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Multi-Residue Determination of Pesticides in Farmed Aquatic Animal Products Using Gas Chromatography-Tandem Mass Spectrometry

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Abstract In the present study, an analytical method was developed and optimized for screening and confirmation of multi-pesticide residues in farmed aquatic animal samples. Target pesticides (organochlorines, organophosphorus, and synthetic pyrethroids) were extracted via the QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach. Ethyl acetate was used for extraction of pesticides from the samples (flatfish, eel, shrimp, and Manila clam), which were then purified using C_{18} and primary secondary amine. Finally, the extracts were filtered through a 0.22-μm polytetrafluoroethylene syringe filter and subsequently analyzed using gas chromatography coupled with mass spectrometry. The target analytes were ionized in the positive mode of electron impact ionization using multiple reaction monitoring. According to the CODEX CAC/GL-71 guideline, accuracy, precision, linearity, and limit of detection were evaluated for all matrices. The accuracy (recoveries) was between 62.4% and 120%, and precision (relative standard deviations) was below 20%. The linearity of the matrix calibration curves was r^2>0.98. The limits of detection and quantification for all pesticides were ≤3 μg/kg and ≤10 μg/kg, respectively. In real sample (n=79) analysis, trifluralin was detected at 67 μg/kg in one Manila clam sample. Based on our results, the proposed method was satisfactory for pesticide residue determination in aquatic animal products.

Key words Analytical method, Fishery products, GC-MS/MS, Pesticide, Residue

Introduction

A wide range of pesticides (insecticide, fungicides, and herbicides) could potentially be transferred into aquatic animal tissue; however, little is known about pesticide accumulation in aquatic animal products. Although most persistent pesticides have been banned since the 1970s, they are still continuously being detected in seafood (Zhao et al., 2016). The organochlorine pesticides have low volatility, high stability, and lipophilic behavior, which are responsible for their persistence in the environment and concentration in fat and tissues. The unintended use of pyrethroids and organophosphorus pesticides is sufficient to reach rivers and the marine environment, thus affecting aquatic animal products. Owing to their metabolic activity in animals, pyrethroids tend to bioaccumulate, becoming a potential source of contamination in foodstuffs. Consequently, pesticide residues have to be monitored in foodstuffs to control food quality and prevent risks to human health (Stefanelli et al., 2009). In addition, a previous study reported that certain pesticides are illegally used in high concentrations for controlling and preventing parasitic and microbial diseases under stressful conditions in fish farms (Sabra & Mehana, 2015). Therefore, pesticide residues should also be monitored and controlled on aquaculture farms and the surrounding environment (Sapozhnikova & Lehotay, 2015).

Due to the structure of pesticides and their chemical properties, pesticide residues are usually analyzed using gas chromatography coupled with electron capture detection (GC/ECD) or using mass spectrometry (GC/MS). GC–MS/

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MS is a selective and sensitive technique that is acceptable for the simultaneous detection of volatile and thermostable pesticide residues in food commodities of animal origin (Raina, 2011). The analytical methods used for the determination of pesticide residues in animal products and food samples (n=60) had a detection rate of 41.7% (Nasiri et al., 2016; Zhao et al., 2016). However, there is a paucity of information on analytical methods used for multi-pesticides in fish using GC-MS/MS (Sapozhnikova & Lehotay, 2013). There are currently no analytical methods for the simultaneous determination of 51 compounds in aquatic animal products.

Pesticide residue analysis in aquatic animal product samples is challenging due to the low concentrations and a wide range of pesticides in a complex matrix (Chan et al., 2012). Therefore, it is necessary to develop rapid, reliable, and effective analytical methods for the simultaneous determination of multiple pesticide compounds (Nasiri et al., 2016). Based on our previous study, we focused on 51 pesticides (including thermostable and strong volatile organochlorines, organophosphorus, and pyrethroids) having the potential to contaminate aquatic animal products. An analytical method was developed and validated for the determination of pesticides in fish (flatfish and eel), shrimp, and Manila clam. The proposed method was applied to aquatic animal samples collected from retail markets.

Materials and Methods

Reagents and chemicals

All pesticide standards were of high purity (>90%) and were purchased from Dr. Ehrenstofer (Augsburg, Germany) and Sigma-Aldrich (Buchs, Switzerland). HPLC grade ethyl acetate, methanol, acetone, and n-hexane were purchased from Merck Inc. (Darmstadt, Germany). Anhydrous magnesium sulfate ($MgSO_4$), sodium chloride, and octadecylsilane (C_{18}) were purchased from Sigma-Aldrich and Waters (Milford, MA, USA), respectively. A filter of 0.22-μm polytetrafluoroethylene (PTFE) was acquired from Teknokroma (Barcelona, Spain).

