

Determination of Chemical Contaminants in Indian prawns by Fast GC-MS/MS using modified QuEChERS as an extraction method

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Introduction

Persistent Organic Pollutants (POPs) like poly-aromatic hydrocarbons (PAH), poly-chlorinated biphenyls (PCB), organochlorines (OC) and agricultural pesticides like organophosphorus (OP) and synthetic pyrethroids (SP) are ubiquitous environmental contaminants due to various anthropogenic activities.

Many of these contaminants in addition to being endocrine disruptors are lipophilic in nature hence capable of bio accumulating in the fatty parts of the organs such as breast milk, blood and fatty tissues. Due to their harmful effects, EU has set strict regulations for these chemicals in all the food products. Since, agricultural runoff, municipality and industrial effluent discharge are the major sources of such contaminants they largely affect the aquatic environment and marine habitat. Hence for marine food products like fish,

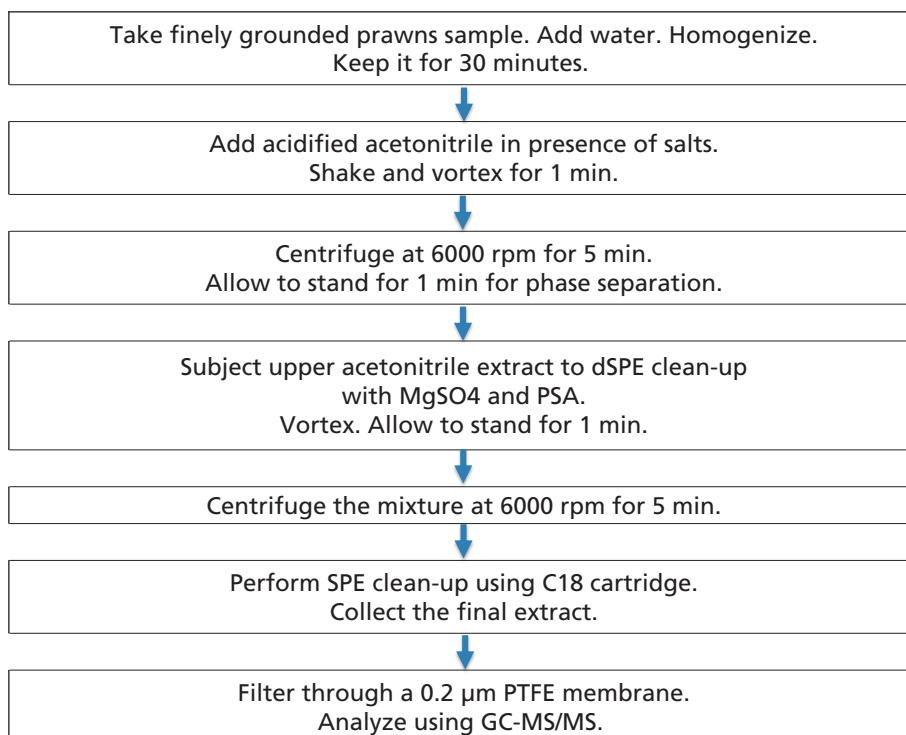
EU-MRLs for most of the contaminants are set as 10 ng/g. Since, in India marine food industry contributes major part of export, it is need of the hour to have validated multi-residue method for qualitative and quantitative monitoring, especially for Indian prawn (*Fenneropenaeus indicus*). Due to inadequate number of approved testing facilities and high number of samples to be tested, sensitive and reliable validated analytical method with high throughput needs to be developed.

This study reports development and validation of a fast method for analysis of 95 analytes (including SP, OC, OP, PAH and PCB) in prawns using GCMS-TQ8040 Triple Quadrupole (GC-MS/MS) system (Figure 1) from Shimadzu Corporation, Japan. Sample preparation method involved QuEChERS extraction, followed by Solid Phase Extraction (SPE) cartridge clean-up.

Methods and Materials

Sample preparation

Extraction of pesticides was done using following method.



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Figure 1. GCMS-TQ8040 Triple quadrupole system by Shimadzu

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.

Method optimization

The fast-GC analysis was carried out using micro-bore column. In such columns, overloading is commonly observed that may lead to many chromatographic issues such as peak merging and distorted peak shapes. This eventually may affect their integration and quantitation. In

order to avoid this, the method was set with optimum split ratio and injection volume as shown in figure 2.

In addition to this, selection of proper target and reference MRM transitions are of utmost importance to remove matrix interference as shown in figure 3.

