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Determination of chemical contaminants in marine fish by GCMS/MS using QuEChERS as an extraction method

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Introduction

As Persistent Organic Pollutants (POPs) are environmental contaminants with a strong potential for bioaccumulation, these chemicals are expected to be present in farm animals and food products of animal origin.[1] In recent years, the occurrence of various POPs, in the marine environment has received much attention with regards to their effect on human health. Especially in India, marine water is contaminated due to uncontrolled disposal from industries and domestic waste. Hence the fish obtained from it has high risk of contamination. Evidently, it becomes necessary to have sensitive, accurate, reliable, reproducible and fast analytical method to quantify these POPs in marine fish at ppb levels.
Fish is a complex matrix and hence requires selective extraction and extensive cleanup such as QuEChERS (Quick Easy Cheap Effective Rugged Safe) to ensure trace level detection with adequate precision and accuracy. In this study, Shimadzu GCMS-TQ8040 was used in Multiple Reaction Monitoring (MRM) mode to analyze POPs like

Figure 1. Prawns

Organo-Chlorine Pesticides (OCP), Polycyclic Aromatic Hydrocarbons (PAH) and Poly-Chlorinated Biphenyls (PCB).
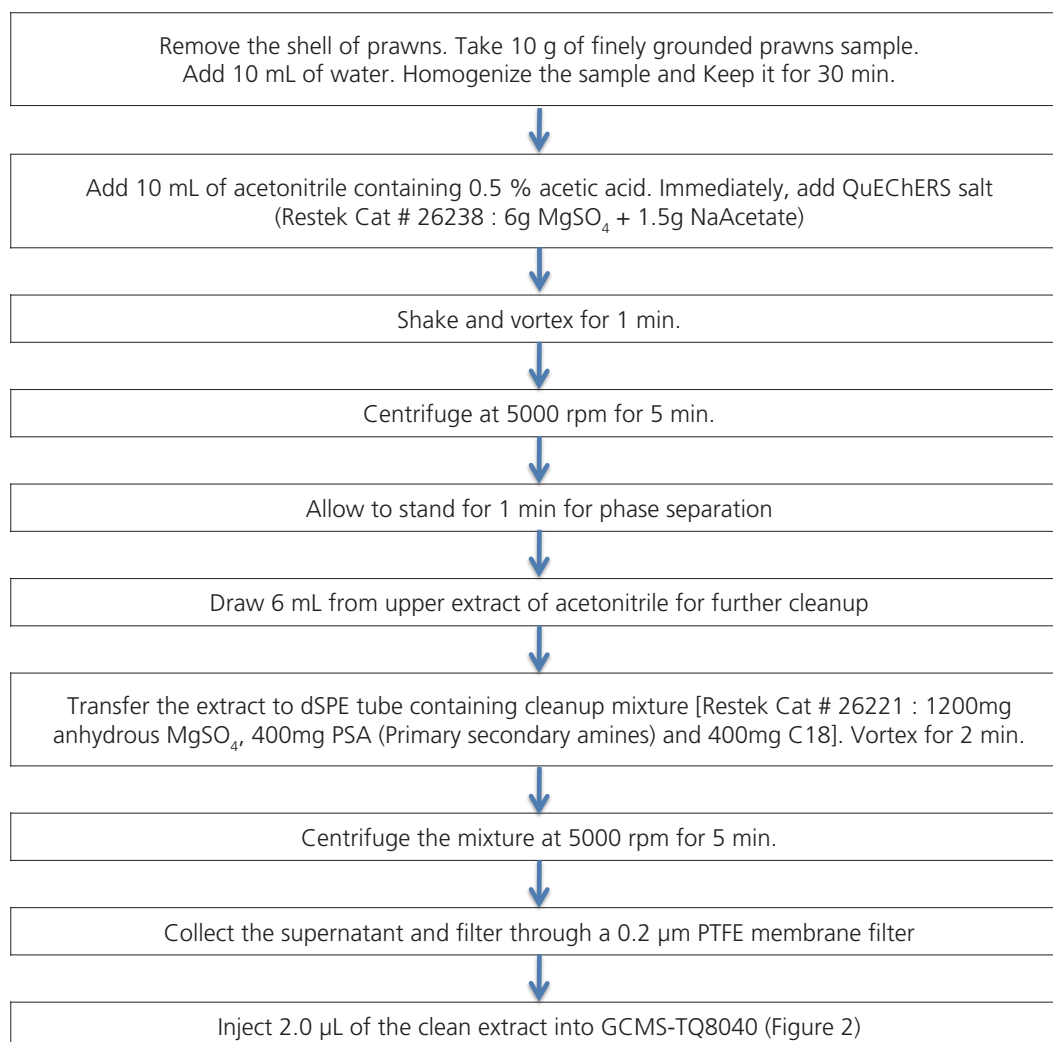Prawns (Figure 1) sample purchased from local market was extracted, spiked and analyzed for obtaining LOD, LOQ, precision and recovery.

