SAARC J. Agri., 16(1): 81-93 (2018) *DOI: http://dx.doi.org/10.3329/sja.v16i1.37425*

DETERMINATION OF MULTIPLE ORGANOCHLORINE PESTICIDE RESIDUES IN SHRIMP USING MODIFIED QuEChERS EXTRACTION AND GAS CHROMATOGRAPHY

M.D.H. Prodhan[*] and S.N. Alam

Pesticide Analytical Laboratory, Entomology Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh

ABSTRACT

Determination of organochlorine pesticide residues in shrimp is very important to ensure the consumer's safety and to fulfill the importer's demand. Therefore, a simple and efficient multiple organochlorine pesticide residues analytical method using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique and Gas Chromatography coupled with Electron Capture Detector (ECD) has been developed and validated for the determination of 19 organochlorine pesticides (α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) in shrimp. The method was validated by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). The average recoveries of the selected pesticides ranged from 84% to 106% with RSDr ≤ 14% in four fortification levels of 0.05, 0.1, 0.2 and 0.3 mg kg^{-1}. The linearity was ≥ 0.996 for all of the selected pesticides with matrix matched calibration standards. The LOD ranged from 0.003 to 0.009 mg kg^{-1} and the LOQ was 0.05 mg kg^{-1}. This method was applied successfully for the residue analysis of 40 shrimp samples collected from different regions in Bangladesh.

Keywords: Shrimp, organochlorine pesticide residues, modified QuEChERS extraction, GC-ECD, method validation.

[*] Corresponding author email: mdhprodhan@gmail.com

Received: 07.02.2018 *Accepted: 14.05.2018*

INTRODUCTION

Fish play a crucial role in the Bangladeshi diet, providing more than 60% of animal source food, representing a crucial source of micro-nutrients, and possessing an extremely strong cultural attachment. Fish (including shrimp and prawn) is the second most valuable agricultural crop in Bangladesh. The culture and consumption of fish therefore has important implications for national food and nutrition security, poverty and growth (Belton et al., 2011). The prime source of high-quality protein is fish, which provides 14–16% of the animal protein consumed worldwide. Over one billion people across the world consume fish as their primary source of animal protein (Helfman et al., 1997). Thus fish either harvested from natural source (s) or cultured artificially and the fish products have great importance as human food worldwide. The fisheries sector especially shrimp production in Bangladesh plays a significant role in providing employment to rural poor, reducing poverty and enhancing export earning. The sector annually contributes 544 million US Dollar to the national economy. The shrimp industry also provides direct employment to over 1 million people. Increasingly stringent standards for food safety rules are being adopted by EU, USA and Japan, the three main importers of shrimp form Bangladesh. Kaphalia et al. (1990) reported that the majority of people were indirect consumers of pesticides through food intake.

In Bangladesh, shrimp production is linked with rice cultivation. For the cultivation of rice, the farmers of our country are using pesticides mostly belonging to organocarbamate, organophosphate and synthetic pyrethroid pesticides. In the long past organochlorine pesticides (OCPs) like endrin were used in rice while other OCPs were used legally in other crops until 1977 when the last OC insecticide heptachlor was banned. The OCPs are lipophilic in nature, their hydrophobicity, low chemical and biological degradation rates have led to their widespread accumulation in food chain (John et al., 2001; Bedi et al., 2005; Aulakh et al., 2006). The exposure of OCPs in humans creates severe health hazards particularly breast cancer, testicular cancer, endocrine dysfunction, births defects, lower sperm count (Brody and Rudel, 2003; Ahmed et al., 1996; Garry et al., 2004; Soto et al., 1998).

Most of the organochlorine pesticides (OCPs) were banned in 1970s for their long persistence in the environment (Annonymous, 1979 and Annonymous, 1989). But because of their long persistence, OCPs are still detectable in fish from various waterways (Zhang et al., 2014; Prodhan et al., 2010; Prodhan et al., 2009; Kaur et al., 2008; Antunes and Gil 2004; Osuna-Flores and Riva, 2001; Chan et al., 1999; Berg et al., 1999; Sapozhnikova et al., 2004). Although not as persistent in the environment as OC pesticides, many pesticides of the other three groups are also suspected to be present in fish samples. As required by importing countries as well as for our own need it is important to know the pesticide residue status in shrimp.

