fishes

MDPI

Article

Simultaneous Determination of Seven Pyrethroid Pesticide Residues in Aquatic Products by Gas Chromatography

Changling Fang, Xiaoyi Lou, Xuan Zhang, Siman Li, Yunyu Tang, Yongfu Shi * and Dongmei Huang

Key Laboratory of Control of Quality and Safety for Aquatic Products, Ministry of Agriculture and Rural Affairs, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China; fangling0334081@163.com (C.F.); huoxingmayi@126.com (X.L.); zhangxuan0430@163.com (X.Z.); lism@ecsf.ac.cn (S.L.); hi_tyy@163.com (Y.T.); hdm2001@126.com (D.H.)

* Correspondence: xyzmn530@sina.com

Abstract: Aquatic products are good sources of essential nutrients, but the presence of pyrethroids (PYRs) as contaminants may pose risks to consumer health as the intricate matrices of PYRs usually obstruct chemical hazard detection. In this study, a gas chromatographic method was established and validated for simultaneously detecting residues of seven PYRs (cyhalothrin, permethrin, cyfluthrin, cypermethrin, tau-fluvalinate, fenvalerate, deltamethrin) in aquatic products. The aim of this method was to assess better the potential risks of pesticide residues in aquatic products. The PYRs in aquatic products were extracted with acetonitrile, purified with n-hexane saturated with acetonitrile, and cleaned up on a Cleanert® Alumina N column. Statistical analysis and orthogonal array experimental design were used to optimize the key parameters. To validate the proposed method, commonly consumed aquatic products (such as carp, crucian carp, whiteleg shrimp, river crab, sea cucumber, and scallop) were obtained from local supermarkets in Shanghai. Satisfied linearity of the calibration curves was achieved in a matrix-matched standard solution, with a correlation coefficient (R^2) larger than 0.995. The average recoveries at five fortification levels varied from 77.0% to 117.2%, with relative standard deviations (RSDs) below 11.1%. Concerning electron capture detection, the limits of detection (LOD) and limits of quantification (LOQ) were 2.0–5.0 μg/kg and 5.0–10.0 μg/kg, respectively. These results demonstrate the high stability and sensitivity of this method for simultaneously detecting PYRs in aquatic products, having great practicability and which can be popularized easily.

Keywords: pyrethroids; aquatic products; gas chromatography; modified SPE pretreatment method; easy application

Key Contribution: Information on specific criteria for maximum residue limits (MRLs) of pyrethroid residues in aquatic products is currently limited domestically as substrates of aquatic products are complex. It is necessary to find a precise, and readily applicable gas chromatography (GC) method for the simultaneous detection of the seven pyrethroid residues. The method was successfully applied to different kinds of aquatic samples, including carp, crucian carp, whiteleg shrimp, river crab, sea cucumber, and scallop. The present method can be employed for routine monitoring and risk assessment research of pyrethroid residues in various kinds of aquatic products.

check for updates

Citation: Fang, C.; Lou, X.; Zhang, X.; Li, S.; Tang, Y.; Shi, Y.; Huang, D. Simultaneous Determination of Seven Pyrethroid Pesticide Residues in Aquatic Products by Gas Chromatography. *Fishes* **2024**, *9*, 79. https://doi.org/10.3390/fishes9030079

Academic Editor: Josef Velíšek

Received: 31 January 2024
Revised: 17 February 2024
Accepted: 17 February 2024
Published: 20 February 2024

1. Introduction

Aquatic products are widely consumed and considered one of the most crucial sources of animal protein with essential nutrients [1,2]. However, the inappropriate use of pesticides and the interference from other factors, such as the accumulation in sediments and aquatic organisms, have become a prominent problem with pesticide residues in aquatic products [3,4]. The pollution of crops and water sources resulting from pesticide misuse can lead to the enrichment of residual pesticides in aquatic products [5,6]. Safety incidents involving pyrethroids (PYRs) continue to emerge due to their inappropriate and excessive

overuse [7,8]. The presence of pesticide residues poses a significant threat to consumer health [9,10], thus, making it necessary to monitor and regulate the use of PYRs to ensure the quality and safety of aquatic products.

Since the 1980s, PYRs have gained popularity for their high efficiency, low toxicity, and biodegradability in preventing and treating crustacean parasites (such as lernaeidae, sinergasilus, and fish lice) in fishery production in China [11–13]. Aquatic organisms would be the most immediate victims if PYRs were to be released into aquatic ecosystems, although they do have satisfactory control effects on pests and external parasites of fish [7]. Due to their lipophilicity, it is difficult to eliminate PYRs once they enter organisms particularly arthropods and fish through food chains [14,15]. Previous studies confirmed that long-term and even low-dose exposure to pyrethroids may result in chronic diseases, since they can act as endocrine disruptors to interfere with the molecular mechanism of vertebrates, especially fish [13,16,17]. PYRs have been found to induce teratogenicity, carcinogenicity, and mutagenicity, and to have toxic effects on the endocrine, immune, and nervous systems of organisms [18,19]. For example, Wang et al. (2020) reported adverse effects on the male reproductive system caused by PYR exposure [20]. Safety concerns arising from pyrethroid residues have attracted widespread attention in recent years, and the determination of pyrethroid residues in aquatic products is vital for ensuring food safety.

