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Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method

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Introduction

Ayurveda is a Sanskrit term, which means 'science of life'[1]. In India, Ayurveda has been long practiced for medicinal purpose. Majority of it's preparations involve presence of plants, herbs etc. (Figure 1). For the normal, disease-free growth of these plants and herbs, pesticides are widely used. Some of these pesticides can act as toxins to animals & humans when consumed even as residues. Hence, it becomes important to quantitate the pesticides at residual levels in orally consumed preparations, especially which involve plant materials such as Ayurvedic cough syrups.

QuEChERS (Quick, Easy, Cheap, Effective, Rugged & Safe) method was used for extraction and clean-up of the sample followed by analysis using GCMS-TQ8040 Triple Quadrupole (GCMS/MS) system (Figure 2) from Shimadzu Corporation, Japan.

Figure 1. Ayurvedic herbs

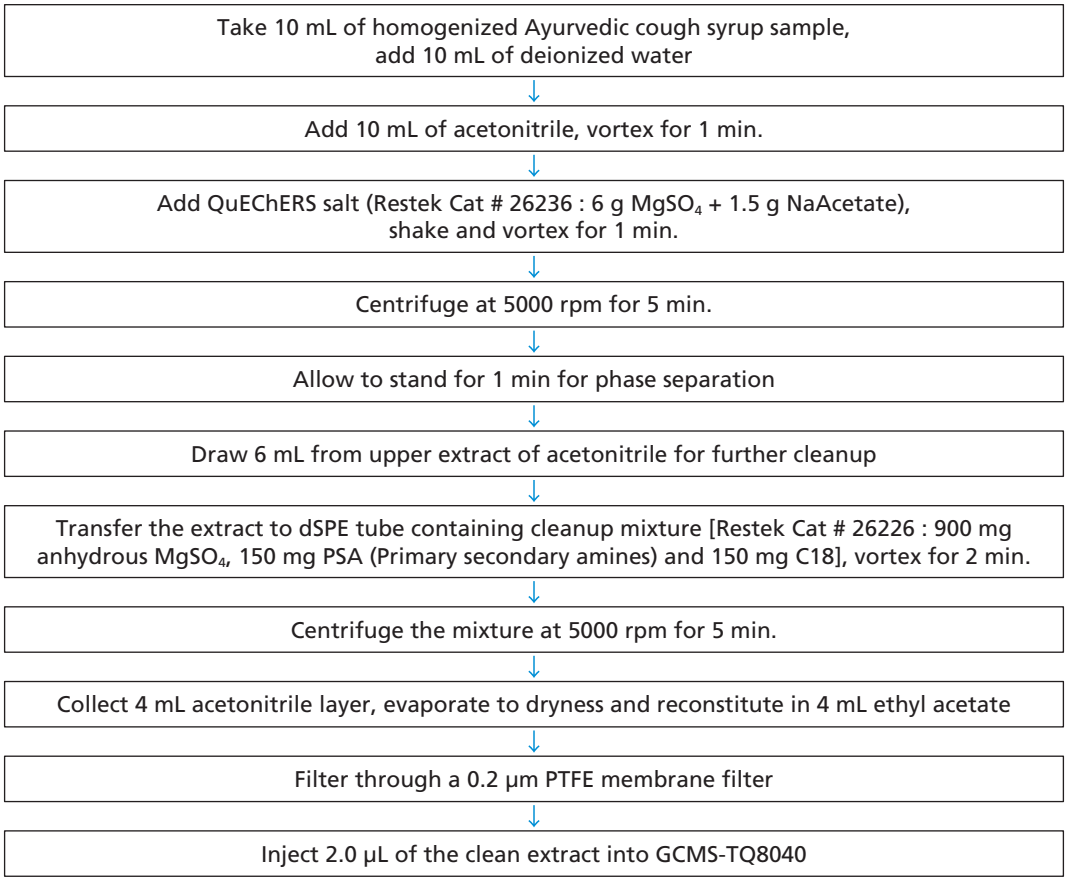
Method of analysis

Sample preparation

Sample extraction

Extraction of pesticides was done using modified AOAC QuEChERS method, as given below[2].

Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method



Preparation of solvent standard calibration levels

Individual pesticides obtained from Sigma-Aldrich® were diluted using ethyl acetate to prepare stock solution of about 1 ppm standard mixture of more than 50 analytes. From this, working stock of 0.1 ppm was prepared. It was then used to make solvent standards at concentration levels of 5 ppb, 10 ppb, 25 ppb, 50 ppb and 100 ppb.