The stock solution of individual analyte (approximately 1000 μg/mL) was prepared in a 50-mL volumetric flask using acetonitrile, methanol, acetone, and n-hexane as solvents. For working standard mixtures, a range of final target concentrations was prepared in acetone from the above stock solution by serial dilution. All stock solutions were stored at -20°C in amber glass bottles to prevent photolysis.

Sample preparation

Aquatic animal product samples were purchased from local markets in Korea. The de-skinned fillets (over 500 g) were homogenized and then stored at -20°C. The blank samples were tested to ensure that it did not contain any of the target pesticides before use as a negative control. The aquatic animal samples (over 500 g) were prepared for analysis using matrix-matched calibration and monitoring. The homogenized samples (2 g) of aquatic animal samples were transferred into a 50 mL centrifuge tube. Thereafter, 10 mL of ethyl acetate was added to each sample, shaken vigorously by hand for 30 s. This was followed by the addition of 500 mg of NaCl and 1 g of anhydrous $MgSO_4$ to each sample, which was then vortexed for 5 min. After vortexing, the extracts were put into a freezer at -20°C for 15 min and then centrifuged at 4500 × *g* at a temperature of 4°C for 10 min. The supernatant was transferred into a 50 mL centrifuge tube. The organic phase was evaporated under nitrogen stream at 50°C and diluted in 10 mL of acetonitrile, after which C_{18} (200 mg), PSA (200 mg), and anhydrous magnesium sulfate (500 mg) were added. The mixture was shaken for 5 min and centrifuged at 4500 × *g*, 4°C for 10 min. The supernatant was trans- ferred into a 15-mL centrifuge tube and evaporated using nitrogen stream at 50°C and reconstituted with 1 mL of 20% acetone in hexane. Finally, the extracts were filtered through a 0.22-μm PTFE syringe filter. The final extracts (5 μL) were injected into the GC-MS/MS system for further analysis.

GC-MS/MS analysis

An Agilent 7890 GC system coupled with an Agilent 7010 GC/MS Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) and a Rxi®-5Sil MS (0.25 mm i.d. × 30 m, 0.50 μm film thickness) capillary column was used for the GC-MS/MS analysis. Electron impact ionization (EI) mass spectra was obtained at 70 eV and monitored from 100 to 600 m/z for full scan mode analysis. The working parameters were as follows: injector temperature was set at 280°C and the carrier gas (He) at 1.0 mL/min. The optimized GC oven temperature was initially 70°C (held for 3 mins), increased to 180°C at a rate of 20°C/min, and then finally to 300°C at 5°C/min (held for 7 mins). The

mass selective detector transfer line was set at 280°C and the ion source at 230°C. The injection mode was splitless, and the injection volume was 1 μL. Data collection was performed in the multiple reaction monitoring (MRM) mode, and the optimized MRM parameters are listed in Table 1.

Method validation

The method was validated according to the Codex guideline (CAC/GL 71, 2009). The blank samples (flatfish, eel, shrimp, and Manila clam) were tested to ensure that they did not contain any interferences and/or target compounds. The measured parameters were the linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy, and precision. The validation study was carried out using tissue samples previously checked to be free of residual pesticides. The LOD was calculated at a signal-to-noise ratio (S/N) of 3, whereas the LOQ value was calculated using an S/N ratio of 10. The linearity was tested using matrix-matched calibrations (blank, 10, 20, 50, 100, 150 μg/kg) that were prepared by adding the appropriate amount (200 μL) of standard mixtures in the solvent into the fish and shrimp samples. The accuracy and precision (expressed as recovery and relative standard deviation, respectively) were determined by analyzing all samples spiked at 10, 20, and 100 μg/kg. The accuracy and precision were validated based on three target concentrations (10, 20, and 100 μg/kg). The accuracy and precision were determined at the three levels in the blank samples in five replicate analyses.

Results and Discussion

Optimization of GC-MS/MS conditions

GC-MS/MS is a valuable approach for the determination of highly hydrophobic and volatile organochlorine pesticides (Hernández et al., 2013; Chatterjee et al., 2016; FSIS, 2018). In the current study, GC-amenable pesticides (organophosphorus, pyrethroids, carbamates insecticides, herbicides, and fungicides) were selected based on their potential use and contamination in fishery products and the aquaculture industry. GC-MS/MS based analytical methods have been preferred for the determination of pesticide residues in fish due to their high sensitivity and selectivity with low interferences (Munaretto et al., 2013; Sapozhnikova & Lehotay, 2013; Manuelmolina-Ruiz et al., 2014; Sahu & Nelapati, 2018; Colazzo et al., 2019).