Residues of pesticides present a critical obstacle to the export of aquatic products, as they pose a risk to the health of consumers and the sustainable development of the fishery economy. Many countries have set a maximum residue limit (MRL) on pyrethroids in food to safeguard public health. For instance, in the US, the limit of deltamethrin (DEL) in fish is set at 10 μg/kg [21]. The European Union (EU) stipulates that the limits of cypermethrin (CYP), fenvalerate (FEN), DEL, cyfluthrin (CYF), and permethrin (PER) in foodstuffs of animal origin (muscle) are 20 μg/kg, 25 μg/kg, 10 μg/kg, 10 μg/kg, and 50 μg/kg, respectively [22]. The Ministry of Agriculture and Rural Affairs (MARA) of the People's Republic of China issued the national standard of MRLs for CYP, FEN, DEL, CYF, cyhalothrin (CYH), and tau-fluvalinate (t-FLU) in food [23]. According to GB31650-2019 [24], the MRL of DEL in fish has been established as 30 μg/kg. However, to the best of our knowledge, specific criteria for MRLs of PYRs in aquatic products have not yet been established in China.

Over the past decades, the determination of some pyrethroids in aquatic products has already been reported [5,11,25–28]. Of them, several determination methods have been involved, including gas chromatography-mass spectrometry (GC-MS) [5,25], and gas chromatography (GC) [28]. GC is generally used to analyze pyrethroids due to their volatile properties which allow the direct determination of the compounds without a derivatization step. CYH, PER, CYF, CYP, t-FLU, FEN, and DEL were chosen in this study, since these seven pyrethroids are widely distributed and used in agriculture and aquaculture, and most of them were detected in 19 species of wild fish from 11 sites on the Pearl River Delta [25]. As far as we know, GB29705-2013 is the only national standard of China for PYRs in aquatic products [29], which provides a determination GC method for three PYRs (cypermethrin, fenvalerate, and deltamethrin) in fish and shrimp. However, this method is not suitable for detection in crab, sea cucumber, and scallop samples [30]. With increasing pesticide consumption and biological accumulation, the existing standard could not meet the monitoring needs [31]. Therefore, it is necessary to find a comprehensive, reliable, and easily accepted method for detection of pyrethroid residues in aquatic products to complement the deficiencies of the existing standard methods.

The goal of this study was to develop and validate a precise, reproducible, and robust method for sample preparation and determination of seven pyrethroid residues in aquatic products. Carp, crucian carp, whiteleg shrimp, river crab, sea cucumber, and scallop, were chosen since these aquatic samples are most commonly consumed by the residents of Shanghai, China [32]. The development of the method focused on wide coverage and easy application, and which can be expected to serve as an analytical tool for regulatory agencies to monitor pyrethroid residues in aquatic organisms.

2. Materials and Methods

2.1. Reagents and Chemicals

The standards of CYF, PER, CYF, CYP, t-FLU, FEN, and DEL were all obtained from Dr. Ehrenstorfer (Augsburg, Germany). All PYRs used in this study were of analytical grade (purity > 98.4%). The structural formulas of seven PYRs are presented in Figure 1. Stock solutions of each PYR at 100 mg/L were prepared by dissolving each compound in benzene. The different concentrations of the mix-standards were prepared by dissolving the seven compounds in n-hexane. High performance liquid chromatographic (HPLC)-grade benzene, n-hexane, ethyl acetate, acetone, and acetonitrile were obtained from J. T. Baker Chemical Company, New Jersey, USA. Three solid phase extraction (SPE) cartridges were chosen for the clean-up procedure: Florisil (500 mg/6 mL, Agela, CA, USA), C18 (500 mg/6 mL, Agela, CA, USA), and neutral alumina (500 mg/6 mL, Cleanert, Agela, CA, USA). Sodium chloride (NaCl) and sodium sulfate anhydrous (Na_2SO_4) were supplied by Sinopharm Group Chemicals Limited (Shanghai, China). NaCl and $NaSO_4$ were heated at 650 °C for 4 h before using them, cooled in a desiccator, and stored in sealed bottles. The n-hexane saturated with acetonitrile was prepared as follows: 200 mL n-hexane was taken into a 250 mL liquid separator funnel and mixed with an appropriate amount of pure acetonitrile, which was then shaken vigorously after distribution equilibrium, and the upper n-hexane was used for the experiment.