Preparation of matrix matched standard calibration levels

Locally purchased Ayurvedic cough syrup was used as a sample. It was extracted as per the flowchart shown in sample extraction to prepare matrix blank. Further it was spiked with above calibration levels to prepare matrix match linearity of 0.5 ppb, 1 ppb, 2.5 ppb, 5 ppb and 10 ppb.

Preparation of pre-extraction spike

In order to study the extraction efficiency of the method, recoveries of the pre-extraction spiked samples were studied. For this, 10 g sample was spiked with 100 ppb solvent standard to prepare pre-extraction spike concentration levels of 0.5 ppb, 1 ppb and 5 ppb. These samples were extracted, analyzed and quantified against matrix match linearity (post-extraction spike) to study their recoveries.

Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method

MRM method development

For MRM method creation, Shimadzu's Smart Pesticide Database Ver.1 was used. For this, retention times of all analytes were predicted with the help of n-Alkane standard mixture. Pre-optimized MRM transitions present in Smart Pesticide Database and accurately predicted retention times, enabled creation of MRM method with overlapping segments resulting in optimum dwell time required for high sensitivity. MRM chromatogram acquired using this method is shown in Figure 3. For each component, minimum two MRM transitions were analyzed.

GCMS/MS Analytical Conditions

The analysis was carried out on Shimadzu GCMS-TQ8040 as per the conditions given in Table 1.

Table 1. Analytical conditions

Chromatographic parameters

Column	: SH-Rxi™-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Splitless
Sampling Time	: 2.00 min
Split Ratio	: 5.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 40.2 cm/sec
Column Flow	: 1.2 mL/min
Injection Volume	: 2.0 µL
Injection Type	: High Pressure Injection
Total Program Time	: 41.87 min

Column Temp. Program :

Rate (°C /min)	Temperature (°C)	Hold time (min)
	70.0	2.00
25.00	150.0	0.00
3.00	200.0	0.00
8.00	280.0	10.00

Mass Spectrometry parameters

Ion Source Temp.	: 230.0 °C
Interface Temp.	: 280.0 °C
Ionization Mode	: EI (Electron Ionization)
Acquisition Mode	: MRM

Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method

Results

Extracted matrix blank sample was screened and none of the pesticides involved in this study were detected. Calibration curve was plotted for matrix matched standards in the range of 0.5 ppb to 10 ppb. Linear response with r^2 more than 0.985 was obtained. Pre-extraction spiked samples were analyzed six times and their % RSD was found to be less than 20 %. Recoveries of the same were in the range of 70 to 120 %. Based on linearity, precision and recovery, the LOQ for each analyte was determined. The statistical results for all analytes are shown in Table 2. Figure 4 depicts the detailed data for one of the pesticides, o,p'-DDD (Sr. No. 28). Summarized LOQ data is listed in Table 3.

Figure 2. GCMS-TQ8040 Triple quadrupole system by Shimadzu

Key Features of GCMS-TQ8040

1. Smart Productivity : Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.

2. Smart Operation : Smart MRM technology creates optimal MRM methods automatically. The "MRM Optimization Tool" automates best MRM transitions for new compounds.

3. Smart Performance : ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest MRM 800 trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.

