Chapter 6

Orientation to hazards - Chemical -II (Contaminants & Pesticide)

Dr.Niladri Sekhar Chatterjee, Dr.DevanandUchoi, Dr. Pankaj Kishore, Dr. Ranjit Kumar Nadella, and Dr.Satyen Kumar Panda
Quality Assurance and Management Division
ICAR-Central Institute of Fisheries Technology

Introduction

Recent studies on marine pollution have reported the presence of multiclass organic contaminants in coastal water as a consequence of the diverse range of anthropogenic activities within their watersheds (Munaron et al., 2012, Sapozhnikova et al., 2007). Chemotherapeutants such as certain organophosphate, carbamate and pyrethroid pesticides are being used in coastal aquaculture to manage pest and disease infestations (Rico et al., 2012). In addition, the marine environment is subject to indirect fluxes of pesticides from widespread agricultural use in nearby crops (García-Rodríguez, Cela-Torrijos, Lorenzo-Ferreira, &Carro-Díaz, 2012). Lipophilic organic contaminants of traditional concern such as organochlorine pesticides (OCs) and polycyclic aromatic hydrocarbons (PAHs) have been monitored widely in fish tissue and the marine environment (Sarkar et al., 2008). In addition to OCs and PAHs, several other classes of pesticides are becoming a point of concern because of their potential bioaccumulation in fish tissue (Chen et al., 2009).

Between 2012 and 2013, India exported 928,215 tons of marine produce with a value of $3.5 billion, which increased by 7.68% this year. The marine products from India are mainly exported to South East Asia (23.12% of the total export), the European Union (22.14%), USA (21.29%), Japan (10.61%), China (7.67%) and the Middle East (5.96%) (Source: MPEDA). Food safety regulations are becoming increasingly stringent worldwide. Japan has specified Maximum Residue Limits (MRL) for a diverse range of pesticides and contaminants (http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/MRLs-p). The EU legislation prohibits the presence of pesticide residues in fish and fishery products, although currently there is no specific MRL recommended (ec.europa.eu/sanco_pesticides/public/index.cfm), a default MRL of 10 ppb is applied.

Marine fishes inherently have high lipid content. During sample preparation, these lipid components often get co-extracted and interfere with the detection and quantification of target analytes by GC–MS. Several approaches have been reported to eliminate these matrix interferences, such as methodologies involving liquid–liquid partitioning, gel permeation chromatography, column chromatography, multi-stage cleanup, and low temperature cleanup (LeDoux, 2011). However these methods are time consuming and labor intensive. So far, there is limited literature available on applications of QuEChERS methodology in fish matrices that include analysis of pyrethrins and pyrethroids and a multiresidue method for 13 pesticides in fish muscle (Lazartigues et al., 2011; Rawn, Judge, & Roscoe, 2010). Recently, a QuEChERS based method was reported for the analysis of 13 flame retardants, 18 pesticides, 14 polychlorinated biphenyl (PCB) congeners, 16 polycyclic aromatic hydrocarbons (PAHs), and 7 polybrominated diphenyl ether (PBDE) congeners in catfish muscle, which uses a proprietary

zirconium-based sorbent for dispersive solid-phase extraction (dSPE) cleanup and low pressure GC–MS/MS (gas chromatography tandem mass spectrometry) for analysis (Sapozhnikova&Lehotay, 2013). QuEChERS methodology when evaluated for other high fat matrixes such as milk, egg and avocado reported high matrix interference and low recovery particularly for non-polar compounds (~27% for hexachlorobenzene) (Lehotay, Mastovska, & Yun, 2005; Wilkowska&Biziuk, 2011). Hence, at present, very few sample preparation methods deal effectively with the challenges of simultaneous analysis of a varied group of chemical contaminants in fatty fish matrix. So far, even the QuEChERS based multiresidue strategies have targeted only a limited number of compounds in fish matrix, and high matrix effect and low recoveries have been reported for several analytes (Munaretto et al., 2013; Norli, Christiansen, &Deribe, 2011). The increasing international trade of seafood and marine produces makes it necessary to screen for a wide variety of chemical contaminants in these matrices.

Definition of Pesticides

As per the World Health Organisation (WHO) 1976, Pesticides are defined as any substance or mixture of substances intended for destroying, preventing or controlling any unwanted species of plants and animals and also includes any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant used for the control of pest during production, storage, transport, marketing or processing of food for man or animal or administered to animal for the control of insect or arachnids.