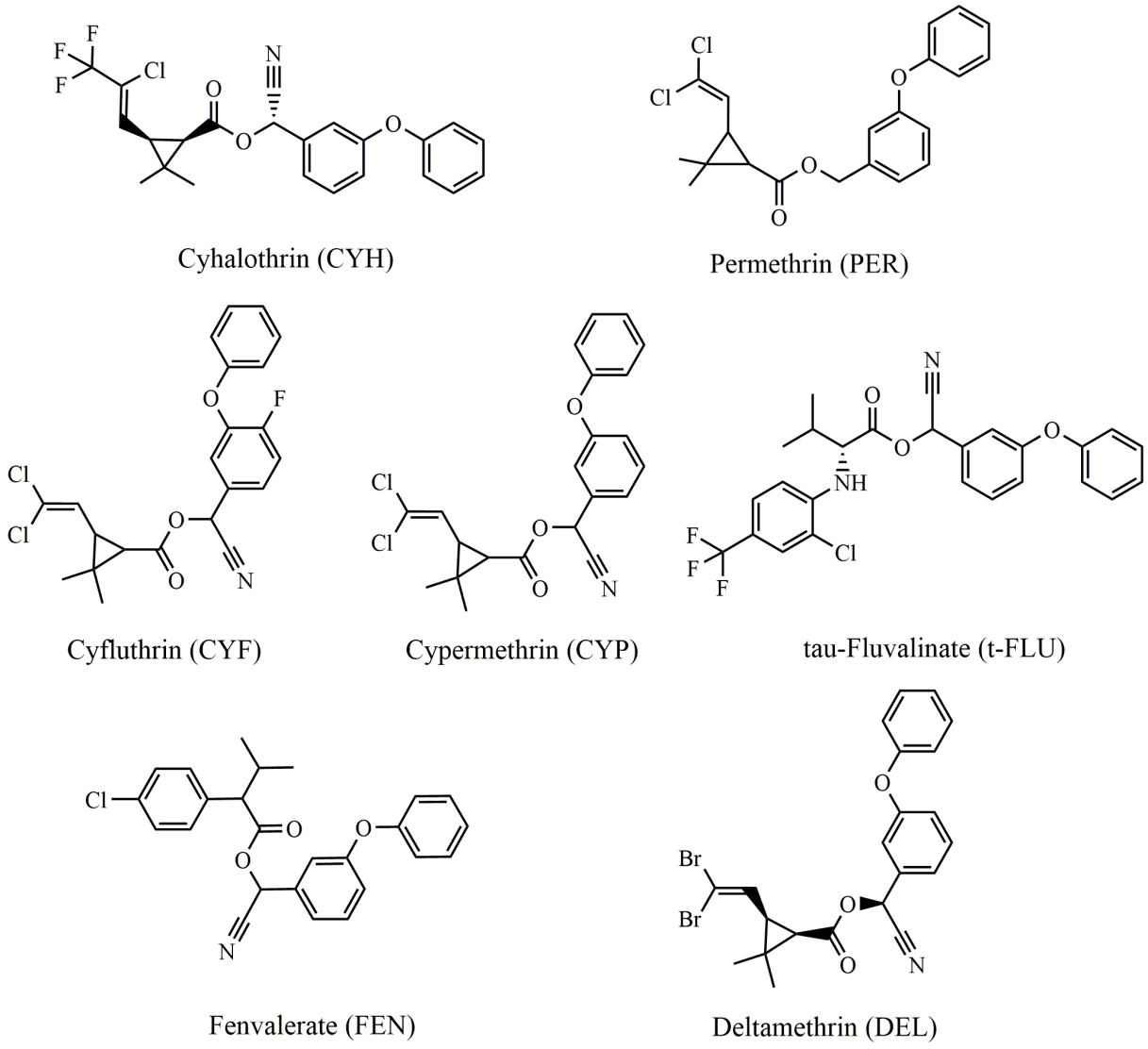

Figure 1. The structural formulas of the seven pyrethroids.

2.2. Sample Collection

Aquatic samples, including carp, crucian carp, whiteleg shrimp, river crab, sea cucumber, and scallops, were purchased from local supermarkets in Shanghai (China). The samples were collected according to the GB/T 30891-2014 standard set by the Ministry of Agriculture of the People's Republic of China (MOA) [33]. The scallop samples weighed at least 700 g, and the other aquatic samples weighed at least 400 g. Carp (edible parts) was used initially as a sample in this study to seek optimization of the analytical conditions. After the confirmation, the final method was applied to quantify pyrethroid residues in other aquatic samples mentioned above to extend the scope.

2.3. Sample Extraction

The method used to extract and purify pyrethroid residues from aquatic samples was a multi-step process involving acetonitrile and n-hexane saturated with acetonitrile combined with solid phase extraction (SPE).

The procedure is depicted in Figure 2 and comprised the following steps. (1) To begin 5.0 g of homogenized sample was placed in a 50 mL polypropylene centrifuge tube and mixed with 10 mL of acetonitrile. (2) Then, 4 g of anhydrous NaCl was added to the mixture, vortexed for 2 min using a Multitube Vortex Mixer (DMT-2500, MIULAB, Hangzhou, China), and centrifuged at 4000 rpm for 5 min using a refrigerated centrifuge (CF16RX, Hitachi, Tokyo, Japan). The resulting supernatant (10 mL) was transferred to a new centrifuge tube, while the remaining residue was extracted again with 5 mL of acetonitrile and the second obtained supernatant (5 mL) was combined with the first one. (3) Following this, 5 g of anhydrous Na_2SO_4 was added to the obtained solution, and the mixture was vortexed for 1 min and centrifuged at 4000 rpm for 5 min. After centrifugation, 5 mL of n-hexane saturated with acetonitrile was added to the mixture, which was shaken for 2 min to homogenize and then centrifuged at 4000 rpm for 5 min. The upper layer of n-hexane was discarded, and the remaining liquid was cleaned once more with 5 mL n-hexane saturated with acetonitrile. (4) The extracts (15 mL) were transferred to Cleanert® Alumina N columns, which were preconditioned with 5 mL of acetonitrile before use. (5) The effluents were collected in 20 mL glass tubes and evaporated to dryness under a stream of nitrogen at 40 °C. (6) The extracted samples were reconstituted with 2.0 mL of n-hexane by homogenization. Finally, 2 µL of the final solution was used for GC analysis.