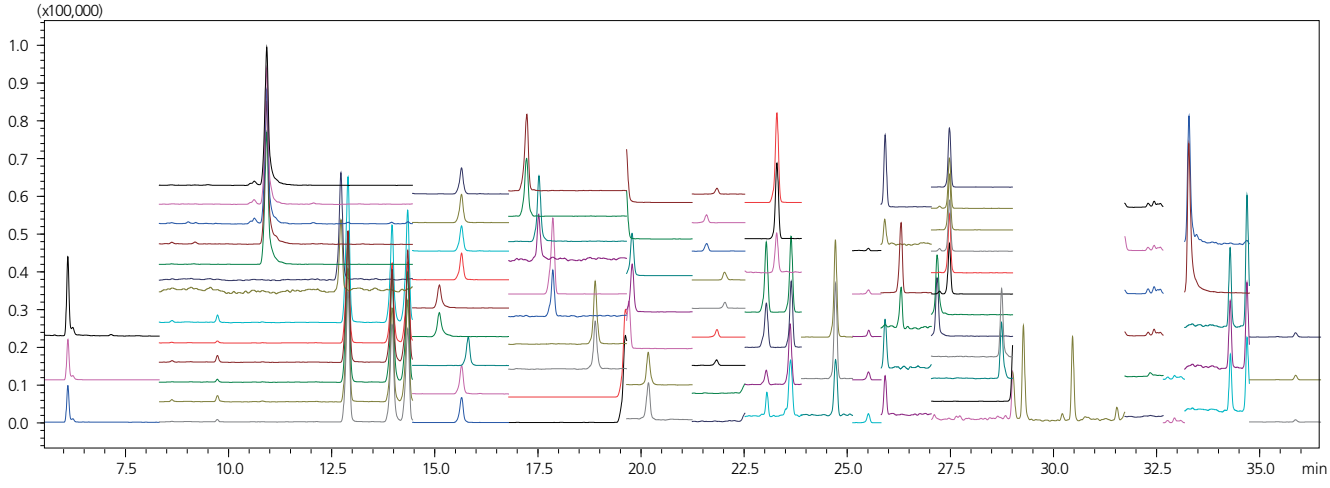


Figure 3. MRM chromatogram for 10 ppb pesticide mixture of 52 pesticides

Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method

Table 2. Quantitation results

Sr. No.	Name	LOQ (ppb)	Retention time (min)	Target MRM (m/z)	r^2	% RSD (n=6)	% Mean recovery at LOQ
1	Dichlorvos	5	6.27	185.00>93.00	0.9991	2.84	94
2	Phorate	0.5	12.78	260.00>75.00	0.9992	8.78	111
3	alpha-BHC	0.5	12.95	218.90>144.90	0.9997	5.75	110
4	beta-BHC	0.5	14.17	180.90>144.90	0.9999	5.40	111
5	gamma-BHC (Lindane)	0.5	14.39	180.90>144.90	0.9995	4.58	111
6	Chlorothalonil	1	15.29	265.90>168.00	0.9960	11.57	87
7	delta-BHC	0.5	15.77	218.90>182.90	0.9997	10.59	116
8	Etrimfos	1	15.88	292.10>181.10	0.9990	6.54	120
9	Chlorpyrifos-methyl	1	17.28	285.90>93.00	0.9998	10.31	114
10	Parathion-methyl	1	17.62	263.00>109.00	0.9982	8.11	115
11	Heptachlor	0.5	17.88	271.80>236.90	0.9999	9.35	112
12	Fenitrothion	1	18.97	277.00>260.00	0.9986	9.18	114
13	Metolachlor	0.5	19.70	238.10>162.10	0.9998	4.29	113
14	Aldrin	1	19.71	262.90>193.00	0.9992	19.31	103
15	Chlorpyrifos	5	19.83	313.90>257.90	0.9986	3.58	112
16	Parathion	5	20.24	291.10>109.00	0.9973	6.27	98
17	Pendimethalin	1	21.63	252.10>162.10	0.9986	8.78	120
18	Heptachlor-endo-epoxide	5	21.86	354.80>253.00	0.9897	9.23	90
19	Heptachlor-exo-epoxide	0.5	21.87	352.80>262.90	0.9985	13.69	107
20	Fipronil	0.5	22.08	366.90>212.90	0.9997	13.06	115
21	Allethrin-3,4 (Bioallethrin)	5	22.60	123.10>81.10	0.9990	3.09	111
22	trans-Chlordane	0.5	23.06	372.80>263.90	0.9998	18.96	113
23	o,p'-DDE	0.5	23.31	246.00>176.00	0.9995	5.09	107
24	alpha-Endosulfan	5	23.64	194.90>160.00	0.9942	16.45	109
25	cis-Chlordane	1	23.66	372.80>263.90	0.9998	9.53	117
26	Isoprothiolane	0.5	24.48	290.10>118.00	0.9981	10.61	119
27	p,p'-DDE	0.5	24.73	246.00>176.00	0.9998	6.00	116
28	o,p'-DDD	0.5	24.95	235.00>165.00	1.0000	4.45	115
29	Oxyfluorfen	5	25.14	361.00>300.00	0.9974	18.41	91
30	Dieldrin	5	25.53	262.90>228.00	0.9996	9.39	93
31	beta-Endosulfan	5	25.94	194.90>160.00	0.9980	7.80	96
32	Endrin	5	25.94	262.90>191.00	0.9960	19.51	94
33	p,p'-DDD + o,p-DDT	0.5	26.27	235.00>165.00	0.9996	4.28	118
34	Edifenphos	0.5	27.18	173.00>109.00	0.9977	12.26	119
35	Endosulfan sulfate	0.5	27.25	271.80>236.90	0.9995	16.14	104
36	Iprodione	5	28.74	314.00>245.00	0.9907	6.09	102
37	Bifenthrin	0.5	29.03	181.10>166.10	0.9994	4.56	121
38	Fenpropathrin	0.5	29.27	265.10>210.10	0.9991	10.76	105
39	lambda-Cyhalothrin	5	29.27	181.10>152.10	0.9923	2.12	87