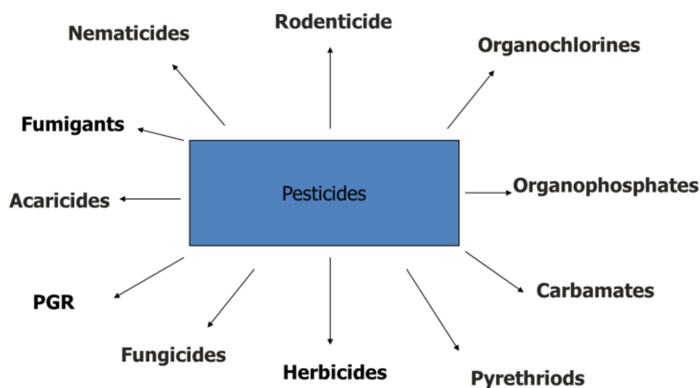

Figure 1. Major types of pesticides

Definition of Pesticide Residue

As per FAO-WHO Codex, "Pesticide residue" means any specified substances in food, agricultural commodities, or animal feed remaining as a result from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance. The term "pesticide residue" includes

residues from unknown or unavoidable sources (e.g., environmental), as well as known uses of the chemical.

Definition of MRL

MRL (Maximum Residues Level) refers to maximum permitted upper limit of a pesticide that may be found in a food or feed commodity. MRLs are not safety limits, and exposure to residues in excess of an MRL does not automatically imply a hazard to health.

Instrumental analysis of PAHs, PCBs, and Pesticides

The analyses of samples are generally performed using a GC equipped with an auto sampler attached to a triple quadrupole mass spectrometer. The analytical separation is performed using a DB-5MS (30 m × 0.25 mm, 0.25 μm) or equivalent capillary column with mid-point back flush set up for the 15 m column towards the injector port end, for which additional helium flow is supplied through a purged ultimate union. A gooseneck liner (78.5 mm × 6.5 mm, 4 mm) needs to be used. The carrier gas (Helium) flow was set at a constant rate of 1.2 mL/min for the first column, and 1.24 mL/min for the second column. The oven temperature program was set at initial temperature of 70 °C (1 min hold), ramped to 150 °C at 25 °C/min (0 min hold), then at 3 °C/min up to 200 °C (0 min hold) and finally to 285 °C at 8 °C/min (9 min hold) resulting in a total run time of 40.49 min. The transfer line temperature was maintained at 285 °C. During a 3 min post-run period, the oven temperature was maintained at 285 °C with the carrier gas flow rate in column 1 set at −3.4 mL/min.

The multi-mode inlet (MMI) is operated in solvent vent mode and 5 μL of sample was injected. The programmable temperature vaporizer (PTV) program was set at the initial temperature of 70 °C (0.07 min hold), raised to 87 °C at 50 °C/min (0.1 min hold) followed by rapid heating at 700 °C/min up to 280 °C (3 min hold). The purge flow to solvent vent was maintained at 50 mL/min, at a pressure of 11.266 psi until 0.17 min after injection. Next, the split vent was closed for 2.7 min to transfer the analytes to the column. Then, the split vent was opened to remove the high boiling matrix compounds from the inlet. The mass spectrometer was operated in MS/MS mode with acquisition starting at 4.4 min. Electron impact ionization (EI+) was achieved at 70 eV and the ion source temperature was set at 280 °C. MRM parameters of each compound have to be optimized.

Sample preparation method

Approximately 2 kg fish meat was separated from bones and skin and crushed thoroughly in a homogenizer. A subsample of 5 g homogenized meat was weighed into a 50 mL centrifuge tube, mixed with 5 mL of distilled water and vortexed for 1 min. Next, 15 mL of acetonitrile (+1% acetic acid) and 2 mL of hexane were added, and the tube was vortexed again for 1 min. Subsequently, 6 g of $MgSO_4$ and 1.5 g of NaAC were added to each tube, followed by vortexing for 2 min and centrifugation at 5000 rpm for 5 min. A portion of the middle organic layer (1.5 mL acetonitrile) was pipetted out of each tube and kept in a 15 mL centrifuge tube at −20 °C for 20 min. Adsorbents (100 mg $CaCl_2$ + 150 mg $MgSO_4$) were added to the tube for dSPE cleanup. The supernatant (1 mL) was further cleaned with 50 mg PSA + 50 mg Florisil + 150 mg C18 + 150 mg $MgSO_4$, vortexed for 1 min and, centrifuged at 10,000 rpm for

5 min. The supernatants from each tube were filtered through a PTFE membrane and analyzed by GC–MS/MS.