Method of analysis

Extraction of pesticides from prawns

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

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Remove the shell of prawns. Take 10 g of finely grounded prawns sample.
Add 10 mL of water. Homogenize the sample and Keep it for 30 min.

Add 10 mL of acetonitrile containing 0.5 % acetic acid. Immediately, add QuEChERS salt
(Restek Cat # 26238 : 6g $MgSO_4$ + 1.5g NaAcetate)

Shake and vortex for 1 min.

Centrifuge at 5000 rpm for 5 min.

Allow to stand for 1 min for phase separation

Draw 6 mL from upper extract of acetonitrile for further cleanup

Transfer the extract to dSPE tube containing cleanup mixture [Restek Cat # 26221 : 1200mg
anhydrous $MgSO_4$, 400mg PSA (Primary secondary amines) and 400mg C18]. Vortex for 2 min.

Centrifuge the mixture at 5000 rpm for 5 min.

Collect the supernatant and filter through a 0.2 μm PTFE membrane filter

Inject 2.0 μL of the clean extract into GCMS-TQ8040 (Figure 2)

MRM method development

Individual mixtures of OCP, PAH and PCB standards were procured from Restek®. For OCP, the MRM transitions existing in Smart Database were used. For PAH, they were obtained from existing application data and imported in Smart Database. In case of PCB, MRMs were optimized. For optimization, about 1 ppm standard mixtures of PCB was analyzed using scan mode. Retention times of individual components were identified and precursor ions were selected. Using selected precursor ion, product ion scan was performed with different Collision Energies (CE). For each component of PCB, MRM transitions with appropriate CEs were determined (Refer Figure 3). All the above steps were simplified with the help of Smart MRM optimization tool. These MRM transitions for PCB were then registered to Smart Database containing OCP and PAH. From this, the final method with optimum segments (Refer Figure 4) and minimum three MRM transitions per compound was generated. The mixture of OCP, PAH and PCB was analyzed using the created method as shown in Table 2 and the MRM chromatogram for the same is shown in Figure 5.

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.

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	: 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	: 1.0 μL
Injector Type	: High Pressure Injection
Total Program Time	: 45.87 min

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

Column Temp. Program :

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

Results

Prawns sample was extracted to prepare matrix blank, which was spiked with various concentration levels of POPs to prepare matrix match or post-extraction spike linearity. Using this linearity parameters like LOD, LOQ, precision were studied. Against this linearity, pre-extraction spike was analyzed to study the recoveries.

Table 2. Method creation using Smart MRM feature of GCMS-TQ8040

Type of compounds	Condition	Intermediate requirement	Step.1	Step.2	Step.3
PCB	No information about transitions	Measure in Scan mode and Determine Pre-cursor ion	**MRM Optimization Tool** Create Batch sequence and Method file of several Collision Energy automatically.	**MRM Optimization Tool** Analyze acquired data files, and select the best transitions and collision energy automatically. And the result can be exported to Smart MRM database on a mouse click.	Method creation using Smart Database
PAH	Known MRM transitions but, Collision energies are not optimized				
OCP	Present in Smart Pesticide Database				

