.70	-16	0.9942	104.60	0.010	120.58	11.35	8.73
23	Mandipropamid	8.568	412.00>328.00	-15	0.9987	101.60	0.010	116.96	5.07	5.92
24	Fluxapyroxad	8.728	382.00>362.05	-14	0.9941	104.20	0.010	80.34	10.51	3.98
25	Fludioxonil	8.796	247.10>180.15	28	0.9949	102.20	0.010	99.42	17.39	12.52
26	Dimethomorph	8.831	388.00>301.00	-21	0.9991	99.60	0.010	73.25	8.01	7.78
27	Permethrin	8.834	391.00>241.05	-22	0.9900	108.60	0.010	94.47	24.43	17.94
28	Linuron	8.869	249.00>181.95	-16	0.9976	101.40	0.010	86.37	12.81	13.18
29	Methoxyfenozide	8.892	369.10>149.05	-18	0.9988	101.60	0.010	100.56	11.83	14.00
30	Myclobutanil	8.900	291.10>70.05	-22	0.9958	95.20	0.010	116.55	15.87	14.14
31	Fluopicolide	8.926	384.90>174.90	-22	0.9972	96.00	0.010	106.98	6.34	8.28
32	Malathion	8.991	348.00>127.15	-13	0.9987	101.00	0.010	105.95	5.05	9.54
33	Chlorpyrifos	9.000	349.75>127.05	-7	0.9985	99.50	0.025	114.45	13.84	3.05
34	Fluopyram	9.097	396.90>207.90	-21	0.9986	102.80	0.010	110.36	3.86	5.49
35	Bifenazate	9.109	301.10>170.10	-10	0.9981	102.20	0.010	113.24	3.56	4.64
36	Spirotetramat	9.152	374.10>216.00	-33	0.9998	100.00	0.010	112.41	4.77	3.58
37	Pyrimethanil	9.188	200.10>107.10	-25	0.9955	104.80	0.010	81.41	9.63	8.69
38	Fenhexamid	9.245	302.10>97.20	-24	0.9975	100.40	0.010	97.12	22.00	15.25
39	Fenbuconazole	9.376	337.00>70.10	-28	0.9913	107.20	0.010	111.61	8.98	8.44
40	Pyriproxyfen	9.393	338.95>69.95	-30	0.9965	96.40	0.010	101.23	14.62	17.60
41	Fipronil	9.420	434.90>330.00	16	0.9997	100.60	0.010	106.16	5.46	6.18
42	Flubendiamide	9.460	680.90>254.10	27	0.9964	100.20	0.010	112.52	6.57	7.15
43	Cyazofamid	9.466	325.00>107.90	-16	0.9848	109.20	0.010	103.53	15.10	9.71
44	Diflubenzuron	9.714	311.00>158.10	-14	0.9938	100.00	0.010	92.69	9.36	13.69
45	Tebuconazole	10.009	308.10>69.95	-24	0.9967	100.40	0.010	107.30	8.31	7.64
46	Spinetoram J	10.239	748.40>142.05	-30	0.9988	101.60	0.010	93.73	3.38	3.74
47	Propiconazole	10.256	342.00>158.90	-27	0.9892	90.60	0.010	113.20	12.12	18.16
48	Diazinone	10.472	305.00>169.10	-21	0.9801	111.80	0.010	93.30	6.90	4.54
49	Pyraclostrobin	10.475	388.00>194.00	-13	0.9942	104.20	0.010	95.61	7.66	6.47
50	Cyprodinil	10.582	226.10>93.10	-37	0.9964	105.20	0.010	80.75	6.90	19.79
51	Indoxacarb	10.611	528.00>150.00	-40	0.9954	99.80	0.010	124.30	17.16	12.25
52	Difenoconazole-1	10.695	406.00>250.90	-25	0.9971	102.60	0.010	103.99	4.93	3.46
53	Difenoconazole-2	10.734	408.00>252.90	-26	0.9965	103.00	0.010	116.62	3.77	3.41
54	Novaluron	10.797	491.00>470.90	13	0.9988	98.80	0.010	103.41	10.25	9.91
55	Spinetoram L	10.799	760.40>142.10	-29	0.9951	105.60	0.010	86.31	6.96	5.01
56	Trifloxystrobin	10.904	409.00>186.00	-20	0.9989	101.20	0.010	105.80	1.80	2.47
57	Triflumizole	11.013	346.10>278.00	-10	0.9972	103.00	0.010	76.48	1.80	1.69
58	Profenofos	11.482	372.80>302.80	-19	0.9898	95.60	0.010	105.20	12.56	16.65
59	Buprofezin	11.694	306.20>201.05	-13	0.9984	102.60	0.010	112.86	3.67	1.92
60	Piperonyl-butoxide	11.969	356.10>177.00	-20	0.9840	98.20	0.010	100.62	7.34	2.29
61	Quinoxifen	12.402	308.00>197.00	-31	0.9967	103.20	0.010	103.62	13.12	14.77
62	Spirodiclofen	12.542	411.10>313.05	-14	0.9902	104.60	0.010	101.34	9.73	16.48
63	Pyridaben	12.989	365.20>147.20	-25	0.9975	100.00	0.010	89.76	3.54	4.48