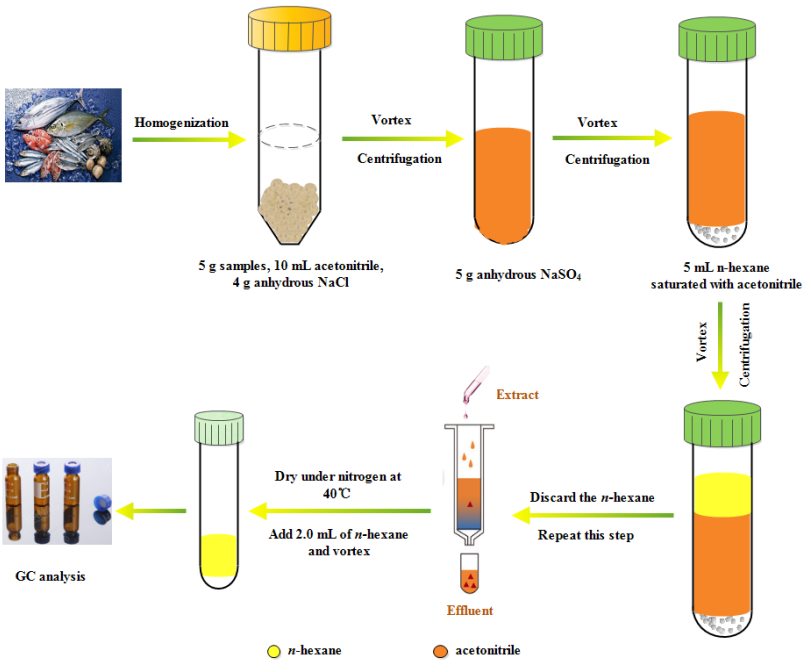

Figure 2. The diagrammatic drawing of the modified method.

2.4. Gas Chromatography Analysis

A gas chromatograph (7890A, Agilent Technologies, CA, USA) equipped with an electron capture detector (ECD) was used to analyze the obtained extracts. Pyrethroid pesticide separations were performed using an HP-35 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). Nitrogen, with a purity of 99.999%, was used as carrier gas under 2.0 mL/min flow. The oven temperature program was initially at 100 °C (held 0.5 min), and ramped to 250 °C with a 30 °C/min rate, then increased up to 280 °C with 3 °C/min rate (held 8 min), with a total run time of 23.5 min [34,35]. Splitless injection was used and the injector and ECD temperatures were set at 280 °C and 300 °C, respectively before use. The external standard technique and matrix matching standard curve were used for quantitation through peak-area comparison.

3. Results and Discussion

3.1. Selection of the Capillary Column

The capillary column is an important factor affecting the separation effect during PYR separation, as well as the carrier gas velocity and the oven temperature. The selection of the appropriate capillary column should be the first thing to be considered when determining the target analytes. To achieve a better separation effect, two different capillary columns were used for the analysis of PYRs [35,36]. They were the HP-5 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) and HP-35 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). As shown in Figure S1, FEN and t-FLU could not be separated using the HP-5 column, since the second peak of FEN coincides exactly with that of t-FLU. The separation chromatogram of the HP-35 capillary column is displayed in Figure 3. It shows that the seven PYRs could be separated under the same conditions with the HP-35 column without the interference of other chromatographic parameters, which was better than that of the HP-5 column in terms of separation effect. Thus, PYR separations were performed using an HP-35 capillary column.

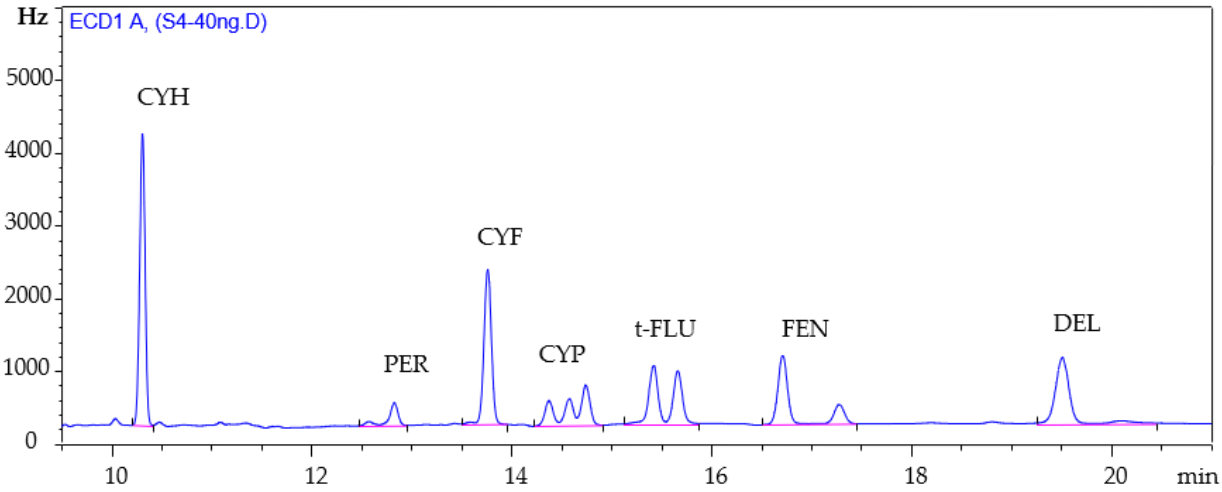

Figure 3. The chromatogram of seven pyrethroid standard solutions using HP-35 capillary column. (40 ng/mL).

3.2. Selection of Extraction Solvents

Aquatic product samples usually contain phospholipid, lipid-soluble pigment, and water-soluble proteins, which can be extracted along with the target substance [37,38]. These impurities further interfere with the determination of pyrethroid residues and then affect the performance and life of the analytical instruments. Therefore, it is necessary to choose a solvent with good solubility of the pyrethroids and low solubility of the impurities.

