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Determination of multiple pesticide residue in green chilli by GC-MS/MS using modified QuEChERS as an extraction method

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Introduction

Pesticides are widely used in agriculture to increase the yield, improve the quality and extend the storage life of food crops by reducing crop destroying pests. However, if used improperly and excessively, pesticides can have significant adverse effect on health and environment. Pesticide residues are the deposits of pesticide active ingredients, its metabolites or breakdown products on crops, in soil and water. It is necessary to develop a sensitive, selective, accurate and reliable method for analysis of multi pesticide residues in food. Chilli (Figure 1) is most commonly used ingredient in different cuisines around the world.

Organo Chlorine (OC), Organo Phosphorous (OP) and Synthetic Pyrethroid (SP) class of pesticides were extracted from chilli matrix using modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged & Safe) method followed by the analysis using GCMS-TQ8040 Triple Quadrupole (GC-MS/MS) system (Figure 2) from Shimadzu Corporation, Japan.

Figure 1. Green chilli

Methods and Materials

Standard/Sample preparation

- **Preparation of standard stock solution**

OC, OP and SP pesticide standards were procured from Restek® and were diluted using ethyl acetate to prepare stock solution of about 10 ppm standard mixture. From this, working stock of 1 ppm was prepared. It was then used to make matrix match standards for linearity.

- **Preparation of matrix match standard calibration level**

Locally purchased green chilli sample was used as matrix. It was extracted as per the flowchart shown in sample extraction to prepare matrix blank. Further it was spiked with above standard stock solution to prepare matrix match linearity of 1 ppb, 5 ppb, 10 ppb, 24 ppb,48 ppb and 91 ppb.

- **Preparation of pre extraction spike sample**

In order to study the extraction efficiency of the method, recoveries of the pre extraction spiked samples were studied. For this, 15 g of sample was spiked with stock solvent standard to prepare pre extraction spike concentration levels of 10 ppb, 50 ppb and 100 ppb. These samples were extracted, analyzed and quantified against matrix match linearity to study their recoveries.

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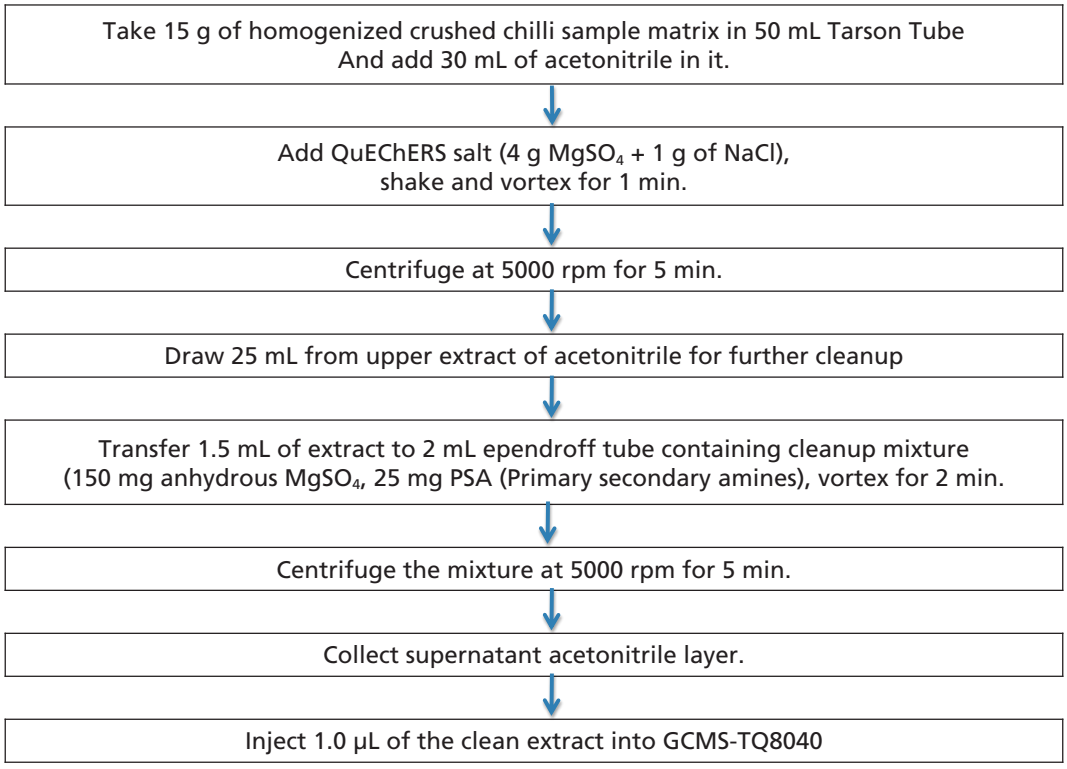
Extraction of pesticides was done using modified QuEChERS method as below.