In order to detect and quantify pesticide residues quickly and easily, multi-residue methods are required. The multi-residue methods used for the analysis of pesticide residues should be validated prior to analyze the samples. In the analysis of pesticide residues, effective extraction and clean-up techniques are essential. Nowadays, the quick, easy, cheap, effective, rugged and safe (QuEChERS) technique, which was first introduced by Anastassiades et al., 2003, is widely used for the extraction and clean-up of food matrices (Anastassiades et al., 2003). Therefore, the QuEChERS extraction techniques followed by GC-ECD were chosen for the determination of OCPs in shrimp. Up until now, few multi-residue methods were developed for the determination of OCPs in shrimp (Osuna-Flores and Riva, 2001; Zhang et al., 2014). However, in this developed method we have incorporated a large number of OCPs that were not incorporated with the previously developed methods. With this view, the present study was initiated to develop and validate an analytical method for the determination of 19 organochlorine pesticide residues in shrimp and to monitor OCPs residues status in shrimp in Bangladesh.

MATERIALS AND METHODS

Chemicals and reagents

Reference standards of Organochlorine Pesicide Mix (α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) were obtained from SIGMA-Aldrich, Germany through SF Scientific, Dhaka, Bangladesh.

Analytical grade Acetonitrile (MeCN), methanol, Sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$) and Primary Secondary Amine (PSA) were also obtained from SIGMA-Aldrich, Germany through SF Scientific, Dhaka, Bangladesh.

Sample preparation procedures

The quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique, which was first introduced by Anastassiades et al. (2003), is widely used for the extraction and cleanup of food matrices. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method modified by Prodhan MDH et al. (2015) was used for the extraction and clean-up of organochlorine pesticide residues from shrimp matrix. The method is described below:

Ten gm of properly homogenized eggplant sample was taken in a 50ml screw-capped polypropylene centrifuge tube and 10 ml acetonitrile (MeCN) was added into the centrifuge tube. The centrifuge tube was closed properly and shaken vigorously for 30 sec. by vortex mixer. Then 4g anhydrous $MgSO_4$, 1g NaCl were added into the centrifuge tube and it was shaken by vortex mixer for 1 minute. Afterwards, the

extract was centrifuged for 5 min at 5000 rpm. An aliquot of 3 ml of the MeCN layer was transferred into a 15 ml micro centrifuge tube containing 600 mg anhydrous $MgSO_4$ and 120 mg Primary Secondary Amine (PSA). The content of the centrifuge tube was thoroughly mixed by vortex for 30 sec. and centrifuged for 5 minutes at 4000 rpm. After centrifuge, a 1 ml supernatant was filtered by a 0.2 µm PTFE filter, and then it was taken in a clean HPLC vial for injection.

Preparation of pesticide standard solution

Mixed pesticide standard stock solutions of α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone were prepared in hexane: toluene (50:50) at a concentration of 200 mg l^{-1} and stored at -20^{0}C until use. An intermediate mixed standard solution of 10 mg l^{-1} in acetone was prepared from the mixed standard solution of 200 mg l^{-1}. Then working standard solutions of 0.5, 1.0, 2.0, 3.0, and 5.0 mg l^{-1} in acetonitrile were prepared by transferring the appropriate amount from 10 mg l^{-1} intermediate mixed standard solution into five separate 5-ml volumetric flasks.

Preparation of matrix matched calibration standard solution

Matrix matched calibration standards were prepared by adding 100 µl of the mixed pesticide standards working solutions of 0.5, 1.0, 2.0, 3.0, and 5.0 mg l^{-1} and 900 µl of the blank extract to reach the final concentrations of 0.05, 0.10, 0.2, 0.3 and 0.5 mg l^{-1}, respectively. Calibration standards in acetonitrile having the same concentrations as in the matrix matched calibration standards were also prepared. All the standard solutions were kept in a freezer at -20^{0}C until use. A typical chromatogram containing 19 organochlorine pesticides prepared with matrix-matched calibration standard is presented in figure 1.

Operating condition of GC

A Gas Chromatograph (GC-2010 Shimadzu) coupled with Electron Capture detector (GC-ECD) was used for the identification and quantification of selected organochlorine pesticides. Separations were done by RTX-CL capillary column (30 m long, 0.25 mm i.d. and 0.25 µm film thicknesses), nitrogen was used as carrier (column flow 1.5 ml/min.) and make up gas as well. The injector and detector temperatures were set to 250 °C and 330 °C, respectively and the column oven temperature was programmed, which was started from 180 °C and went up to 220°C with incremental rate of 5 °C (12 min hold), then it raised to 260°C with incremental rate of 5 °C. All the injections (1 µl) were done in spit mode. The total run time was 28 min. Identification of the analyte in the samples was done by comparing the retention time of the corresponding matrix matched calibration standard and quantification was done by external calibration curves maid with 5 point matrix matched calibration standard.