The precursor ions, product ions, and collision energies were optimized for the best intensity of target compounds (Table 1). Based on a full scan spectrum, precursor ions were selected; then, the collision energy was adjusted to generate the product ions. MRM transitions with the highest intensities with related collision energies as well as retention times for all the pesticides were selected for quantification. The most abundant precursor ion with the highest m/z value was designated as the quantification ion,

Table 1. MRM transition and optimized parameters of GC-MS/MS for 51 target compounds

Compounds	Formula	Retention time (min)	Molecular weight (g/mol)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
				263[a)]	**193**	40
Aldrin	$C_{12}H_8Cl_6$	15.9	364.9	263	191	40
				255	220	15
				136	93.0	20
Allethrin	$C_{19}H_{26}O_3$	17.1	302.4	**123**	**81.0**	10
				123	79.9	20
				219	183	5
alpha-HCH	$C_6H_6Cl_6$	12.1	290.8	217	181	5
				181	**145**	15
				219	183	10
beta-HCH	$C_6H_6Cl_6$	12.7	290.8	219	147	30
				181	**145**	10
				344	159	5
Carbophenothion	$C_{11}H_{16}ClO_2PS_3$	21.1	342.9	342	199	5
				342	**157**	5

Table 1. continued

Compounds	Formula	Retention time (min)	Molecular weight (g/mol)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
				375	266	20
Chlordane_*cis*	$C_{10}H_6Cl_8$	18.2	409.8	**373**	**266**	20
				373	264	20
				375	266	20
Chlordane_*trans*	$C_{10}H_6Cl_8$	17.7	409.8	**373**	**266**	20
				373	264	20
				213	171	10
Chlorpropham	$C_{10}H_{12}ClNO_2$	11.6	213.7	213	127	5
				171	**127**	15
				314	258	20
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	15.7	350.6	199	171	15
				197	**169**	15
				286	271	20
Chlorpyrifos_methyl	$C_7H_7Cl_3NO_3PS$	14.4	322.5	**286**	**93.0**	20
				-	-	-
				219	147	30
delta-HCH	$C_6H_6Cl_6$	13.6	290.8	181	146	10
				181	**145**	10
				255	174	5
Deltamethrin	$C_{22}H_{19}Br_2NO_3$	31.4	505.2	253	174	5
				253	**93.0**	15
				250	139	15
Dicofol	$C_{14}H_9Cl_5O$	16.2	370.5	**139**	**111**	15
				139	75.0	35
				277	241	10
Dieldrin	$C_{12}H_8Cl_6O$	18.1	380.9	277	206	20
				263	**193**	20
				241	206	20
Endosulfan_alpha	$C_9H_6Cl_6O_3S$	18.2	406.9	239	204	20
				207	**172**	20
				241	206	15
Endosulfan_beta	$C_9H_6Cl_6O_3S$	20.0	406.9	207	172	15
				205	**170**	15
				274	237	25
Endosulfan_sulfate	$C_9H_6Cl_6O_3S$	20.2	406.9	272	237	20
				239	**204**	15
				263	**193**	40
Endrin	$C_{12}H_8Cl_6O$	19.0	380.9	263	191	35
				245	173	30
				317	281	10
Endrin keton	$C_{12}H_8Cl_6O$	22.9	380.9	317	245	20
				317	**101**	15
				169	**141**	5
EPN	$C_{14}H_{14}NO_4PS$	23.2	323.3	169	77.0	20
				157	77.0	30