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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	: Rxi-5Sil MS (30 m L x 0.25 mm I.D. x 0.25 µm)
Injection Mode	: Splitless
Sampling Time	: 1.00 min
Split Ratio	: 5.0
Carrier Gas	: Helium
Flow Control Mode	: Linear Velocity
Linear Velocity	: 47.2 cm/sec
Column Flow	: 1.69 mL/min
Injection Volume	: 1.0 µL
Total Program Time	: 36.50 min
Column Temp. Program	:

Rate (˚C/min)	Temperature (˚C)	Hold time (min)
	50.0	1.00
25.00	125.0	0.00
10.00	300.0	15.00

Mass Spectrometry parameters	
Ion Source Temp.	: 200.0 ˚C
Interface Temp.	: 250.0 ˚C
Ionization Mode	: EI (Electron Ionization)
Acquisition Mode	: MRM
CID Gas	: Argon

MRM Method development

For MRM method creation, Shimadzu's Smart Pesticide Database was used. Pre-optimized MRM transitions present in Smart Pesticide Database and retention times from scan data, enabled creation of MRM with overlapping segments, optimum dwell time (required for high sensitivity). For each component, minimum two MRM transitions were analyzed.

Figure 2. GCMS-TQ8040 Triple quadrupole

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Key Features of GCMS-TQ8040

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.

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

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

Results

Extracted sample matrix was screened using developed MRM method. None of the pesticides involved in this study were detected. Calibration curve was plotted for matrix matched standards in the range of 1 ppb to 91 ppb. Linear response with $r^2 > 0.985$ were obtained. Post-extraction spiked samples at 10 ppb level were analyzed six times and their % RSD was < 14 %.

Recoveries of the pre extraction spike levels were in the range of 70 % to 120 %. The statistical results for all analytes are shown in Table 2.
Quantitative chromatogram and calibration curve for one of the compound Chlorpyrifos (Sr. No. 15 in Table 2) is shown in figure 3.