GC-MS/MS Analytical Conditions

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

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Table 1. Analytical conditions

Chromatographic parameters			
Column	: Rxi™-5Sil MS (10 m L x 0.10 mm I.D. x 0.10 µm)		
Injection Mode	: Split		
Split Ratio	: 4.0		
Carrier Gas	: Helium		
Flow Control Mode	: Linear Velocity		
Linear Velocity	: 44.4 cm/sec		
Column Flow	: 0.5 mL/min		
Injection Volume	: 1.0 µL		
Total Program Time	: 18.00 min		
Column Temp. Program	:		
	Rate (°C/min)	Temperature (°C)	Hold time (min)
		50.0	1.00
	40.00	200.0	0.00
	15.00	280.0	15.00
Mass Spectrometry parameters			
Ion Source Temp.	: 250.0 °C		
Interface Temp.	: 280.0 °C		
Ionization Mode	: EI (Electron Ionization)		
Acquisition Mode	: MRM		
CID Gas	: Argon		

Results

This fast-GC method was able to reduce the run time by almost 2.5 times. The total time saved per analysis can be calculated as shown in Table 2.

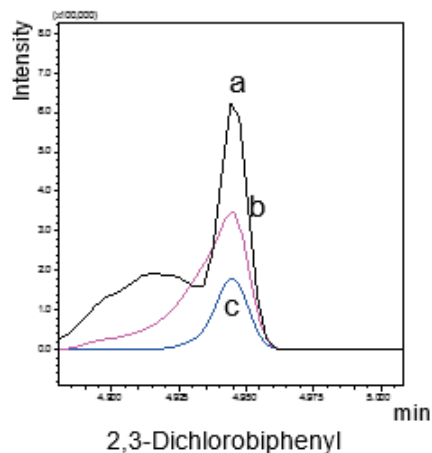
Although the reduction in analysis run time resulted in 10 times sharper peak widths; ultra-fast mass spectrometer was able to obtain sufficient data points showing good repeatability (RSD_r) for all the analytes as depicted graphically in figure 4 and 5.

The results obtained were studied w.r.t. parameters like linearity, specificity, accuracy and precision (repeatability

and reproducibility). Graphical representation of the data for accuracy (Average % recovery), repeatability (RSD_r) and reproducibility (RSD_{wr}) is shown in figure 4 and 5. Among 95 compounds, 60 compounds at 5 ng/mL and 10 compounds at 10 ng/mL level showed recoveries in the range of 70 to 120%.

Out of 95 compounds, 91 showed RSD_r<20% at 5 ng/mL level and remaining 4 compounds showed RSD_r< 20% at 10 ng/mL level.

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a :- Splitless injection @ 1 μ L
b :- Split injection with Split ratio of 1:5 @ 2 μ L
c :- Split injection with Split ratio of 1:4 @ 1 μ L

Figure 2. Overlay of chromatograms indicating effect of sample loading on peak shape

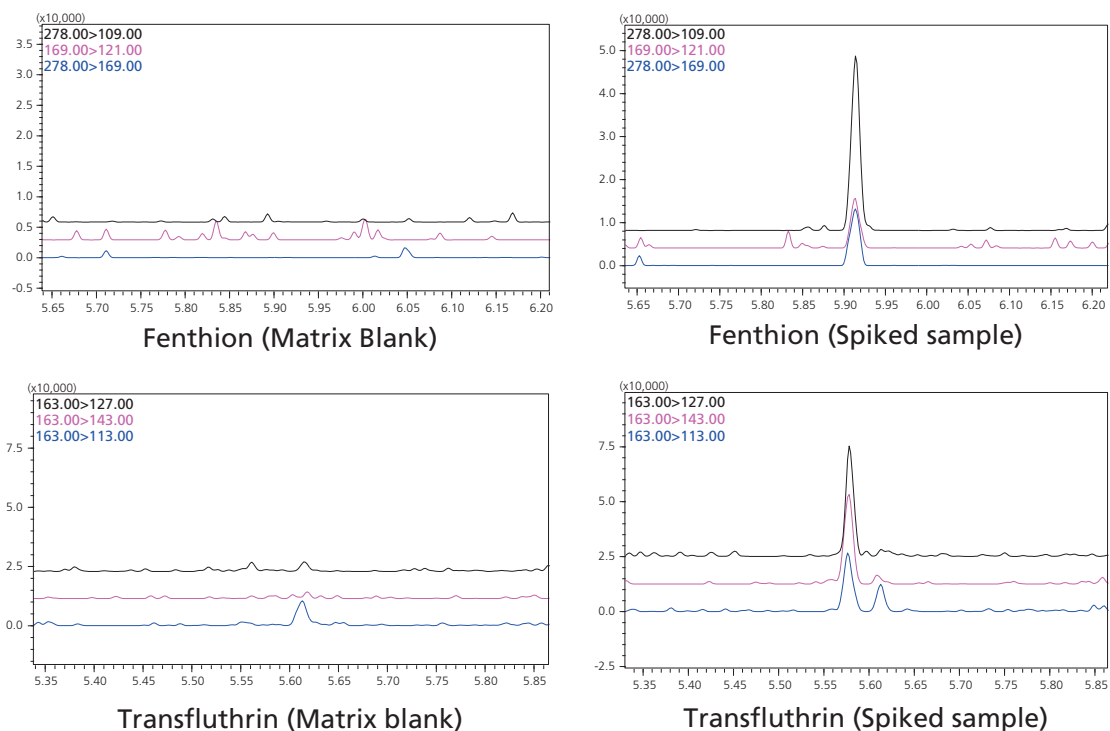


Figure 3. MRM Chromatograms of matrix blank and spiked sample at 5 ng/mL level showing minimum interference