Table 1. continued

Compounds	Formula	Retention time (min)	Molecular weight (g/mol)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
				163	135	10
Etofenprox	$C_{25}H_{28}O_3$	28.9	376.5	**163**	**107**	20
				163	77.0	30
				265	**210**	10
Fenpropathrin	$C_{22}H_{23}NO_3$	23.5	349.4	265	89	40
				209	116	15
				367	255	30
Fipronil	$C_{12}H_4Cl_2F_6N_4OS$	16.8	437.1	**367**	**213**	30
				351	255	15
				383	**255**	30
Fipronil sulfone	$C_{12}H_4Cl_2F_6N_4O_2S$	18.7	453.1	383	241	15
				255	228	15
				233	**165**	20
Flusilazole	$C_{16}H_{15}F_2N_3Si$	19.0	315.4	233	152	20
				233	91.0	30
				219	183	10
gamma-HCH	$C_6H_6Cl_6$	13.0	290.8	219	147	30
				181	**145**	10
				274	237	20
Heptachlor	$C_{10}H_5Cl_7$	14.8	373.3	**272**	**237**	20
				237	119	40
				355	265	15
Heptachlor_epoxide_a	$C_{10}H_5Cl_7O$	17.0	389.3	**353**	**263**	15
				237	143	25
				253	183	30
Heptachlor_epoxide_b	$C_{10}H_5Cl_7O$	17.1	389.3	**217**	**181**	30
				183	119	30
				284	249	25
Hexachlorbenzene	C_6Cl_6	12.3	284.8	**284**	**214**	40
				-	-	-
				218	203	20
Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	31.1	527.8	**203**	**134**	20
				203	106	30
				131	130	15
Kresoxim_methyl	$C_{18}H_{19}NO_4$	19.1	313.3	131	89.0	35
				116	**89.0**	15
				159	131	20
Mecarbam	$C_{10}H_{20}NO_5PS_2$	17.1	329.4	**131**	**86.0**	15
				131	74.0	10
				238	**162**	10
Metolachlor	$C_{15}H_{22}ClNO_2$	15.7	283.8	162	133	15
				162	117	40
				164	98.0	10
MGK_264	$C_{17}H_{25}NO_2$	16.4	275.4	**164**	**93.0**	10
				164	80.0	30
				409	**300**	15
Nonachlor cis	$C_{10}H_5Cl_9$	20.2	444.2	407	300	30
				407	298	30

Table 1. continued

Compounds	Formula	Retention time (min)	Molecular weight (g/mol)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
				409	**300**	25
Nonachlor_trans	$C_{10}H_5Cl_9$	18.3	444.2	407	300	15
				-	-	-
				187	123	20
Oxychlordane	$C_{10}H_4Cl_8O$	17.0	423.7	187	87.0	35
				187	**84.9**	35
				291	**109**	20
Parathion	$C_{10}H_{14}NO_5PS$	15.9	291.3	291	80.9	30
				186	140	5
				265	**194**	10
Pentachloroaniline	$C_6H_2Cl_5N$	14.1	265.3	265	192	5
				-	-	-
				183	**168**	20
Permethrin_cis	$C_{21}H_{20}Cl_2O_3$	26.8	391.3	183	155	10
				183	154	20
				308	70.0	15
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$	26.9	376.7	**180**	**138**	10
				180	69.0	20
				285	96.0	5
Procymidone	$C_{13}H_{11}Cl_2NO_2$	17.3	284.1	**283**	**96.0**	5
				283	68.0	20
				135	**107**	20
Propargite	$C_{19}H_{26}O_4S$	22.1	350.5	135	94.9	20
				135	77.1	30
				175	147	15
Propyzamide	$C_{12}H_{11}Cl_2NO$	13.1	256.1	**173**	**145**	20
				173	109	35
				197	141	10
Tefluthrin	$C_{17}H_{14}ClF_7O_2$	13.4	418.7	177	137	20
				177	**127**	20
				336	218	20
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	16.0	372.1	**336**	**204**	40
				336	164	30
				265	**250**	15
Tolclofos_methyl	$C_9H_{11}Cl_2O_3PS$	14.6	301.1	265	220	25
				265	93.0	30
				299	271	20
Trichloronate	$C_{10}H_{12}Cl_3O_2PS$	16.2	333.6	**297**	**269**	20
				297	223	20
				306	**264**	10
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	11.6	335.3	306	160	20
				264	160	10
				285	212	10
Vinclozolin	$C_{12}H_9Cl_2NO_3$	14.5	286.1	**187**	**124**	20

[a)]The bold text expressed as quantification ion.

whereas the least intense product ion was designated as the qualifier ion. Due to the co-eluting sample interfering with the analytes, two precursor or additional product ions were used as qualifiers to prevent possible false-positives.

Optimization of extraction and purification

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach was applied to this method because of its versatility (de Oliveira et al., 2019). The analytical method was developed and validated using GC-MS/MS based on QuEChERS. The optimization of purification was carried out using a salting-out solvent extraction step and a d-SPE clean-up step to remove matrix components (e.g., fatty acid). For the extraction step, salts that are easily electrolyzed in an aqueous solution were used as reagents to achieve separation of the ethyl acetate of nonpolar pesticides in an organic solvent (Sapozhnikova, 2014; Cao et al., 2015; FSIS, 2018). For the purification step, $MgSO_4$, PSA, and C_{18} were used. $MgSO_4$ was used for moisture removal (Perović et al., 2018). PSA provided polar adsorption and weak anion exchange, which removed polar compounds such as organic acids, fatty acids, carbohydrates, and sugars, whereas the C_{18} hydrocarbon chains eliminated fatty acids and nonpolar interfering substances (Sapozhnikova & Lehotay, 2013; Shin et al., 2018; Kim et al., 2020). Based on previous studies, the combination of $MgSO_4$ (500 mg), C_{18} (200 mg), and PSA (200 mg) was adopted for multi-pesticide detection in fishery products.