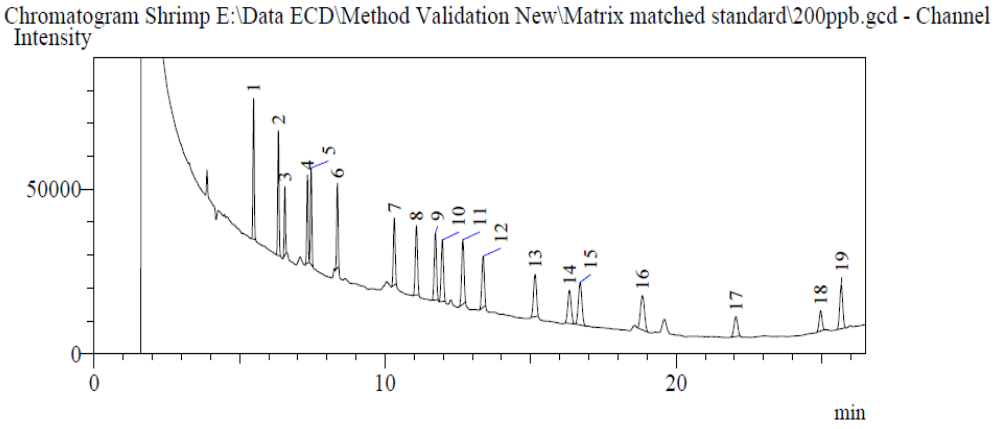

Figure 1. GC-ECD chromatogram of matrix matched standard of organochlorine pesticides in shrimp matrix: 1) α- BHC, 2) δ- BHC, 3) β- BHC, 4) γ- BHC, 5) Heptachlor, 6) Aldrin, 7) Heptachlor Epoxide, 8) γ- Chlordane, 9) α- Chlordane, 10) α- Endosulfan, 11) 4,4 DDE, 12) Dieldrin, 13) Endrin, 14) 4,4 DDD, 15) β- Endosulfan, 16) 4,4 DDT, 17) Endosulfan sulphate, 18) Methoxychlor, and 19) Endrin Ketone.

Method validation

The method was validated by evaluating the accuracy, precision, linearity, limit of detection and the limit of quantification.

Accuracy and precision

The accuracy of the method was calculated as percent recovery of pesticides from spiked samples. A 10-g homogenized sample was spiked prior to the extraction procedure by the addition of a mixed pesticide standard working solution to reach the final fortification levels of 0.05, 0.10, 0.20 and 0.30 mg kg^{-1}. For each level of fortification, five replicates were analyzed. After fortification, the sample was equilibrated by shaking and then allowed to settle for 30 min prior to the extraction procedures in order to ensure the sufficient contact of the analytes with the whole matrix. Then, the samples were prepared according to the method described earlier. Precision in case of repeatability (RSD_r) was determined at four fortification levels of 0.05, 0.10, 0.20 and 0.30 mg kg^{-1} with 5 replicates on the same day. Precision in case of Reproducibility (RSD_R) was determined at two fortification levels of 0.05 and 0.20 mg kg^{-1} with 5 replicates during a period of 2 months interval.

Limit of detection (LOD) and limit of quantification (LOQ)

The Limit of Detection (LOD) was calculated according to EURACHEM guidelines (EURACHEM 1998). In order to determine the LOD of each analyte 10 independent blank samples fortified at the lowest acceptable concentration of 0.05 mg kg^{-1} were processed and the LOD was expressed as the analyte concentration corresponding to 3 times the standard deviation. LOQ was determined according to the European Commission (EC) document number SANTE/11945/2015 (European commission 2015). LOQ was set as the lowest fortification level for each pesticide giving an acceptable accuracy (mean recoveries for individual pesticides being in the range of 70- 120%) and precision (RSD r $\leq$20%).

