Application News

GCMS-TQ™ 8050 NX

Multiresidue Pesticides Analysis in Solvent Extract of Annatto (Natural Color Additive) Using GCMS-TQ8050 NX

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User Benefits

- Accurate and reproducible trace level quantitation of pesticides in highly pigmented complex matrix using Ultra-fast GCMS-TQ8050 NX and QuEChERS extraction
- Single-laboratory partial validated method for LOQ determination meeting regulatory guidelines
- Ease of instrumental method optimization using GCMS Smart Pesticides Database™ Ver.2 from Shimadzu Corporation

1. Introduction

Annatto is an orange-red condiment and food colorant derived from the seeds of the achiote tree (*Bixa Orellana*) (Fig. 1). It is often used to impart a yellow to red-orange color to foods, but sometimes also for its flavor and aroma. Similar effects can be obtained by extracting some of the color and flavor principles from the seeds, which are then added to the food. Annatto and its extracts are now widely used in an artisanal or industrial scale as a coloring agent in many processed food products, such as cheeses, butter, baked goods, potatoes, snack foods, breakfast cereals, smoked fish etc. In these uses, Annatto is a natural alternative to synthetic food coloring compounds.

Owing to its large culinary uses and other diverse applications, use of chemical pesticides for its production in large quantities is imperative. Dye extraction process may result in concentration of pesticides and in turn contribute to adverse impact on human health when incorporated in various preparations. Hence quantitation of residual pesticides in Annatto extract becomes very important. As the oleoresin is a complex matrix, it is required to develop a rugged, sensitive and efficient method for extraction of residual pesticide and its analysis.

This study reports a highly sensitive method for simultaneous quantification of multiple pesticides in highly pigmented complex matrix of Annatto using modified QuEChERS[1] with triple quadrupole gas chromatography (GC-MS/MS) system.

Fig. 1 *Annatto* Seeds

2. Materials and Methods

For this study, customized reference standard mixture of most commonly observed pesticides in Annatto seeds was procured from Restek Corporation.

The calibration standards were analyzed from 0.5 to 1000 µg/L. Linearity was plotted by external standard method and using weighted regression of $1/C^2$. Sample was spiked at 3 levels i.e., 10, 25 and 100 µg/kg. Recovery samples were prepared in 6 replicates at each level.

GCMS-TQ8050 NX (Fig. 2), manufactured by Shimadzu Corporation Japan, was used for quantitation. LabSolutions Insight™ was used for data processing, which helped in evaluating validation parameters with ease.

Fig. 2 Shimadzu GCMS-TQ™8050 NX

2.1. Sample preparation

For extraction, modified QuEChERS method approach was adopted. Sodium chloride (AR grade), anhydrous magnesium sulphate (AR grade) salts were used in optimized 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, Graphitized carbon black (GCB), Primary secondary amine (PSA) and anhydrous magnesium sulphate. This helped to minimize matrix interference, reduce instrument contamination and achieve lower LOQs.

After clean up, the remaining aliquot of acetonitrile was evaporated and reconstituted in ethyl acetate such that final sample was diluted 10 times.

All samples were analysed using GC-MS/MS as per conditions shown in Table 1.

2.2. Analytical Conditions

Table 1 Instrument Configuration and Analytical Conditions

System Configuration	
Model	: GCMS-TQ8050 NX
Auto-injector	: AOC^{TM}-20i + s
Column	: SH-I-5Sil MS (30 m × 0.25 mm I.D., df = 0.25 µm) (P/N: 221-75954-30)
Liner	: Topaz Liner, Splitless Single Taper w/Wool (P/N: APARES-23322)
GC	
Injector temp.	: 250 °C
Column oven temp	: 80 °C (2 min), 20 °C/min to 180 °C (0 min), 5 °C/min to 300 °C (3 min)
Run time	: 34 min
Injection mode	: Splitless (High pressure at 250 kPa)
Injection volume	: 2 µL
Carrier gas	: He
Linear Velocity	: 40.4 cm/sec (Constant mode)
MS	
Ionization mode	: EI
Ion source temp.	: 230 °C
Interface temp.	: 280 °C
Solvent cut time	: 5.0 min
Loop Time	: 0.3 sec

3. Result and Discussion

Based on the latest SANTE guidelines [2], validation parameters like linearity, recovery and precision were studied to determine LOQs (Limit of quantitation) and the results obtained are shown in Table 2.