The selection of suitable solvents is very important to obtain good sensitivity and better extraction efficiency of the PYRs [39,40]. Commonly, the extraction solvents are n-hexane,

ethyl acetate, acetone, and acetonitrile. N-hexane/acetone (1:1) and acetonitrile were used for the extraction of spiked carp samples with 20 μg/kg of each pyrethroid in this study. The ethyl acetate reagent was excluded because of its impurity peaks and large baseline noise, which could disturb the accuracy and quantification of the target. As can be seen in Figure 4, several PYRs achieved high recoveries when N-hexane/acetone (1:1) was applied, such as CYH (88.7%), CYF (88.5%), and CYP (92.1%), while the recoveries of FEN and DEL were only 58.9% and 62.1%. Satisfactory recoveries (>83%) were obtained when acetonitrile was used to extract the PYRs as presented in Figure 4. Using acetonitrile as extraction agent has less interference on the extraction process, which could not only shorten the extraction process, but also cater to the requirements of the recoveries. Therefore, acetonitrile was selected as the extraction reagent in this study.

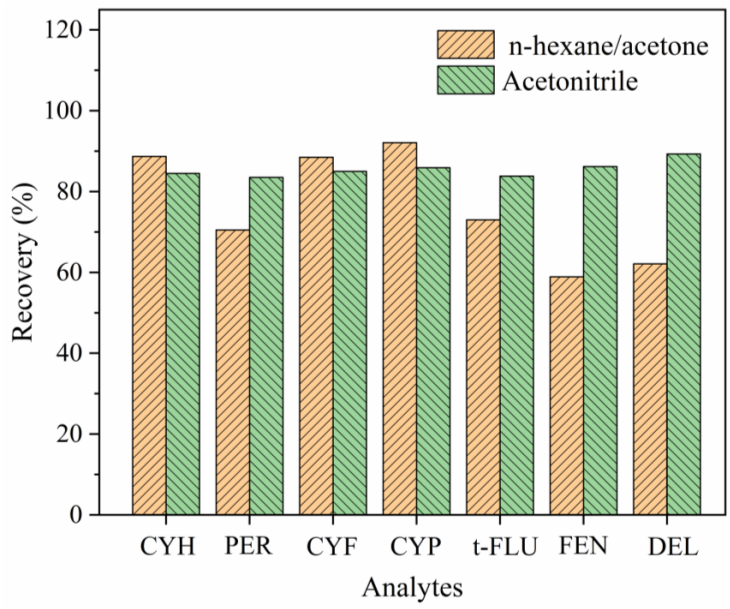

Figure 4. The recoveries of seven pyrethroids with different extraction solvents.

3.3. Selection of Clean-Up Methods

The qualitative and quantitative analysis of PYRs can be interfered with due to the complexity of the aquatic product matrix, which might further pollute the capillary and detector. Acetonitrile denatures the protein and causes turbidity in the extract. Although protein can be removed by centrifugation, fats and phospholipids contained in aquatic products will be extracted along with the target PYRs, further causing interference with the separation of the target PYRs. Therefore, it is necessary to effectively purify the acetonitrile extract. Solid phase extraction (SPE) is a commonly used purification method of pesticide extract [41,42].

In this study, three different commercial SPE cartridges (Florisil, C18 and neutral alumina) were evaluated by analyzing spiked carp samples with 20 μg/kg of PYRs, to obtain better recoveries for each PYR. Figures 5 and S2 showed the effect of three SPE cartridges on the removal of matrix interference and the recoveries of the seven PYRs. As presented in Figure S2, better elimination of the matrix effect was obtained when using the neutral alumina cartridge, since the matrix effects were still obvious after purification with Florisil and the C18 cartridge. Significant differences were observed in the spiked recoveries of the seven PYRs when purified by three different SPE cartridges. The spiked recoveries of PER, CYP, and DEL were relatively low without SPE cartridge purification, indicating that the three pesticides had matrix effects, which needed to be further weakened. As shown in Figure 5, three analytes (CYF, FEN, and DEL) obtained high recoveries (more than 85%) using Florisil cartridges, but the recoveries of PER, CYP, and t-FLU were less than 53%. Acceptable recoveries were achieved using the C18 cartridge, since CYH and FEN (93.4% and 89.7%) were higher than when using the neutral alumina cartridge (84.5% and

86.2%). However, the recoveries of other PYRs were all lower than that using the neutral alumina cartridge, especially CYF (65.7%) and CYP (64.9%). Due to the polarity of their chemical structure, PYRs are difficult to retain on the neutral alumina cartridge, which is commonly used to remove aromatic and aliphatic compounds [43,44]. The results verified that all seven PYRs could flow out completely with recoveries above 83% when a neutral alumina cartridge was used. The observed results showed that neutral alumina cartridges are relatively more stable, and better than the other two SPE cartridges when considering the purification effects. Therefore, the neutral alumina cartridge was finally used as the pass-through cleanup cartridge for pyrethroid purification in this study.