RESULTS

Method validation

Accuracy and precision

A very good accuracy and precision was found for all of the analytes at four fortification levels of 0.05, 0.10 0.2, and 0.30 mg kg^{-1}. The average recoveries ranged from 84 to 106% with relative standard deviations (RSD_r) $\leq$ 14% for all of the analytes (Table 1). Reproducibility (Interday accuracy and precision) was determined at two fortification levels of 0.05 and 0.20 mg kg^{-1} with 5 replicates. A very good accuracy and precision was also found. The average recoveries ranged from 85.92 to 104.63% and RSD_R were $\leq$ 5 % for all of the analytes (Table 2).

Calibration curve and linearity

Five point calibration curves were prepared by matrix matched standards and analyzed in triplicate. Calibration curves were made by plotting the mean peak area of the selected pesticides versus concentration. Linearity was evaluated by calculating the correlation coefficient, intercept and slope of the regression line. Linearity was very good and coefficients of determination were $\geq$ 0.996 for all of the selected pesticides with matrix matched calibration standards. The correlation coefficients for all of the selected pesticides are summarized in table 3.

Table 1. Mean recovery (%) and RSD (%) of the selected pesticides in shrimp matrix at different fortification levels

Name of Pesticide	Fortification level							
	0.05 mg kg^{-1}		0.1 mg kg^{-1}		0.2 mg kg^{-1}		0.3 mg kg^{-1}	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
α- BHC	98.86	3.52	96.44	4.90	89.64	6.01	90.25	2.59
δ- BHC	88.31	3.40	94.12	3.86	89.63	6.75	90.08	2.74
β- BHC	103.85	4.62	95.22	3.88	89.34	5.50	90.97	3.58
γ- BHC	102.68	2.98	84.07	5.71	87.42	11.33	88.52	4.78
Heptachlor	90.90	4.44	96.85	4.13	90.33	4.31	89.79	2.61
Aldrin	95.60	4.36	94.12	3.86	86.18	4.53	85.78	4.02
Heptachlor Epoxide	100.36	2.19	96.61	2.46	93.67	6.48	90.61	3.21
γ- Chlordane	90.66	6.60	99.07	2.07	93.01	3.87	90.13	3.35
α- Chlordane	98.32	3.32	98.32	3.32	88.75	4.83	90.35	3.62
α- Endosulfan	97.04	11.93	94.59	3.79	90.65	4.78	90.05	3.55
4,4 DDE	86.24	4.51	98.73	3.84	90.47	3.84	90.20	3.90
Dieldrin	103.12	9.52	87.20	4.12	88.32	5.85	89.34	3.52
Endrin	84.01	5.79	96.57	6.02	89.81	2.94	90.39	3.70
4,4 DDD	102.76	8.05	100.95	5.69	88.68	6.39	91.80	2.74
β- Endosulfan	106.16	10.29	99.79	5.60	89.75	3.62	90.93	3.40
4,4 DDT	97.40	13.68	99.22	8.30	89.23	5.16	88.12	3.44
Endosulfan sulphate	90.51	10.35	96.90	3.37	86.91	14.27	86.83	7.38
Methoxychlor	98.78	13.16	100.55	10.45	94.55	5.16	89.54	3.47
Endrin ketone	94.45	6.77	95.54	4.25	89.08	4.93	90.23	4.27

Table 2. Interday accuracy [Mean Recovery (%)] and precision [RSD_R (%)] of the selected pesticides in shrimp matrix at different fortification levels at different days

Pesticides	Fortification level			
	0.05 mg kg^{-1}		0.2 mg kg^{-1}	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)
α- BHC	99.25	0.78	91.06	1.65
δ- BHC	93.80	2.28	91.56	1.07
β- BHC	102.78	1.14	94.15	2.44
γ- BHC	100.59	1.24	93.10	1.38
Heptachlor	96.31	2.10	95.82	1.00
Aldrin	99.01	1.20	90.00	1.09
Heptachlor Epoxide	99.25	1.55	101.12	0.77

Pesticides	Fortification level			
	0.05 mg kg^{-1}		0.2 mg kg^{-1}	
	Mean (%)	RSD (%)	Mean (%)	RSD (%)
γ- Chlordane	98.76	1.16	100.62	0.70
α- Chlordane	104.63	3.15	94.30	1.76
α- Endosulfan	98.83	1.91	99.13	1.21
4,4 DDE	94.49	2.95	98.45	0.55
Dieldrin	101.34	0.67	93.97	4.14
Endrin	85.92	3.25	97.59	1.04
4,4 DDD	100.51	1.02	94.89	1.24
β- Endosulfan	100.90	2.55	98.49	1.00
4,4 DDT	98.25	2.30	98.19	1.25
Endosulfan sulphate	98.67	2.06	92.13	2.50
Methoxychlor	97.39	2.37	103.49	1.40
Endrin ketone	98.28	4.45	99.24	1.65