As per SANTE guidelines, for exceptional cases, mean recoveries outside the above range can be accepted, but shall lie within 30-140 %, and only when the precision criteria for repeatability (RSD_r) are met.

3.1. Linearity study

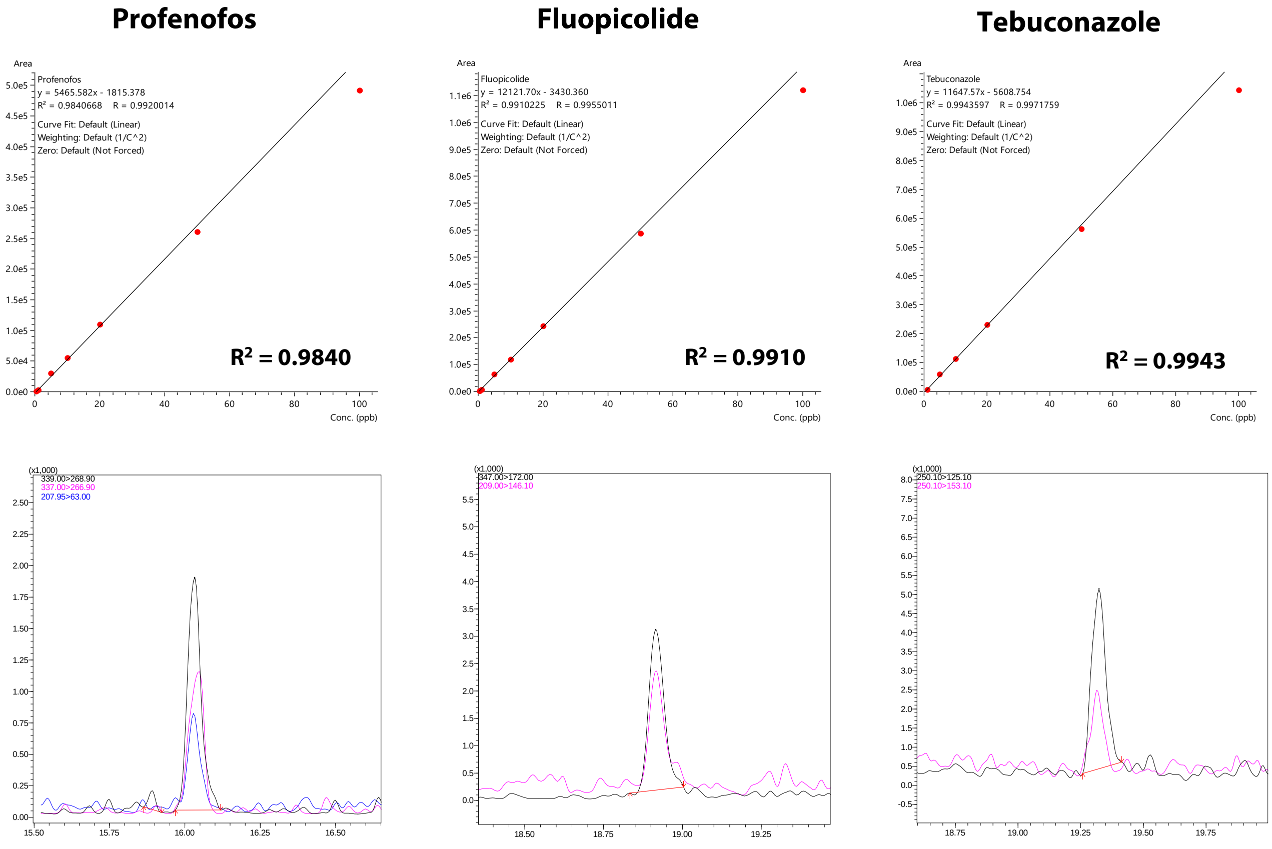
For linearity study, matrix match calibration standards were used. Calibration curve was plotted in the range of 0.5 to 1000 µg/L. All calibration standards were found within 80 to 120 % accuracy as per requirement of SANTE guidelines.