Multi Pesticides Residue analysis in Ayurvedic cough Syrup by GCMS/MS using QuEChERS' extraction method

Table 2 (Continued). Quantitation results

Sr. No.	Name	LOQ (ppb)	Retention time (min)	Target MRM (m/z)	r^2	% RSD (n=6)	% Mean recovery at LOQ
40	Permethrin-1	1	31.53	183.10>168.10	0.9975	13.76	119
41	Permethrin-2	1	31.71	163.10>127.10	0.9996	14.31	88
42	Cyfluthrin-1	5	32.29	163.10>127.10	0.9952	7.11	111
43	Cyfluthrin-2	5	32.56	163.10>127.10	0.9987	2.75	108
44	Cyfluthrin-3	1	32.76	163.10>127.10	0.9965	15.91	96
45	Cyfluthrin-4	5	33.08	163.10>127.10	0.9989	9.94	102
46	Etofenprox	0.5	33.27	163.10>135.10	0.9999	6.51	102
47	Fenvalerate-1	5	34.28	225.10>119.10	0.9980	5.07	106
48	tau-Fluvalinate-1	5	34.54	250.10>200.10	0.9926	10.39	96
49	tau-Fluvalinate-2	5	34.68	250.10>200.10	0.9995	13.52	95
50	Fenvalerate-2	1	34.68	225.10>119.10	0.9985	3.49	115
51	Deltamethrin-1	1	35.41	252.90>93.00	0.9947	16.71	82
52	Deltamethrin-2	5	35.86	252.90>93.00	0.9959	9.28	96

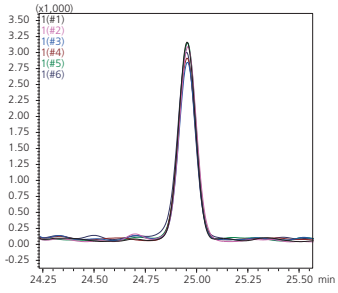
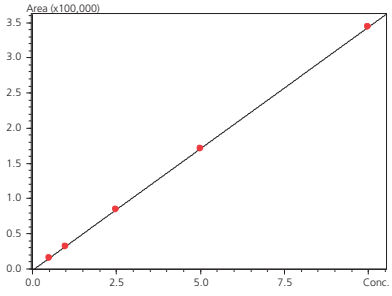
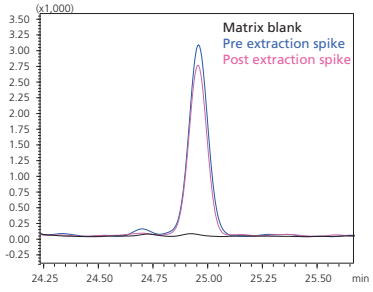
Overlay of precision data	Calibration curve	Recovery overlay
		
% RSD at 0.5 ppb LOQ (n=6)	Linearity (r^2)	% Mean recovery at 0.5 ppb LOQ
4.45	0.9999	115

Figure 4. Detailed data for o,p'-DDD

Table 3. LOQ summary result

LOQ Level (ppb)	0.5	1	5
No. of compounds	20	13	19

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Conclusion

- Smart Pesticide Database feature enabled automatic creation of MRM method with overlapping segments and optimum dwell times for achieving high sensitivity at trace level.
- All the analytes involved in the study showed LOQ less than or equal to 5 ppb.
- The MRM method developed for these pesticides can be used for screening various Ayurvedic syrups.

References

[1] Vaidya Dash, Acarya Manfred, A Handbook of Ayurveda, (1983), 1.
[2] Pesticide Residue in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate (AOAC Official Method 2007.01), (2007), 06.

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