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) of each analyte is presented in table 3. The LOD ranged from 0.003 to 0.009 mg kg^{-1}. The Limit of Quantification (LOQ) for all of the selected pesticides was set to 0.05 mg kg^{-1}which was achieved the acceptable accuracy (mean recoveries for individual pesticides in the range of 84% to 106% and precision (RSD r ≤14%).

Application of the method for real sample analysis

The proposed method was used for the analysis of shrimp samples collected from different market places in Bangladesh. A total of 40 samples were analyzed. Among the analyzed samples 38 (95% of the total no. of samples) contained no detectable residues of the pesticides sought and 2 (5% of the total no. of samples) had pesticides residues. None of the sample was found contaminated at a level above the EU-MRLs (European commission 2005). The detected pesticide was 4, 4 DDT. The ranges of the detected residues were 0.057-0.95 mg kg^{-1}.

DISCUSSION

The described method in this study is an efficient and effective multi-residue analytical method using Gas Chromatography coupled with Electron Capture Detector (GC-ECD) for the determination of 19 organochlorine pesticide residues in shrimp. A very good accuracy and precision was found for all of the analytes using this proposed method.

Table 3. Retention Time (RT), Limit of Detection (LOD), Limit of Quantification (LOQ) and Coefficient of determination (R^2) of the selected pesticides for Shrimp matrix

Pesticides	RT	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	R^2
α- BHC	5.48	0.003		0.996
δ- BHC	6.33	0.007		0.997
β- BHC	6.55	0.005		0.996
γ- BHC	7.33	0.009		0.997
Heptachlor	7.46	0.005		0.998
Aldrin	8.36	0.004		0.999
Heptachlor Epoxide	10.31	0.006		0.999
γ- Chlordane	11.07	0.007		0.9998
α- Chlordane	11.73	0.005		0.997
α- Endosulfan	11.97	0.007		0.999
4,4 DDE	12.67	0.008		0.999
Dieldrin	13.37	0.006		0.998
Endrin	15.15	0.007		0.998
4,4 DDD	16.33	0.006		0.998
β- Endosulfan	16.69	0.004		0.996
4,4 DDT	18.83	0.009		0.999
Endosulfan sulphate	22.05	0.008		0.999
Methoxychlor	24.96	0.007		0.999
Endrin ketone	25.67	0.004	0.05	0.999

The average recoveries ranged from 84 to 106% with $RSD_r \leq 14\%$ and $RSD_R \leq 5\%$, thus fulfilling the requirement set by SANTE document no. SANTE/11945/2015 for accuracy and precision (European commission 2015). Ninteen organochlorine pesticides (α- BHC, δ- BHC, β- BHC, γ- BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ- Chlordane, α- Chlordane, α- Endosulfan, 4,4 DDE, Dieldrin, Endrin, 4,4 DDD, β- Endosulfan, 4,4 DDT, Endosulfan sulphate, Methoxychlor, and Endrin Ketone) were incorporated in this method that helps the scientist/ analysts for quick determination of multiple pesticide residues in shrimp. In addition, this analytical method was applied successfully to monitor the selected organochlorine pesticide residues in 40 shrimp samples collected from different regions of Bangladesh. Among the analyzed samples, 2 (5% of the total no. of samples) had pesticides residues. None of the sample was found contaminated at a level above the EU-MRLs (European commission 2005). The detected pesticide was 4, 4 DDT. The ranges of

the detected residues were 0.057-0.95 mg kg^{-1}. Thus the proposed method can be used successfully to monitor multiple organochlorine pesticide residues in shrimp.

The findings of the present study are in a good agreement with the observation of Sankar et al. (2006). They have collected fish from five different locations from the Caligut region, India and analyzed for the quantification of organochlorine (OC) insecticides and heavy metal (HM) residues. The highest concentrations of OC insecticides detected in the edible portion of fish were 10.47, 70.57 and 28.35 ng g^{-1} in marine, brackish water and freshwater, respectively. BHC and heptachlor epoxide formed the major share of OC insecticides in the marine fish while BHCs contributed to the major share in the freshwater and brackish water fish. The DDT ranged from 0.05 to 80 ng g^{-1} in the samples irrespective of the habitat. The concentrations of OC insecticides and HMs in the samples, in general, were below the EU-MRLs (European Commission, 2005).