The linearity graphs of few representative pesticides are shown in Fig. 3.

3.2. Recovery study

Six spiked samples of each 10, 25 and 100 µg/kg were analyzed, and their mean recovery was evaluated. As mentioned, spiked samples were diluted 10 times.

Except Captan and Etoxazole, all other pesticides showed mean recovery within 60-120 % with reproducibility (RSD_R) < 30 % at LOQ level (Refer Table 3). Captan and Etoxazole showed mean recovery between 50 to 60 %, but the RSD_r was found to be within acceptable limit of 20 %.



Profenofos

Fluopicolide

Tebuconazole

Fig. 3 Representative Linearity Graphs and Chromatograms at LOQ Level

Table 2 Summary Results

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	Diazinon	10.641	304.10>179.10	10	0.9908	0.01	84.45	110.50	2.98	5.13
2	Pyrimethanil	10.922	198.10>118.10	30	0.9875	0.01	80.36	89.90	7.17	8.71
3	Metalaxyl (Mefenoxam)	12.269	249.20>190.10	8	0.9998	0.025	99.43	76.58	12.14	16.40
4	Malathion	12.901	157.95>125.00	9	0.9980	0.01	93.44	100.29	12.04	13.46
5	Chlorpyrifos	13.109	313.95>257.90	17	0.9912	0.025	117.66	102.22	7.92	8.48
6	Cyprodinil	14.168	224.15>222.10	24	0.9930	0.025	116.44	93.49	13.22	4.78
7	Fipronil	14.516	367.00>255.00	29	0.9905	0.01	84.69	104.13	4.37	4.23
8	Captan	14.646	149.05>70.00	24	0.9970	0.1	94.14	57.94	6.13	10.77
9	Triflumizole	14.813	278.05>73.10	8	0.9906	0.01	80.31	75.68	13.53	14.55
10	Flutriafol	15.719	219.10>123.10	21	0.9983	0.025	105.54	83.67	8.48	5.84
11	Fludioxonil	16.272	248.05>127.10	27	0.9955	0.01	102.30	82.07	16.78	16.36
12	Profenofos	16.032	339.00>268.90	15	0.9841	0.01	83.13	115.54	8.39	9.53
13	Myclobutanil	16.437	179.05>125.00	18	0.9954	0.01	98.25	119.91	5.24	5.11
14	Buprofezin	16.387	172.10>57.10	21	0.9908	0.01	83.14	81.85	22.85	15.94
15	Chlorfenapyr	16.737	247.00>227.00	14	0.9841	0.025	118.58	118.19	12.78	8.37
16	Trifloxystrobin	18.502	206.10>116.10	5	0.9909	0.01	86.38	119.99	11.38	10.89
17	Propiconazole-1	18.574	172.95>109.00	25	0.9938	0.025	115.81	99.55	8.39	12.48
18	Quinoxyfen	18.568	306.95>237.10	24	0.9939	0.01	87.26	92.99	6.52	3.96
19	Propiconazole-2	18.774	172.95>109.00	25	0.9973	0.025	104.71	97.16	10.81	10.78
20	Fenhexamid	18.824	177.00>113.00	17	0.9927	0.01	89.49	102.64	4.16	4.55
21	Fluopicolide	18.917	347.00>172.00	19	0.9910	0.01	87.50	110.71	2.62	5.15
22	Tebuconazole	19.333	250.10>125.10	21	0.9953	0.025	112.67	91.87	4.51	4.53
23	Piperonyl butoxide	19.515	176.10>131.10	12	0.9918	0.025	112.32	104.65	13.14	11.43
24	Iprodione	20.256	314.00>56.10	25	0.9892	0.01	81.32	100.76	9.21	8.76
25	Fluxapyroxad	20.488	159.05>139.00	12	0.9881	0.01	82.71	111.63	5.10	6.00