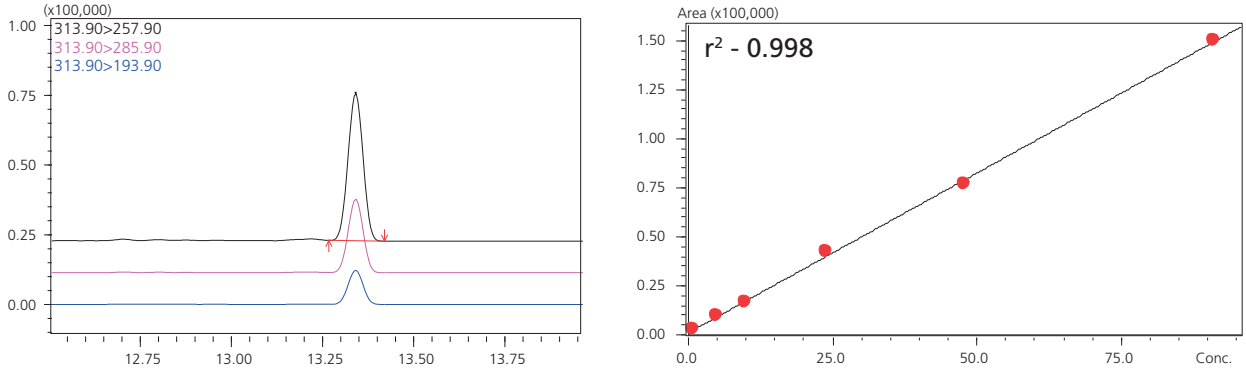

Figure 3 Quantitative chromatogram & calibration curve of Chlorpyrifos

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Table 2 Quantitation result

Sr. No.	Compound name	Retention time (min)	Target MRM (m/z)	% RSD for 10 ppb (n=6)	r^2 (10 - 91 ppb)	% recovery at		
						10 ppb	50 ppb	100 ppb
1	Dichlorvos	5.806	185.00>93.00	13.5	0.997	88	100	94
2	Mevinphos-1 & 2	7.575	192.00>127.00	11.3	0.998	94	99	90
3	Ethoprophos	9.847	200.00>158.00	6.5	0.998	87	87	85
4	Phorate	10.463	260.00>75.00	7.7	0.999	88	89	87
5	alpha-BHC	10.570	218.90>182.90	3.9	0.998	91	97	95
6	beta-BHC	11.093	218.90>182.90	2.2	0.998	105	96	92
7	gamma-BHC (Lindane)	11.249	218.90>182.90	2.9	0.998	95	99	98
8	Diazinon	11.465	304.10>179.10	6.7	0.998	81	90	85
9	Disulfoton	11.672	186.00>153.00	2.8	0.998	84	88	86
10	Tefluthrin	11.748	177.00>127.10	4.3	0.999	84	94	97
11	delta-BHC	11.814	218.90>182.90	4.4	0.998	110	101	101
12	Parathion-methyl	12.525	263.00>109.00	2.8	0.999	82	91	88
13	transfluthrin	12.620	163.00>142.90	4.2	0.999	97	91	91
14	Fenchlorphos	12.749	284.90>269.90	1.5	0.999	88	85	88
15	Chlorpyrifos	13.341	313.90>257.90	3.0	0.998	86	81	83
16	Fenthion	13.417	278.00>109.00	2.8	0.999	85	84	82
17	Aldrin	13.420	262.90>191.00	6.1	0.997	75	82	82
18	Trichloronat	13.415	297.00>269.00	3.7	0.999	78	79	79
19	Heptachlor-exo-epoxide	13.677	352.80>262.90	2.8	0.997	99	90	89
20	trans-Chlordane	14.174	372.80>263.90	1.8	0.998	85	89	90
21	Tetrachlorvinphos	14.628	328.90>109.00	1.7	0.998	114	101	101
22	alpha-Endosulfan	14.704	338.90>160.00	8.9	0.996	98	87	90
23	cis-Chlordane	14.884	372.80>263.90	1.8	0.999	95	87	86
24	Prothiofos	14.881	309.00>238.90	2.8	0.999	78	76	79
25	p,p'-DDE	15.127	246.00>176.00	2.4	0.999	76	77	79
26	Dieldrin	15.296	276.90>241.00	5.8	0.998	73	91	89
27	Endrin	15.403	262.90>193.00	2.7	0.997	109	93	95
28	Fensulfothion	15.799	293.00>153.00	9.2	0.998	76	81	84
29	beta-Endosulfan	15.956	338.90>160.00	7.8	0.999	90	81	86
30	p,p'-DDD	16.000	235.00>165.00	2.8	0.998	90	88	88
31	o,p'-DDT	16.089	235.00>165.00	2.8	0.998	92	89	89
32	Sulprofos	16.089	322.00>156.00	5.5	0.998	82	82	82
33	Endosulfan sulfate	16.377	386.80>288.80	8.6	0.999	104	99	116
34	p,p'-DDT	16.721	235.00>165.00	7.5	0.995	77	68	80
35	Tetramethrin-1	16.796	164.10>77.00	1.2	0.995	103	90	88
36	Endrin ketone	17.569	317.00>281.00	5.1	0.986	73	115	120
37	Bifenthrin	17.601	181.10>166.10	0.9	0.998	80	88	87
38	Tetramethrin-2	17.672	164.10>107.10	3.7	0.995	86	98	94
39	Methoxychlor	17.731	227.10>169.10	6.0	0.995	80	85	88
40	Acrinathrin-1 & 2	17.818	289.10>93.00	6.0	0.999	83	90	93
41	lambda-Cyhalothrin	18.831	163.10>127.00	1.7	0.999	91	84	82
42	Permethrin-1	19.587	183.10>168.10	1.0	0.999	90	82	83
43	Permethrin-2	19.461	183.10>168.10	2.2	0.998	90	84	85
44	Coumaphos	19.588	362.00>109.00	3.2	0.996	100	98	97
45	Cyfluthrin-1	19.597	226.10>206.10	3.4	0.997	81	85	88
46	Cyfluthrin-2	19.995	226.10>206.10	5.9	0.998	89	85	84
47	Cyfluthrin-3 & 4	20.093	226.10>206.10	3.5	0.999	95	91	89
48	Flucythrinate-1	20.160	199.10>157.10	2.2	0.999	91	88	88
49	Flucythrinate-2	20.481	199.10>157.10	2.8	0.998	86	87	86
50	Fenvalerate-1	20.675	419.10>225.10	3.9	0.998	83	86	88
51	tau-Fluvalinate-1	21.204	250.10>200.10	3.7	0.998	80	82	89
52	tau-Fluvalinate-2	21.324	250.10>200.10	7.3	0.998	73	84	84
53	Fenvalerate-2	21.386	419.10>225.10	5.8	0.997	74	83	82
54	Deltamethrin-1	21.408	252.90>93.00	2.0	0.999	90	89	90
55	Deltamethrin-2	21.961	252.90>93.00	3.1	0.997	72	77	82

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Conclusion

- Shimadzu GCMS-TQ8040 with Smart MRM feature was not only able to optimize MRM transitions with ease but also create method with optimum segments leading to increased dwell-time resulting in achieving high sensitivity for trace level quantitation of food contaminants in complex matrix like prawns.
- This optimized extraction and MRM method for these pesticides can be used for screening various food matrices

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