Battu et al. (1984) have detected OC insecticides in fresh water fish in Ludhiana, India and residues of both DDT and HCH in all the samples with the maximum levels of DDT at 3.02 mg kg^{-1}, while Kannan et al. (1992) reported mean levels of HCH and DDT at 0.002 and 0.015 mg kg^{-1}, respectively in fish. In Pakistan, Saqqib et al. (2005) have detected DDE, aldrin and dieldrin residues in fish tissues while Jabber et al. (2001) reported DDT, aldrin, dieldrin, lindane and heptachlor in different organs of fish (muscle, liver, gut and egg samples) in Bangladesh. Among the analyzed four organs, they have found residues in the following order: egg > gut > muscle > liver. Higher levels of residues have found during the dry season due to high lipid content in fishes. They have also observed a positive correlation between pesticide residues and lipid contents of fish. The concentrations of pesticide residues in muscle, liver and gut were below the FAO/WHO (1993) recommended permissible limit except in eggs.

Pesticides residues remain in fish including other food item have become a consumers' safety issue. The exposure of OCPs in food products to the consumers creates severe health hazards particularly breast cancer, testicular cancer, endocrine dysfunction, births defects, lower sperm count (Garry et al., 2004; Brody and Rudel, 2003; Ahmed et al., 1996; Soto et al., 1998). Thus, the food safety issues concerning pesticide residues needs to be considered along with food production and an effort including integrated pest management and stringent quality control system comprising rational use of pesticides and their regular monitoring in the environmental samples including fish should be ensured.

CONCLUSION

The described method in this study is an efficient and effective multi-residue analytical method using GC-ECD for the determination of 19 organochlorine

pesticide residues in shrimp. A very good accuracy and precision was found for all analytes using this proposed method. The average recoveries ranged from 84 to 106% with $RSD_r \leq 14\%$ and $RSD_R \leq 5\%$, thus fulfilling the requirement set by SANTE document number SANTE/11945/2015 for accuracy and precision (European commission 2015). Ninteen organochlorine pesticides were incorporated in this method that helps the scientist/ analysts for quick determination of multiple pesticide residues in shrimp. In addition, this analytical method was applied successfully to monitor the selected organochlorine pesticide residues in shrimp in Bangladesh. Thus the proposed method can be used successfully to monitor multiple organochlorine pesticide residues in shrimp.

REFERENCES

Ahmed, S., Ajmal, M.S., and Nomani, A.A. (1996).Organochlorines and polycyclic hydrocarbons in the sediments of Ganga River, India. *Bulletin of Environmental Contamination and Toxicology*, 57, 794–802.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., and Schenck, F.J. (2003).Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International*, 86, 412-431.

Anonymous. (1979).DDT and its derivatives. In "Environmental Health criteria 9". World Health Organization. Geneva.

Anonymous. (1989).DDT and its derivatives-environmental aspects. In "Environmental Health criteria 83". World Health Organization. Geneva.

Antunes, P., and Gil, O. (2004).PCB and DDT contamination in cultivated and wild sea bass form Ria de Aveiro, Portugal. *Chemosphere*, 54, 1503-1507.

Aulakh, R.S., Gill, J.P., Bedi, J.S., Sharma, J.K., Joia, B.S., and Ockerman, H.W. (2006). Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. *Journal of the Science of Food and Agriculture*, 86(5), 741–744.

Battu, R. S., Gupta, S.C., Chawla, R.P., and Kalra, R.L. (1984).Residues of DDT and BHC in market samples of meat of various animals. *Indian Journal of Ecology*, 2, 177-182.

Bedi, J.S., Gill, J.P.S., Aulakh, R.S., Joia, B.S., and Sharma, J.K. (2005).Contamination levels of DDT and HCH residues in different caprine tissues in Punjab, India. *Indian Journal of Animal Science*, 75(1), 11–13.

Belton, B., Karim, M., Thilsted, S., Murshed-E-Jahan, K., Collis, W., and Phillips, M. (2011). Review of aquaculture and fish consumption in Bangladesh. Studies and Reviews 2011. The World Fish Center.