Method validation

Specificity was evaluated through the analysis of the four different fishery product samples against a reagent blank. No interference was observed at the same retention time as the analyte. The validation process was performed by determining the linearity, LOD, LOQ, accuracy, and precision based on the CODEX guidelines (CODEX, 2014). The chromatograms of the target compounds are shown in Figure 1. The linearity (expressed as correlation coefficients, r^2) of the matrix calibration curves was >0.98 for all target compounds. Our results showed good linearity and allowed for the coverage of all target compounds. The LOD and LOQ were ≤3 and ≤10 μg/kg, respectively. The accuracy (expressed as recovery, %) and precision (expressed as RSD, %) of the target compounds were evaluated in spiked blank samples at three concentrations (10, 20, and 100 μg/kg). The overall recoveries for all the target compounds ranged from 62.4% to 120%. The precision was observed at 20.7% (Table 2). Three compounds (i.e., chlorothalonil, iprodione, and terbufos) were excluded before the start of method validation because of their inconsistent recoveries and/or unsatisfactory linearity of the calibrations. Some pesticides cannot be appropriately assessed using the buffered QuEChERS method (Lehotay et al., 2005; Cho et al., 2016).

Application and real sample monitoring

The applicability of the method was evaluated through the analysis of the target pesticides in 79 fishery product samples purchased from the local markets in Korea. Trifluralin was detected in one sample (1%) at a concentration of 67 μg/kg in the Manila clam, while its residue in flatfish was below LOQ. Trifluralin is frequently detected in aquatic animal samples. The residue of trifluralin was reported to be above 1 μg/kg in shrimp produced in Asian countries (Chan et al., 2012). Trifluralin residues (35–204 μg/kg) were detected in 11 pangasius fillet imported from Vietnam in 2011 (Chan et al., 2013). Previous studies have revealed that the trifluralin residues in Manila clam and flatfish (<LOQ) indicated the presence of pesticide runoff into the aquaculture environment (Shin et al., 2011). Trifluralin has been reported to mostly appear in runoff water in agricultural fields (Antoniouds, 2012).

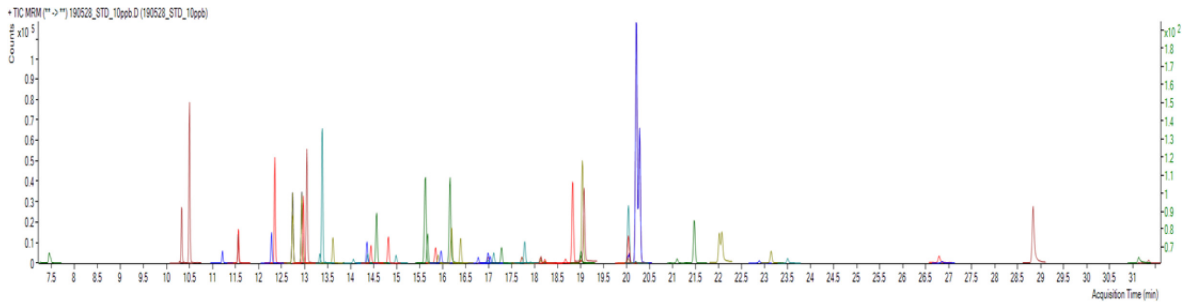

Fig. 1. GC-MS/MS Chromatogram of a spiked sample with 51 pesticides at 10 μg/kg.

Table 2. Accuracy and precision at three testing levels in fishery products, shrimp and manila clam