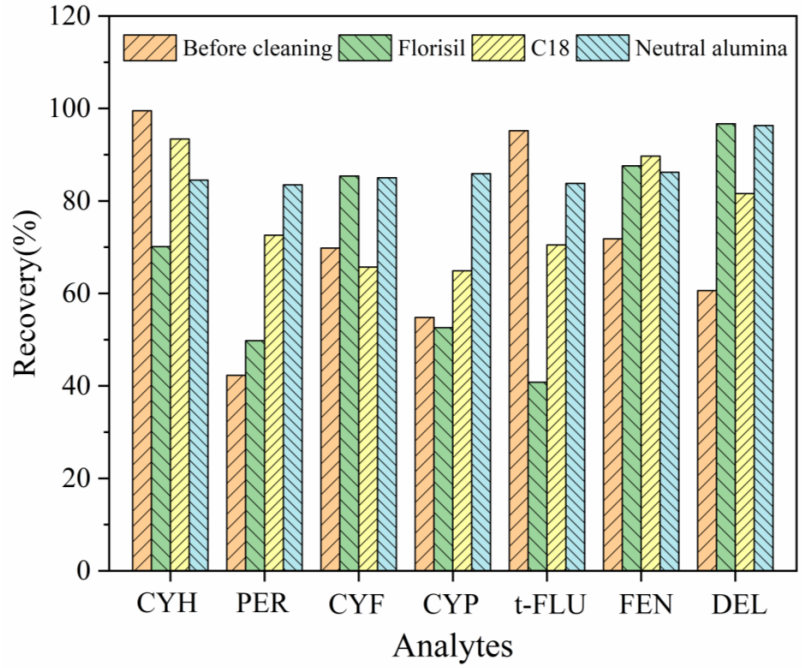

Figure 5. The recoveries of the seven pyrethroids purified by three pass-through SPE cartridges.

3.4. Method Validation

Optimum experimental conditions were conducted to evaluate the modified GC method for quantifying the seven PYRs using the standard addition method. The method was validated in terms of selectivity, linearity, limit of detection (LOD) and quantitation (LOQ), accuracy, and precision.

3.4.1. Selectivity

Under the selected chromatographic conditions, the samples were not interfered with by other substances in the retention times of the pesticides analyzed. The selectivity of the analytical method was verified by comparison of the chromatogram of a sample without the addition of the pesticide standard with the same sample fortified with the standards of the compounds analyzed at a concentration of 40 ng/mL for each PYR.

3.4.2. Linearity

The linearity of the analytical method was demonstrated using matrix-matched calibration standards. Six different matrix-matched calibration curves (carp, crucian carp, whiteleg shrimp, sea cucumber, scallop, and river crab) were conducted. The calibration curves were prepared in the range of (2.0–100) μg/L for carp, crucian carp, whiteleg shrimp, sea cucumber, and scallop analytes, and (4.0–200) μg/L for the river crab analytes. By constructing calibration curves, all PYRs showed good linearity with R^2 values greater than 0.995. Table 1 shows strong linear correlation between the concentration and the peak areas of the compounds analyzed, enabling the quantification of pesticides in aquatic products.

Table 1. Linearity and analysis limits of pyrethroids.

Analytes	Aquatic Products	Calibration Curve	Correlation Coefficient (R^2)	Linear Range (μg/L)	LOD (μg/kg)	LOQ (μg/kg)	Intraday RSD (%)	Interday RSD (%)
CYH	A [a]	y = 423.605x − 872.080	0.9981	2.0–100	2.0	5.0	3.23~5.70	1.01~11.1
	B [b]	y = 426.009x − 1046.744	0.9982				2.55~7.72	1.13~4.47
	C [c]	y = 474.335x − 582.351	0.9992				5.89~7.48	2.59~10.5
	D [d]	y = 503.486 x − 530.919	0.9958				3.34~6.02	4.80~10.1
	E [e]	y = 414.387x − 596.557	0.9997				6.10~9.55	3.26~9.39
	F [f]	y = 1004.350x + 651.708	0.9996	4.0–200	5.0	10.0	5.39~6.70	3.31~10.5
PER	A	y = −50.169x + 110.006	0.9978	2.0–100	2.0	5.0	3.16~7.49	1.60~10.2
	B	y = 50.289x + 132.365	0.9958				1.06~4.02	1.73~3.82
	C	y = 64.363x + 4.265	0.9980				3.03~6.99	1.56~10.9
	D	y = 56.578x + 236.658	0.9956				4.47~8.88	2.84~8.63
	E	y = 51.278x + 300.166	0.9952				7.31~9.39	4.65~10.6
	F	y = 115.010x + 321.741	0.9954	4.0–200	5.0	10.0	6.24~8.34	1.40~8.77
CYF	A	y = 355.083x + 272.933	0.9990	2.0–100	2.0	5.0	2.93~8.82	2.35~9.44
	B	y = 356.444x + 174.074	0.9990				1.05~5.68	0.44~5.55
	C	y = 405.200x − 494.986	0.9991				5.83~7.66	1.68~8.96
	D	y = 406.796x − 226.775	0.9995				4.03~6.76	1.54~8.14
	E	y = 293.246x − 72.026	0.9997				4.28~8.47	2.92~4.73
	F	y = 859.599x − 2361.579	0.9948	4.0–200	5.0	10.0	5.98~8.17	2.99~10.1
CYP	A	y = 228.655x + 112.484	0.9966	2.0–100	2.0	5.0	2.45~5.53	3.24~10.3
	B	y = 229.841x +26.293	0.9966				3.05~6.57	1.96~8.18
	C	y = 250.180x +104.107	0.9995				4.51~7.86	2.51~10.8
	D	y = 232.540x + 302.113	0.9970				4.47~6.60	2.45~7.06
	E	y = 218.375x + 876.602	0.9971				3.62~9.55	2.83~10.1
	F	y = 536.696x − 1227.487	0.9956	4.0–200	5.0	10.0	3.17~10.99	3.79~11.1
t-FLU	A	y = 312.937x + 357.533	0.9965	2.0–100	2.0	5.0	4.41~10.9	0.82~10.3
	B	y = 315.477x + 173.000	0.9967				1.44~4.44	1.35~7.89
	C	y = 364.654x − 293.698	0.9993				4.95~6.54	2.53~9.37
	D	y = 333.022x − 27.745	0.9998				4.19~10.8	3.72~10.6
	E	y = 307.854x + 556.203	0.9966				6.41~9.08	2.09~4.84
	F	y = 785.214x − 1659.751	0.9937	4.0–200	5.0	10.0	6.17~8.33	1.56~10.5
FEN	A	y = 265.370x − 197.144	0.9957	2.0–100	2.0	5.0	3.54~7.28	2.46~9.95
	B	y = 267.353x − 341.241	0.9957				2.89~5.05	2.75~6.18
	C	y = 320.138x − 35.931	0.9998				3.72~8.70	1.60~10.9
	D	y = 282.965x + 160.786	0.9991				5.77~7.68	4.33~10.4
	E	y = 297.034x + 790.323	0.9988				4.26~9.91	1.26~7.74
	F	y = 669.215x − 1342.672	0.9959	4.0–200	5.0	10.0	4.20~8.29	1.42~6.58
DEL	A	y = 282.223x − 570.911	0.9983	2.0–100	2.0	5.0	4.13~9.87	1.70~9.98
	B	y = 283.967x − 697.655	0.9984				1.69~6.81	2.04~5.10
	C	y = 342.853x − 504.689	0.9992				6.76~8.96	3.96~10.0
	D	y = 306.851x − 19.581	0.9995				5.15~6.86	5.06~9.53
	E	y = 281.446x − 94.842	0.9982				3.27~8.52	2.25~5.40
	F	y = 645.332x − 1049.162	0.9964	4.0–200	5.0	10.0	3.80~8.82	2.15~6.90