26	Bifenthrin	20.414	181.05>179.10	7	0.9907	0.1	85.86	71.20	13.86	14.33
27	Etoxazole	20.716	359.15>187.20	21	0.9955	0.025	108.59	52.50	14.08	12.87
28	Fenpropathrin	20.760	265.05>210.10	12	0.9890	0.01	87.92	115.08	5.25	3.30
29	Pyriproxyfen	21.909	136.10>96.00	16	0.9927	0.01	109.86	89.58	8.28	7.42
30	lambda-Cyhalothrin	22.331	208.00>181.00	8	0.9924	0.01	83.79	118.42	15.92	16.85
31	Spirodiclofen	23.485	312.00>259.10	12	0.9902	0.025	100.01	94.96	14.18	12.30
32	Permethrin-1	23.766	162.95>127.00	9	0.9962	0.1	96.31	70.23	6.74	10.49
33	Permethrin-2	24.026	162.95>127.10	9	0.9983	0.1	102.74	73.21	11.43	13.94
34	Fenbuconazole	24.864	198.10>129.10	12	0.9948	0.025	114.12	86.11	8.37	10.91
35	Cyfluthrin-1	24.879	163.10>127.10	6	0.9931	0.025	115.73	87.85	12.61	14.17
36	Cyfluthrin-2	25.094	163.10>127.10	6	0.9907	0.025	118.78	98.93	10.18	11.83
37	Cyfluthrin-3	25.192	163.10>127.10	6	0.9918	0.025	117.10	103.76	9.84	10.64
38	Cyfluthrin-4	25.291	163.10>127.10	6	0.9943	0.025	109.38	89.84	13.46	15.09
39	Cypermethrin-1	25.481	163.10>127.10	6	0.9927	0.025	118.84	91.99	14.44	13.83
40	Cypermethrin-2	25.700	163.10>127.10	6	0.9945	0.025	114.54	92.99	9.41	11.36
41	Cypermethrin-3	25.791	163.10>127.10	6	0.9955	0.025	111.62	81.80	12.18	16.93
42	Cypermethrin-4	25.887	163.10>127.10	6	0.9983	0.025	106.52	75.79	7.34	6.51
43	Boscalid	25.593	342.10>140.10	14	0.9889	0.01	83.18	93.27	6.53	6.54
44	Difenoconazole-1	28.046	323.00>265.00	14	0.9865	0.01	81.91	105.73	10.45	7.80
45	Difenoconazole-2	28.170	323.00>265.00	14	0.9903	0.01	82.16	99.51	6.25	4.92
46	Indoxacarb	28.463	264.05>176.00	15	0.9878	0.01	82.17	119.60	0.89	2.12
47	Azoxystrobin	28.977	344.10>156.10	30	0.9994	0.025	102.82	77.53	3.43	3.53
48	Dimethomorph-1	29.191	387.10>301.10	12	0.9964	0.01	94.77	99.62	8.39	8.89
49	Dimethomorph-2	29.780	301.10>165.10	14	0.9933	0.01	87.36	99.73	9.86	10.80

3.3. Precision study

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

RSD_r : Repeatability experiment was performed by injecting 6 replicates of spiked samples at 10, 25 and 100 µg/L concentration levels. The % RSD for 6 injections at their respective LOQ levels was found to be less than 20 % (Refer Table 3).

RSD_R : Reproducibility experiment for recoveries was performed on 6 different spiked samples at 10, 25 and 100 µg/L concentration levels. The % RSD of 6 spiked samples at their respective LOQ level was found to be less than 30 % (Refer Table 3).

Trend graphs for recovery and precision data obtained are shown in Fig. 4.

3.4. LOQ

The concentration level which satisfied the criteria of validation parameters was considered as method LOQ for the respective pesticide.