Method Validation

The performance of the analytical method was assessed as per the DG-SANCO guidelines for the validation of the analytical methods (Document No. SANCO/10684/2009). The following parameters were considered during the validation process.

Sensitivity

The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantification (LOQ) of the test compounds. The LOD was determined by considering a signal to noise ratio (S/N) of 3 with reference to the background noise obtained for an unspiked matrix blank. LOQs were determined by considering a S/N of 10 with the qualifier SRM having S/N $\geqslant$ 3:1.

Matrix effect (ME)

The ME was evaluated by comparing peak areas of the matrix matched standards (peak area of post-extraction spike) with the corresponding peak areas of standards in solvent at 25 μg/kg in ten replicates. The ME was quantified as the average percent suppression or enhancement in the peak area using the following equation:

$$\mathrm{ME}\ (\%) = \frac{\text{Peak area of matrix matched standard} - \text{peak area of solvent standard}) \times 100}{\text{Peak area of matrix matched standard}}$$

A negative value of ME signifies matrix induced signal suppression, whereas a positive value signifies an enhancement in signal intensity.

Accuracy-recovery experiments

The recovery experiments were carried out by spiking the homogenized fish meat (5 g) in six replicates with the test analytes under study at three concentration levels: 10, 25 and 50 μg/kg. These samples were processed following the optimized protocol and analyzed using GC–MS/MS. The quantification was performed using external calibration standards (matrix matched).

Precision

The precision in the conditions of repeatability (three different analysts prepared six samples each on a single day) and the intermediate precision (a single analyst prepared six samples each on three different days) were estimated separately at 25 μg/kg. Precision was expressed as the ratio of the reproducibility standard deviation (RSDR) to the predicted relative reproducibility standard deviation (PRSDR) and repeatability standard deviation (RSDr) to the predicted repeatability standard deviation (PRSDr) for the assessment of reproducibility and repeatability, respectively. According to Horwitz, the ratio between the calculated and the predicted values should be $\leqslant$2 (known as the HorRat value) (Horwitz & Albert, 2006). This is also applicable for the Thompson equation which suggests that at concentration below 120 μg/kg, PRSDR = 22.0 and PRSDr = 0.66 PRSDR. The Thompson equation is

claimed to be better able to account for the precision at an analyte concentration below120 μg/kg and hence in this study, the Thompson equation was followed (Thompson, 2000).

Assessment of uncertainty

The combined uncertainty was assessed as per the statistical procedure described in EURACHEM/CITAC Guide CG 4 in the same way as reported earlier (http://www.measurementuncertainty.org). The following variables were evaluated for all the test compounds: uncertainty associated with the calibration graph (u1), day-wise uncertainty associated with precision (u2), analyst-wise uncertainty associated with precision (u3), day-wise uncertainty associated with accuracy/bias (u4), and analyst-wise uncertainty associated with accuracy/bias (u5). The combined uncertainty (U) was calculated as follows:

$$U = \sqrt{u_1^2 + u_2^2 + u_3^2 + u_4^2 + u_5^2}$$

The combined uncertainty (U) was reported in relative measures as expanded uncertainty, which is twice the value of the combined uncertainty. Relative uncertainty represents the ratio of uncertainty value at a given concentration to the concentration at which the uncertainty is calculated.

Conclusion

Chemical hazards in seafood are highly important from trade, health & safety perspectives. Regulatory agencies all over the world stringently monitor residues of pesticides and persistent organic pollutants (POPs) in different food commodities. Presence of residue often results in economic loss for the exporting country. Hence it is important to develop rugged analytical methods for regulatory control and monitoring of these contaminants in seafood. Multiresidue methods can save time and money by simultaneously analyzing hundreds of compounds in a single method. However, some specific pesticides like Glyphosate, 2, 4-D, Paraquat, Diquat etc. are difficult to measure in multiresidue methods and for them single residue methods should be developed.

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