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Table 2. Calculation of sample analysis time

Technique	Sample extraction technique	Time required for extraction (A)	Time taken by GC-MS/MS run (B)	Number of runs required* (C)	Total analysis time per sample = A+ (B × C)
Conventional GC-MS/MS method	Standard QuEChERS	60 min	45 min	12 runs	600 min
Fast GC-MS/MS method	Modified QuEChERS with cartridge clean up.	105 min	18 min	12 runs	321 min
Total time saved per analysis					279 min

* 2 × Blank matrix + 6 Calibration runs + 2 × Blank matrix + Sample in duplicate

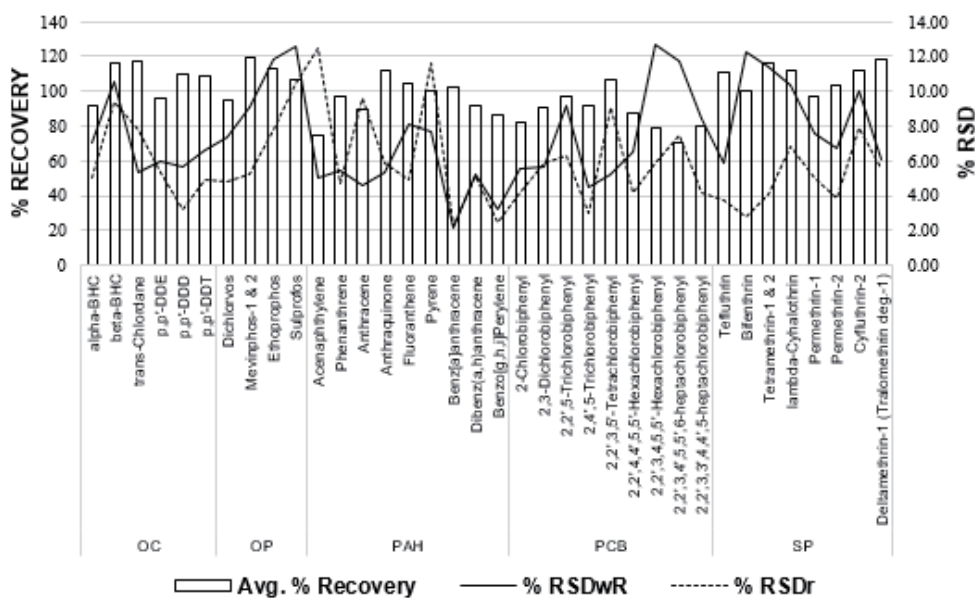


Figure 4. Recovery, repeatability and reproducibility of selected compounds at 5 ng/mL spike recovery study

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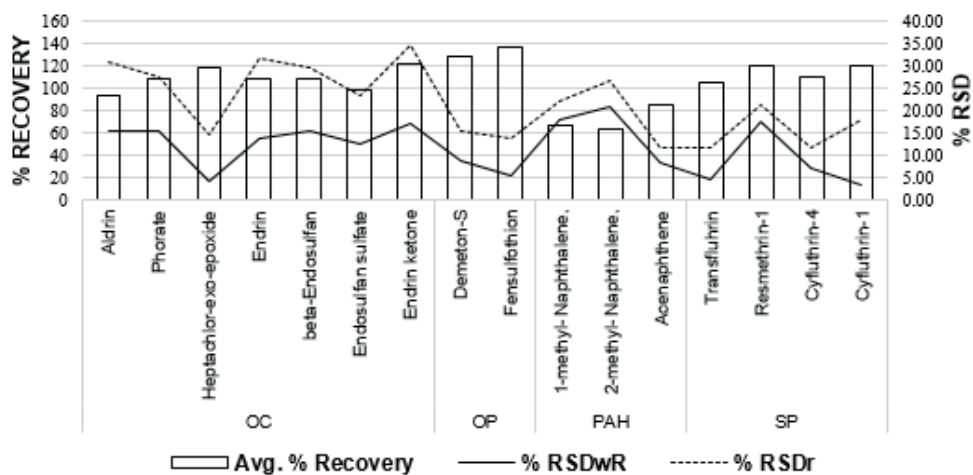


Figure 5. Recovery, repeatability and reproducibility of selected compounds at 10 ng/mL spike recovery study.

Conclusion

- This fast GC-MS/MS method can be easily adopted for testing export samples to EU, as achieved LOQ levels for more than 80% of analytes meet the EU-MRL requirements.
- 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.

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