Compounds	Target testing level (μg/kg)	Flatfish (n=5)		Eel (n=5)		Shrimp (n=5)		manila clam (n=5)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Aldrin	10	108	4.8	86.7	8.1	99.1	8.4	88.4	14.8
	20	102	6.2	95	5.2	104	13.8	96.7	8.6
	100	100	2.5	95.1	5	102	10.2	92.5	18.1
Allethrin	10	108	6.3	92.1	17.2	106	16.6	110	16.9
	20	103	10.2	91.4	14.5	100	19.2	110	9.8
	100	88.1	11	90.9	12.3	96.8	15.7	98.4	6
Carbophenothion	10	109	4.2	86.5	7.8	94.5	11.1	73.5	11.6
	20	104	6.1	94.5	6.2	105	7.5	86.2	5.5
	100	102	1.6	95.4	4.1	106	8.7	86.9	13.4
Chlordane-cis	10	108	1.4	85.6	5.2	100	9.5	97.3	6.7
	20	102	4.3	94.4	4.2	104	4.7	97.3	6.8
	100	98.2	2.5	96.9	3.7	104	6	98.9	12.3
Chlordane-trans	10	110	2.7	89.6	5.1	98.8	7.9	72.7	12.2
	20	102	4.4	95	4.1	102	5.7	92.1	6.9
	100	100	2.1	95.5	4	105	6.8	104	9.3
Chlorpropham	10	117	11.6	88.9	8.7	99	11.6	80.7	15.2
	20	105	13.3	88.5	8.7	95.4	14	90.4	11
	100	107	4.3	86	6.7	92.5	12.4	96.8	16.4
Chlorpyrifos	10	118	6.6	92.3	5.5	94.9	11	93.1	13
	20	108	6.6	97	5.9	104	6.2	102	8.1
	100	103	2	94.8	4.1	103	9.8	103	15.2
Chlorpyrifos methyl	10	115	9	88.3	7.3	99	12	94.2	13.9
	20	106	11.1	93.1	8.4	98.8	8.4	96.5	9.2
	100	103	4.1	91.9	4.8	102	19.4	92.8	13
Deltamethrin	10	112	7.5	90.7	9.6	92.2	20.1	103	19.3
	20	103	6.9	93.9	7.4	107	12.1	108	14.3
	100	101	4.4	93.2	3.9	119	13.8	109	6.6
Dicofol	10	120	4.3	94.9	8.3	91	9	102	8.3
	20	116	4.7	94.2	7.3	97.3	5.9	108	5.7
	100	113	2.7	97.7	3.2	94.3	7.3	118	14.3
Dieldrin	10	104	4.4	88.6	7.4	103	7.1	79.8	19.1
	20	100	6.2	93.7	4.3	104	4.4	89.9	8.9
	100	98.9	2.7	95.7	4.6	104	6.4	100	9.8
Endosulfan α	10	109	2.8	91	5.9	108	16.5	95.7	6.9
	20	104	5.3	98.2	4.3	107	4.6	98.3	3.5
	100	103	2.7	96.1	3	112	16.4	97.1	13.9
Endosulfan β	10	106	2.1	87.3	5.6	100	6	96.5	6.9
	20	101	4.1	96.4	3.7	103	4.4	97.6	3.9
	100	98.8	2.4	98.5	4.3	104	5.7	98.8	11.1
Endosulfan sulfate	10	107	4.2	83.9	7.8	101	6.2	89.1	7.3
	20	100	5.2	94.9	3.9	103	3.8	93.6	2
	100	102	2	98.5	3.9	103	4.8	92.4	6.6
Endrin	10	105	2.7	84.3	5.6	100	6.4	75.2	9.3
	20	100	4.5	95.8	3.4	104	3.7	94.7	7.9
	100	97.7	2	98.6	4.8	105	4.5	107	13