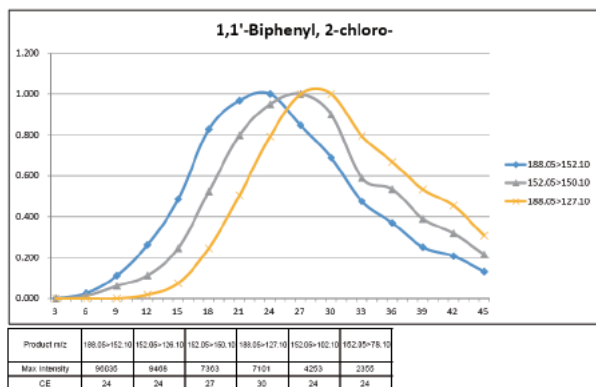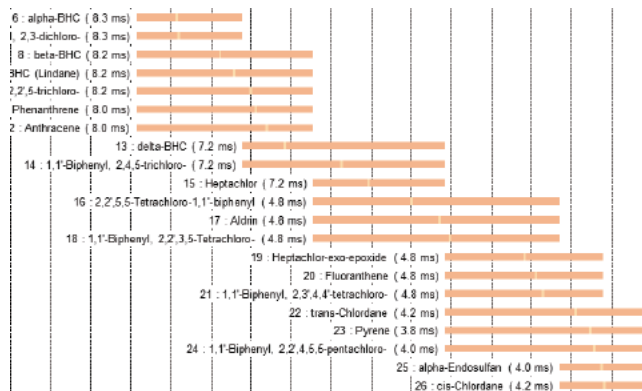Figure 3. CE Optimization using Smart MRM optimization tool

Figure 4. Optimum segmented method created using Smart Database

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Relative Standard Deviation (% RSD) for 5 ppb standard solution (n=5) was less than 15 % for all components. Calibration plot of matrix