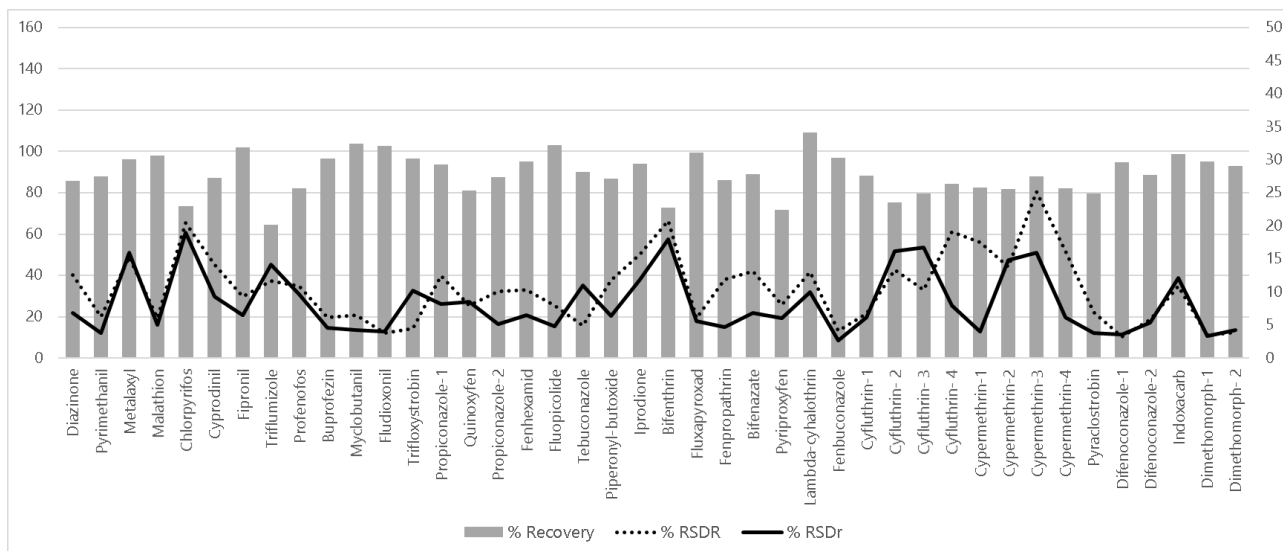


Fig. 6 Trend graph of summary results on GC-MS/MS

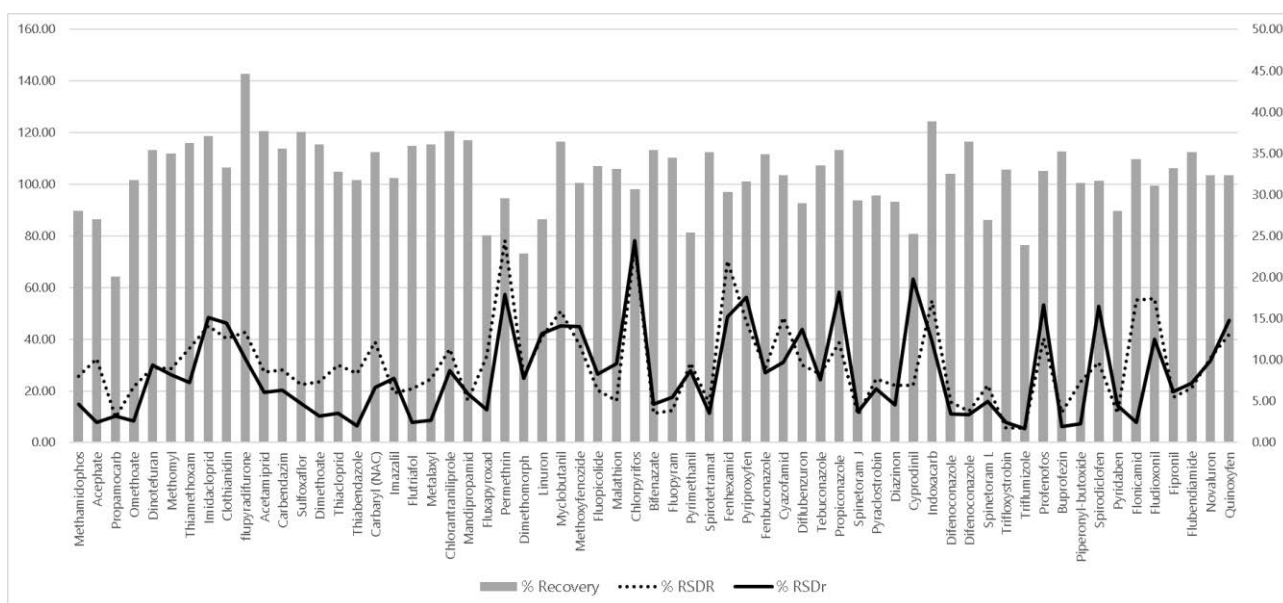


Fig. 7 Trend graph of summary results on LC-MS/MS

4. Conclusion

This study shows that the modified QuEChERS method combined with GC-MS/MS and LC-MS/MS achieved consistent pesticides monitoring in carrot oleoresin sample. Although oleoresin sample is 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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