Table 2. continued

Compounds	Target testing level (μg/kg)	Flatfish (n=5)		Eel (n=5)		Shrimp (n=5)		manila clam (n=5)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Endrin keton	10	106	1.8	90.4	4.9	100	6.3	79.5	2.3
	20	102	3.3	95.3	3.7	104	3.9	76.1	5.8
	100	102	1.8	96.2	4.4	105	5.4	78.3	14
EPN	10	102	2.6	89	4.6	98.3	9.8	107	3.1
	20	97.6	5.4	89.1	4.2	104	6.6	98.1	2.6
	100	103	1.1	91.5	4.3	109	8.1	94.3	4.7
Etofenprox	10	114	5.3	84.2	8.1	92.7	9.6	119	6.1
	20	107	5.8	95.9	7.1	101	5.5	117	5
	100	101	3.4	97.3	4.6	105	8.1	109	8.2
Fenpropathrin	10	108	3.8	89	6.4	96.1	10.4	86.3	5.8
	20	101	4.9	96	5.3	105	7	95.4	5.5
	100	99.2	1.7	94.8	4.5	107	8.6	102	6.3
Fipronil	10	110	2	93.7	7.3	95.8	17.4	105	4.1
	20	101	4.6	96.3	6.6	100	11	100	3.5
	100	101	3.7	95.6	4	101	16.8	91.6	5.3
Fipronil sulfone	10	106	2.8	86.9	5.7	117	12.8	100	4
	20	100	3.9	94.5	4.3	80.7	14.6	95.2	4.4
	100	98	3.4	96.2	3.6	83.3	12.5	89.3	5.5
Flusilazole	10	106	2.8	87.7	6.2	108	13	99.1	5.3
	20	101	5	97.7	4.3	91.6	15.7	100	4
	100	97	1.6	96.6	3.4	94.3	14.7	95.5	5.8
alpha-HCH	10	112	9.6	81.2	7	103	14.1	78.6	17.4
	20	105	11.8	91.1	6.2	116	12.6	94.7	11.5
	100	106	5.2	88.6	8.5	93.5	19.9	86	19.3
bata-HCH	10	110	3.4	79.8	8.2	104	6.5	84.8	13.2
	20	97.5	7.4	91.1	4.6	107	7.9	99.1	7.4
	100	86.5	3.9	95.4	3.9	112	12.9	109	14.6
delta-HCH	10	109	4	86.5	6.2	103	6.6	81.4	8.6
	20	104	4.3	93.6	5.3	100	6.7	93.9	5.6
	100	101	1.8	96.7	4.5	106	14.9	106	12.8
gamma-HCH	10	113	6.8	86.8	5.7	106	9.2	83.4	17.1
	20	105	7.6	92.5	5.5	107	16.1	83.5	11.3
	100	105	3.3	91.3	5.1	98	12.2	81.6	12.7
Heptachlor	10	112	5.3	82.9	7	104	8.6	76.5	17.8
	20	104	8.2	92	6.1	105	15.6	79.7	10.1
	100	104	2.3	93.2	5.3	99	12.4	71.7	13.5
Heptachlor epoxide a	10	109	2.7	90.5	6.8	101	7.3	97.4	7.1
	20	101	5.3	95.2	4.2	103	6.5	96.4	6.9
	100	100	1.7	95.3	3.9	105	5.4	96	13.4
Heptachlor epoxide b	10	112	5.4	93.8	16.8	86.6	8	89.8	17.4
	20	98.6	7.6	96.8	9.1	100	6.3	102	15.8
	100	100	4.1	92.4	2.7	106	5.9	100	15.2
Hexachlorbenzene	10	111	7.4	118	7.8	100	17	75.1	11.5
	20	95.4	7.3	104	7.5	103	16.1	90.6	15.1
	100	96.2	1.8	107	1.8	105	17.8	74.5	14.9

Table 2. continued

Compounds	Target testing level (μg/kg)	Flatfish (n=5)		Eel (n=5)		Shrimp (n=5)		manila clam (n=5)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Indoxacarb	10	105	5.6	83.5	9.9	96.7	8.9	100	8.6
	20	100	3.5	95.8	5.5	99.3	4.8	98.6	4.5
	100	96.4	3.1	98.4	2.9	98.2	9.6	96.5	6.4
Kresoxim methyl	10	110	2	78.5	5.6	95.9	9.4	96.7	4.7
	20	103	4.4	94.6	4.6	105	4.7	103	3
	100	97.3	2.1	100	4.2	105	7.8	107	8.2
Mecarbam	10	113	4	86.6	6.4	99.2	15	98.9	7.8
	20	106	5.5	93.7	6.5	104	9.6	101	3.2
	100	103	2.8	92.9	4.1	102	13.4	108	10.8
Metolachlor	10	108	2.7	87.1	5.7	95.9	10.7	80.4	9.5
	20	102	4.9	96.6	4.5	102	5.8	94.8	4.5
	100	96.8	1.3	95.9	4.5	101	9.7	102	12.1
MGK-264	10	117	3.8	83.7	6.8	96.9	9.8	100	8.2
	20	104	4.6	96.4	5.1	103	6.2	103	4.3
	100	101	1.4	97	3.8	103	9.3	104	12.6
Nonachlor cis	10	110	3.6	89.3	6.1	101	7.7	88.7	6.1
	20	105	4.5	96.2	4	105	5.6	92.3	4.1
	100	102	1.8	98	4	105	5.4	98.5	6.6
Nonachlor trans	10	117	2.4	81.6	7.7	97.5	8.1	81.2	10.7
	20	117	3.4	95.6	3.7	103	5.5	92.6	7
	100	115	2.8	102	4.6	106	6.2	104	11.6
Oxychlordane	10	110	3.2	85.9	4.4	101	6.7	98.9	7.1
	20	102	4.6	95.8	3	104	6.8	96.5	5.6
	100	100	1.9	96.2	3.8	103	6.6	97.1	14.1
Parathion	10	108	3.7	90.5	5.8	98.1	12.6	77.8	9.2
	20	100	6.6	91.1	5.1	101	8	88.7	6.5
	100	104	3	89.6	4.7	105	13.3	99.3	13.9
Pentachloroaniline	10	111	6.7	86	9.9	97.8	9.8	87.9	14.1
	20	103	8.5	94.8	8	100	7.7	98.3	10
	100	103	3.1	95.3	5	104	14.7	104	18.7
Permethrin cis	10	103	3.1	87.7	5.7	92	9.1	115	8.4
	20	105	6.2	94.2	6.8	102	7.2	111	8.5
	100	104	2.1	95.7	4.2	106	8.7	110	10.3
Prochloraz	10	112	7.8	94.1	7.5	92.7	16.5	109	4.6
	20	107	6.8	93.5	8	103	7.3	100	5.6
	100	106	2.2	94.1	5.6	106	12.2	79.9	15.4
Procymidone	10	109	3.9	88.3	5.8	98.9	9.9	88.2	8.3
	20	103	5.6	96.2	5	103	5.8	98.1	5.3
	100	101	1.8	95.4	3.8	102	8.8	103	9.8
Propargite	10	110	1.8	81.5	6.8	94.8	8.3	85.3	6.4
	20	106	4.1	98	4.6	106	4.5	100	2.5
	100	95.2	1.9	98.3	3.6	104	4.9	107	6.6
Propyzamide	10	115	7.4	87.4	7.9	96.4	11.4	76.4	11.2
	20	107	9	93.3	8.5	97.4	8.1	93.4	5.2
	100	103	3.8	93.1	4.6	97.2	10.1	109	14.1