match standards ranging from 1 ppb to 50 ppb concentration level showed linear response with r2 more than 0.995. Recoveries for the prawns sample spiked with 5 ppb standard mixture were in the range of 70 to 130 %. On the basis of statistical data obtained as shown in Table 3, the method was proved to be highly selective, sensitive and accurate.
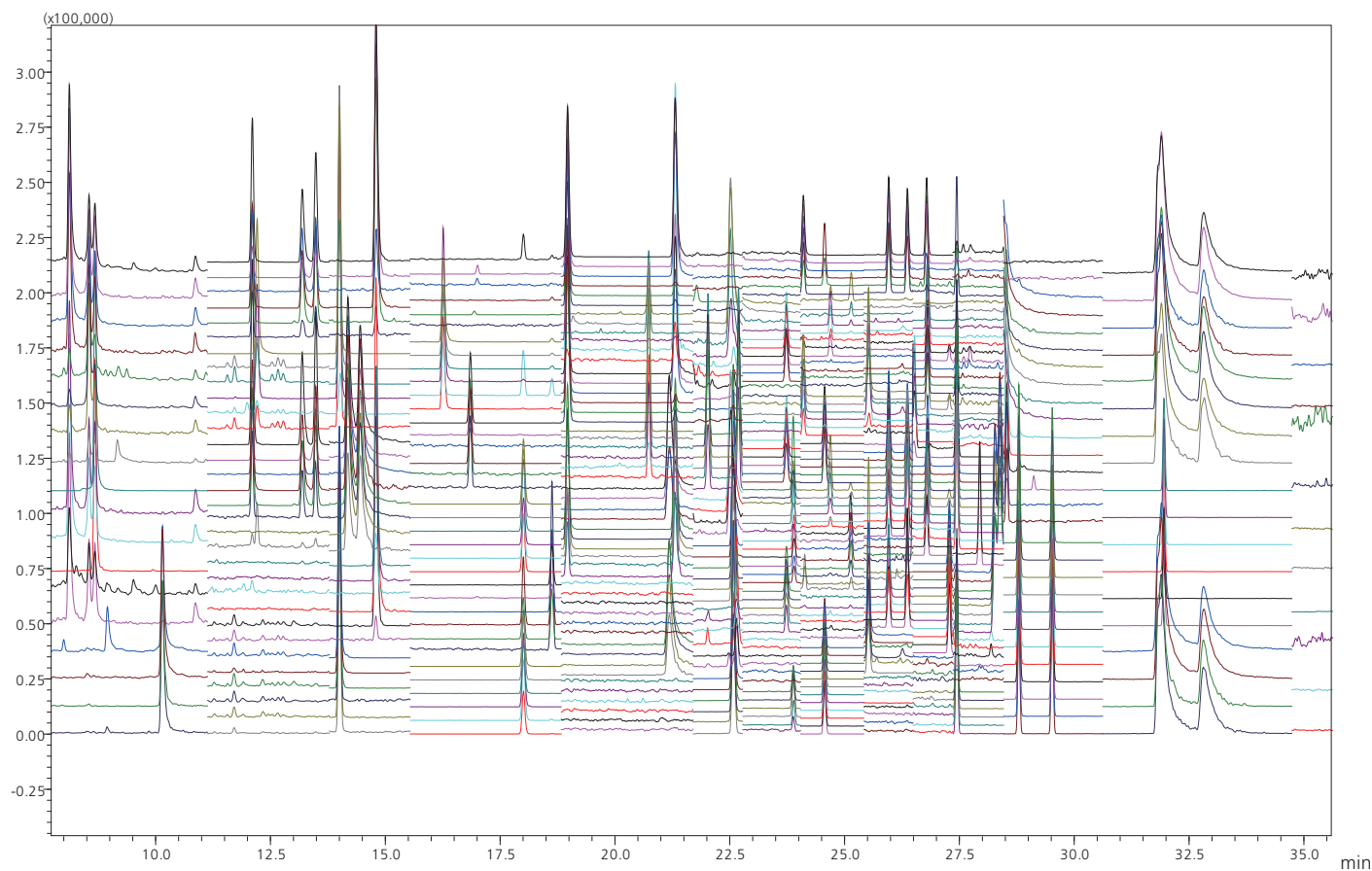