[a] A, Carp. [b] B, Crucian carp. [c] C, Whiteleg shrimp. [d] D, Sea cucumber. [e] E, Scallop. [f] F, River crab.

3.4.3. Limits of Detection and Quantification

The minimum concentration of each analyte determines the values of LOD and LOQ, where the signal-to-noise ratio (S/N) is greater than 3 and 10, respectively [2,45]. As summarized in Table 1, the LOD and LOQ of the seven PYRs in carp, crucian carp, whiteleg shrimp, sea cucumber, and scallop analytes, were 2.0 μg/kg and 5.0 μg/kg. The LOD and LOQ of the seven PYRs in the river crab analytes were 5.0 μg/kg and 10.0 μg/kg. These above results reconfirmed that an analytical method could be used to determine PYRs in aquatic samples.

3.4.4. Accuracy and Precision

The accuracy and precision of the method were determined by spiking of PYRs at five different concentrations. The spiking of PYRs in river crab were different from the other five analytes, since there are different matrix effects between different kinds of aquatic product samples [46]. Recovery experiments are generally used to evaluate the accuracy of the analytical method (Table 2). It is possible to observe that the recoveries of the seven PYRs in six different analytes ranged from 77.0% to 117.2% at all five spiking levels. To evaluate the precision of the method, the intraday RSD values were calculated by analyzing spiked samples on the same day with the same instrument and operator. The inter-day RSD results were obtained using the identical method on three separate days with the same instrument and operator (Table 1). The method showed a satisfactory precision for the seven pyrethroids analyzed in six different analytes, since the RSDs ranged from 1.06% to 11.1% and were lower than 15%.

Table 2. Accuracy and precision of the seven pyrethroids in different aquatic products (n = 3).