Table 2. continued

Compounds	Target testing level (μg/kg)	Flatfish (n=5)		Eel (n=5)		Shrimp (n=5)		manila clam (n=5)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Tefluthrin	10	115	8.1	83	7.9	96.5	10.8	86.9	18.1
	20	104	12.7	92	8.1	101	9.5	98.5	9.8
	100	103	4.1	91.1	6	101	13.6	92.4	14.6
Tetraconazole	10	107	3	87.8	6.5	95	12.6	97.4	4.3
	20	101	3.6	94.3	4.8	101	6	98.3	4.7
	100	97	2.1	96.6	3.2	102	11.4	92.2	6.9
Tolclofos methyl	10	115	7.1	87.7	7.4	98.1	11.2	86.4	14.6
	20	105	9.5	94.2	7.8	101	7.6	93.1	7.5
	100	103	2.7	92.4	4.8	105	16.1	93.1	12.1
Trichloronate	10	111	2.8	84.6	7.2	95	10.4	94.9	9.8
	20	103	5.5	95.8	5.2	104	6.4	95.8	7.4
	100	99.1	2.1	96.2	4.7	103	10.1	98.3	13.3
Trifluralin	10	104	11.3	87.3	6.9	100	6.5	79.1	18.6
	20	95.8	15.8	84.7	7.9	108	7.6	81	13.1
	100	104	6.2	79.9	8.6	82.6	16.7	73.6	12.9
Vinclozolin	10	111	6.3	88.3	7.3	96.9	9.9	62.4	16.6
	20	105	7.6	95	7	99.4	5.9	89.8	7
	100	103	2.6	94.9	4.5	100	9.2	116	17.9

Furthermore, trifluralin residues in shrimp are associated with its use in the control of fungi and parasites in aquaculture farms and the surrounding environment. Further studies are needed to more clearly interpret the pesticide residues found in aquatic animal species.

The aquaculture industry has been overwhelmed by a wide range of parasitic and bacterial diseases affecting cultured species (Bondad-Reantaso et al., 2005). In order to prevent or treat these diseases, several chemicals have been used in high-density aquatic farms (Kang et al., 2018). Moreover, non-compliant samples in farmed aquatic animals are increasing due to the unintended and overuse of chemical compounds (Park et al., 2020). Further investigations are required to assess the dietary exposure to ethoxyquin residues and their health risks associated with the dietary intake of the farmed aquatic animals (Choi et al., 2020).

Conclusions

In this study, a multi-residue pesticide analysis method was developed and optimized for 51 pesticides in fishery products based on the QuEChERS approach combined with GC-MS/MS. The developed method was both selective and sensitive. The method was successfully tested on 79 fishery product samples purchased from the local markets in Korea, proving to be suitable for routine multi-residue analyses of target pesticides for monitoring purposes. Trifluralin was detected in one sample (1%). The proposed method was successfully validated and applied for the identification and confirmation of pesticides in fishery products. These findings indicate that these compounds do not need to be as persistent as pesticides to accumulate in fishery products. Additionally, more extensive monitoring studies are needed to understand the potential of these compounds to bioaccumulate and assess their runoff from river water into aquaculture farms.

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Conflict of interest

The authors declare that they have no conflict of interest.

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