The method successfully achieved LOQs in the range of 10 to 100 µg/kg for 49 pesticides. Out of 49 pesticides, 10, 25 and 100 µg/kg LOQ was achieved for 24, 21 and 4 pesticides, respectively. Refer to summary Table 2. Representative chromatograms of pesticides at their LOQ levels are shown in Fig. 3.

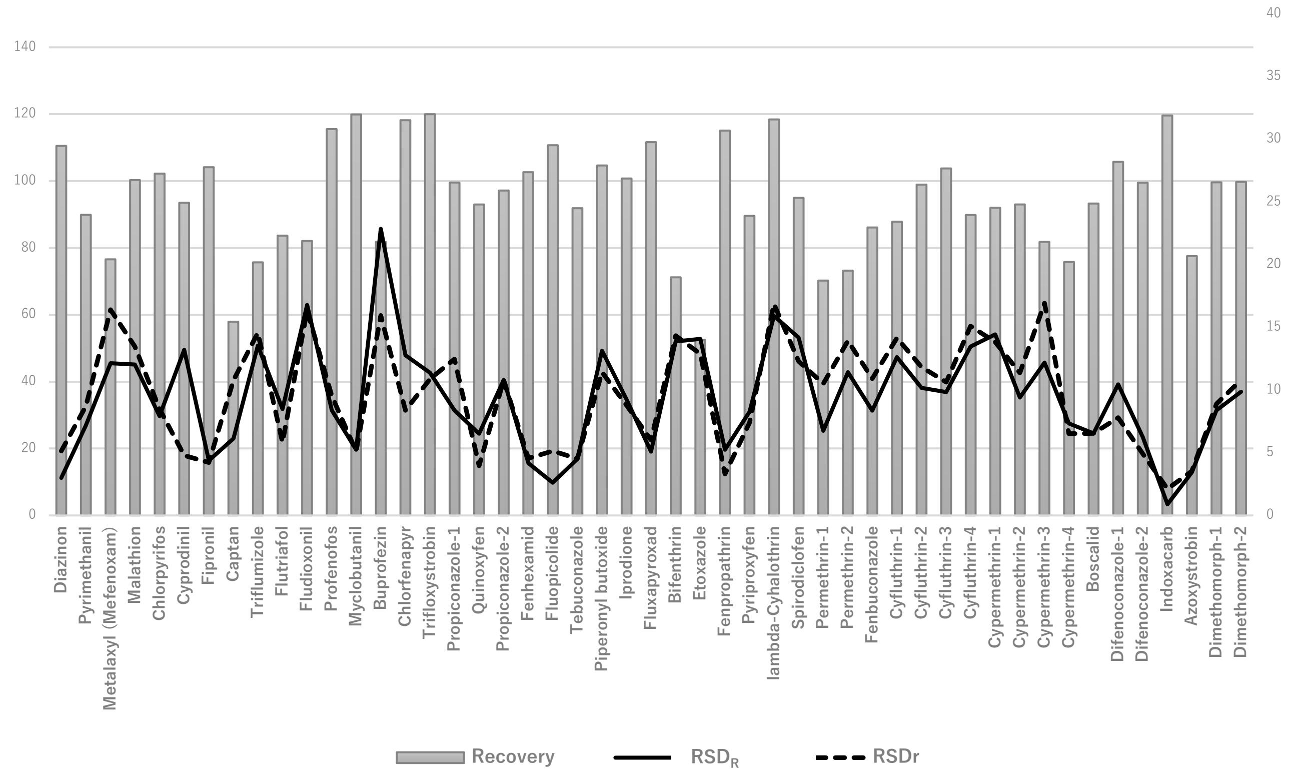


Fig. 4 Trend Graphs for Recovery and Precision Study

4. Conclusion

This study shows that the modified QuEChERS method combined with GC-MS/MS system is a reliable and efficient tool to quantify residual pesticides in Annatto extract sample. Although Annatto extract is a highly pigmented complex matrix, the modified QuEChERS method significantly reduces interference. Also, extremely sensitive Shimadzu GC-MS/MS allows trace level detection even after multifold dilution of sample. This helps in reducing contamination and enhancing ruggedness resulting in reproducible detection of analytes.

5. References

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