Figure 5. MRM Chromatogram for 10 ppb POPs mixture in matrix

Table 3. Quantitation results

ID	POPs	Retention time (min)	Target MRM (m/z)	r^2	S/N at 5 ppb LOQ level	% RSD at LOQ level (n=5)	% Recovery at LOQ
1	Naphthalene	5.41	128.10>128.10	0.9993	2261.7	3.97	117
2	Acenaphthylene	8.09	152.10>152.10	0.9994	349.37	5.46	94
3	Acenaphthene	8.53	153.10>153.10	0.9999	16.05	1.74	104
4	Fluorene	10.11	166.10>166.10	0.9996	98.46	8.96	96
5	Phenanthrene	14.12	178.10>178.10	1.0000	22.5	3.00	111
6	Anthracene	14.39	178.10>178.10	1.0000	31.09	5.13	95
7	Fluoranthene	21.23	202.10>202.10	1.0000	112.84	5.22	107
8	Pyrene	22.59	202.10>202.10	1.0000	55.95	7.10	102
9	Benz[a]anthracene	28.44	228.10>228.10	0.9950	17.2	4.25	124
10	Chrysene	28.44	228.10>228.10	0.9948	21.66	4.47	131
11	Benzo[k]fluoranthene	32.00	252.10>252.10	0.9999	999.8	4.80	85
12	Benzo[b]fluoranthene	32.01	252.10>252.10	0.9998	303.42	7.99	91
13	Benzo[a]pyrene	32.97	252.10>252.10	0.9993	88.39	9.18	79
14	Indeno[1,2,3-cd]pyrene	37.37	276.10>276.00	0.9996	22.22	7.19	70
15	Dibenz(a,h)anthracene	37.45	278.10>278.10	0.9948	36.15	12.28	94
16	Benzo[g,h,i]Perylene	38.42	276.10>276.10	0.9996	918.4	9.98	70
17	1,1'-Biphenyl, 2-chloro-	8.67	188.05>152.10	0.9998	3181.75	3.25	95
18	1,1'-Biphenyl, 2,3-dichloro-	12.19	222.00>152.10	0.9999	691.78	1.48	92
19	1,1'-Biphenyl, 2,2',5-trichloro-	13.99	255.95>186.00	0.9999	1516.47	1.01	93
20	1,1'-Biphenyl, 2,4,5-trichloro-	16.25	255.95>186.00	0.9999	582.39	2.17	85
21	2,2',5,5-Tetrachloro-1,1'-biphenyl	18.00	289.90>220.00	0.9999	1326.79	2.16	86
22	1,1'-Biphenyl, 2,2',3,5-Tetrachloro-	18.96	289.90>220.00	0.9998	597.52	4.12	87
23	1,1'-Biphenyl, 2,3',4,4'-tetrachloro-	21.29	289.90>219.90	1.0000	117.87	2.57	80
24	1,1'-Biphenyl, 2,2',4,5,5-pentachloro-	22.57	325.90>255.90	0.9999	1487.66	5.86	73
25	1,1'-Biphenyl, 2,2',3,4,5-pentachloro-	23.73	325.90>255.90	0.9998	263.7	6.44	75
26	2,3,3',4,6'-Pentachloro-1,1'-biphenyl	24.09	325.90>255.80	0.9997	303.94	1.99	80
27	2,2',3,5,5',6'-Hexachloro-1,1'-biphenyl	24.56	359.85>289.80	0.9999	635.77	4.58	70
28	alpha-BHC	12.15	180.90>144.90	0.9999	740.26	4.36	106
29	beta-BHC	13.23	180.90>144.90	0.9999	424.32	1.78	113
30	gamma-BHC (Lindane)	13.57	180.90>144.90	0.9999	484.21	3.37	106
31	delta-BHC	14.83	180.90>144.90	0.9997	132.15	4.84	100
32	Heptachlor	16.92	271.80>236.90	1.0000	376.67	7.29	98
33	Aldrin	18.71	262.90>193.00	0.9997	62.23	3.17	78
34	Heptachlor-exo-epoxide	20.82	352.80>262.90	0.9998	132.2	6.57	114
35	trans-Chlordane	22.10	372.80>263.90	0.9999	424.26	7.84	90
36	cis-Chlordane	22.69	372.80>263.90	1.0000	388	7.73	82
37	alpha-Endosulfan	22.74	194.90>160.00	0.9999	11.86	11.71	97
38	Dieldrin*	23.95	262.90>193.00	0.9997	162.75	10.58	78
39	p,p'-DDE	23.99	246.00>176.00	1.0000	205.29	3.00	86
40	Endrin*	24.78	262.90>191.00	1.0000	184.28	8.87	107
41	beta-Endosulfan*	25.22	194.90>160.00	0.9988	24.94	11.52	107
42	p,p'-DDD	25.63	235.00>165.00	1.0000	365.71	4.30	109
43	Endosulfan sulfate	26.61	271.80>236.90	0.9988	183.35	11.75	95
44	p,p'-DDT	26.86	235.00>165.00	1.0000	101.05	5.24	103

Note * : For these compounds LOQ was 10 ppb   Key : RAH PCB OCP

Conclusion

- Shimadzu GCMS-TQ8040 with Smart MRM feature was able to optimize MRM transitions with ease.
- New Smart Database tool creates method with optimum segments leading to increased dwell-time, which resulted in achieving high sensitivity for trace level quantitation of POPs in complex matrix like prawns.
- The MRM method developed for POPs can be used for screening of pesticides in various marine fish products. For 90 % of the POPs, LOQ of 5 ppb was achieved.

References

[1] Weiss, J., Paepke, O. and Bergman, A. 2005. A wordwide survey of polychlorinated dibenzo-p-dioxins, dibenzofurans, and related contaminants in butter. Ambio 34(8):22-30.
[2] Pesticide Residue in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate (AOAC Official Method 2007.01), (2007), 06.

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