Berg, V., Polder, A., and Skaare, J.U. (1999).Oragnochlorines in deep-sea fish from the Bordfjord. *Chemosphere*, 38, 175-182.

Brody, J.G., and Rudel, R.A.(2003).Environmental pollutants and breast cancer. *Environmental Health Perspectives*, 111, 1007–1019.

Chan, H.M., Chan, K.M., and Dickman, M. (1999).Organochlorines in Hong Kong Fish. *Marine Pollution Bulletin*, 39, 346-351

EURACHEM. (1998).The fitness for purpose of analytical methods.www.eurachem.com.

European commission. (2015).Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed. Document no. SANTE/11945/2015

FAO/WHO. (2003).Report of the joint meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO core assessment group on pesticide residues.

Garry, V.F. (2004). Pesticide and children. *Toxicology and Applied Pharmacology*, 198, 152–163.

Helfman, G., Collette, B., and Facey, D. (1997).The Diversity of Fishes, Blackwell Publishing, p 3, 1997, ISBN 0-86542-256-7

Jabbar, S.A., Khan, Y.S., and Rahman, M.S. (2001).Levels of organochlorine insecticide residues in some organs of the Ganges perch, Lates calcarifer, from the Ganges-Brahmaputra-Meghna estuary, Bangladesh. *Marine Pollution Bulletin*, 42(12), 1291-1296.

John, P.J., Bakore, N., and Bhatnagar, P. (2001).Assessment of organochlorine pesticide residues levels in dairy milk and buffalo milk from Jaipur city, Rajasthan, India. *Environmental Interntional*, 20(4), 231–236.

Kaphalia, B.S., Takroo, R., Mehrotra, S., Nigam, U., and Seth, T.D. (1990).Organochlorine pesticide residues in different Indian cereals, pulses, spices, vegetables, fruits, milk, butter, deshi ghee, and edible oils. *Journal of AOAC International*, 73, 500-512.

Kaur, M., Sharma, J.K., Gill, J.P., Aulakh, R.S., Bedi, J.S., and Joia, B.S. (2008). Determination of organochlorine pesticide residues in freshwater fish species in Punjab, India. *Bulletin of Environmental Contamination Toxicology*, 80, 154–157.

Osuna-Flores. and Riva, M.C. (2002).Organochlorine pesticide residue concentrations in shrimps, sediments, and surface water from Bay of Ohuira, Topolobampo, Sinaloa, Mexico. *Bulletin of Environmental Contamination Toxicology*, 68, 532–539.

Prodhan, M.D.H., Papadakis, E.N., and Papadopoulou-Mourkidou, E. (2015).Determination of multiple pesticide residues in eggplant with Liquid Chromatography Mass Spectrometry. *Food Analytical Methods*, 8, 229–235.

Prodhan, M.D.H., Rahman, M.A., Ahmed, M.S., and Kabir, K.H. (2010).Pesticide residues in fish samples collected from different fish cultivation regions of Bangladesh. *SAARC Journal of Agriculture*, 8 (2), 53-64.

Prodhan, M.D.H., Rahman, M.A., Ahmed, M.S., and Kabir, K.H. (2009). Quantification of Organophosphorus and Organochlorine insecticide residues from fish samples using simple GC technique. *Bangladesh Journal of Agriculturist*, 2 (2), 197-204.

Sankar, T.V., Zynudheen, A.A., Anandan, R., and Nair, P.G.V. (2006).Distribution of organochlorine insecticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. *Chemosphere*, 65(4), 583-590.

Saqqib, T.A., Nagvpo, S.N., Sidiqui, P.A., and Azmi, M.A. (2005).Detection of insecticide residues in muscles, liver and fat of 3 species of Lobeo found in Kalri and Haliji lakes. *Journal of Environmental Biology*, 26(2), 433-438.

Spozhikova, Y., Bawardi, O., and Schlenk, D. (2004).Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. Chemosphere, 55, 797-809.

Soto, A.M., Michaelson, C.L., Preehti, N.V., Weill, B.C., Sonnenschein, C., Serrano, F., and Olea, N. (1998).Assays to measure estrogen and androgen agonists and antagonists. *Advances in Experimental Medicine and Biology*, 444, 9-23.

Zhang, G., Pan, Z., Bai, A., Li, J., and Li, X. (2014).Distribution and bioaccumulation of organochlorine pesticides (OCPs) in food web of Nansi Lake, China. *Environmental Monitoring and Assessment,* 186, 2039–2051.