Analytes	Spiking Level (μg/kg)	Recovery/% (RSD/%)					
		Carp	Crucian Carp	Whiteleg Shrimp	Sea Cucumber	Scallop	River Crab
CYH	2	107.6 (3.86)	102.1 (3.96)	101.4 (6.00)	103.0 (6.02)	98.8 (7.12)	/
	5	82.7 (5.34)	82.4 (5.58)	95.9 (6.23)	107.1 (5.23)	78.0 (9.55)	93.2 (5.46)
	10	86.2 (3.23)	83.5 (5.71)	92.5 (5.89)	85.7 (3.34)	77.1 (7.00)	88.5 (6.70)
	20	84.5 (3.67)	92.7 (7.72)	89.6 (5.97)	87.7 (3.41)	78.7 (6.10)	85.9 (6.37)
	50	85.5 (5.70)	88.7 (2.55)	91.9 (7.48)	86.2 (3.61)	82.3 (8.38)	85.1 (6.29)
	100	/	/	/	/	/	84.5 (5.39)
PER	2	89.3 (7.49)	101.2 (4.02)	101.1 (6.99)	93.5 (8.55)	94.7 (7.93)	/
	5	85.4 (6.14)	96.4 (1.06)	91.6 (5.57)	97.9 (8.88)	81.0 (9.88)	94.3 (7.32)
	10	86.8 (3.53)	96.7 (2.47)	92.9 (3.03)	101.6 (4.47)	88.0 (7.31)	92.8 (6.85)
	20	83.5 (4.48)	84.9 (2.03)	86.9 (4.63)	88.0 (4.75)	83.9 (9.39)	93.6 (8.34)
	50	87.1 (3.16)	86.2 (3.88)	89.3 (3.62)	85.2 (4.64)	79.6 (6.08)	91.4 (6.72)
	100	/	/	/	/	/	91.0 (6.24)
CYF	2	102.2 (8.82)	102.3 (1.05)	108.7 (6.57)	85.6 (4.03)	91.8 (6.68)	/
	5	83.1 (2.93)	98.6 (5.27)	96.3 (7.66)	92.9 (6.76)	82.2 (8.36)	84.6 (6.06)
	10	83.4 (6.00)	89.5 (5.68)	95.7 (6.33)	84.7 (4.42)	77.0 (6.99)	77.8 (8.17)
	20	85.0 (3.90)	87.4 (1.73)	91.8 (5.93)	87.3 (4.77)	80.5 (8.47)	84.0 (5.98)
	50	82.3 (4.25)	84.9 (2.80)	93.7 (5.83)	87.9 (5.54)	78.3 (4.28)	86.2 (6.52)
	100	/	/	/	/	/	87.1 (7.32)
CYP	2	105.7 (2.45)	101.1 (3.06)	103.4 (5.07)	93.0 (6.08)	90.6 (6.14)	/
	5	88.2 (4.37)	85.8 (6.57)	89.7 (7.58)	101.5 (6.60)	84.1 (3.62)	90.9 (6.95)
	10	89.3 (4.22)	84.0 (3.21)	90.1 (4.68)	87.4 (6.57)	79.9 (7.92)	90.9 (10.2)
	20	85.9 (3.65)	84.3 (5.49)	89.7 (4.51)	87.9 (4.47)	82.1 (9.55)	87.6 (11.1)
	50	87.4 (5.53)	88.6 (3.05)	88.7 (7.86)	90.8 (4.57)	78.6 (8.73)	85.5 (7.94)
	100	/	/	/	/	/	90.5 (5.76)

Table 2. *Cont.*

Analytes	Spiking Level (μg/kg)	Recovery/% (RSD/%)					
		Carp	Crucian Carp	Whiteleg Shrimp	Sea Cucumber	Scallop	River Crab
t-FLU	2	106.8 (7.56)	98.3 (3.85)	101.5 (4.96)	97.5 (10.8)	89.5 (6.89)	/
	5	88.4 (4.41)	91.6 (2.17)	97.5 (6.54)	105.3 (5.42)	82.3 (7.09)	88.8 (8.24)
	10	84.2 (5.45)	83.7 (1.44)	92.1 (4.95)	97.6 (4.19)	83.2 (9.08)	89.2 (6.85)
	20	83.8 (4.81)	84.7 (4.02)	90.3 (6.44)	100.1 (5.52)	83.0 (8.93)	82.8 (8.33)
	50	81.7 (10.9)	84.9 (4.44)	90.2 (5.47)	97.3 (6.32)	82.4 (6.41)	83.3 (8.17)
	100	/	/	/	/	/	86.0 (6.17)
FEN	2	107.9 (6.11)	97.4 (3.64)	97.1 (7.82)	99.9 (7.68)	88.4 (6.24)	/
	5	117.2 (3.54)	83.9 (4.51)	96.9 (7.90)	102.3 (5.77)	89.4 (9.91)	89.7 (8.29)
	10	82.6 (7.22)	87.1 (2.89)	99.9 (3.72)	95.2 (5.92)	87.1 (4.26)	89.0 (6.43)
	20	86.2 (4.70)	86.2 (3.88)	91.2 (8.70)	90.0 (6.69)	85.0 (6.92)	85.5 (7.69)
	50	80.0 (7.28)	80.9 (5.05)	91.6 (4.84)	90.6 (7.09)	80.8 (6.35)	83.4 (8.21)
	100	/	/	/	/	/	86.5 (4.20)
DEL	2	90.8 (5.43)	95.3 (6.81)	103.5 (8.96)	88.1 (6.04)	82.9 (7.79)	/
	5	87.8 (5.34)	89.3 (1.69)	96.6 (7.86)	92.8 (6.86)	85.0 (7.47)	88.8 (6.98)
	10	89.5 (9.18)	81.8 (6.57)	94.5 (7.09)	87.4 (5.15)	83.0 (8.52)	87.0 (8.82)
	20	89.3 (4.13)	88.7 (2.55)	92.6 (6.76)	89.1 (5.40)	82.6 (5.31)	87.0 (5.72)
	50	83.2 (9.87)	84.1 (2.53)	93.7 (8.55)	86.7 (5.68)	83.8 (3.27)	87.7 (5.87)
	100	/	/	/	/	/	86.6 (3.80)

3.5. Application in Real Samples

The validated method was applied to determine the seven pyrethroid residues in six aquatic organisms, purchased from Jiangsu, Shanghai, and Liaoning Province, after obtaining the optimum conditions for the extraction and purification. To achieve reliable results, each experiment was performed in triplicate, and spiked samples at a concentration of 20 μg/kg were also carried out. The PYR contents in carp, crucian carp, whiteleg shrimp, sea cucumber, scallop, and river crab are listed in Table S1. PER was detected in crucian carp, whiteleg shrimp, and scallop samples in concentrations of 0.54 μg/kg, 0.32 μg/kg, and 0.56 μg/kg, respectively (Figure S3, Table S1). Lower amounts of DEL were also detected in whiteleg shrimp and river crab samples, which were 0.45 μg/kg and 0.73 μg/kg, respectively. All the pyrethroid residues detected in the six aquatic organisms were less than the LOQ, and were lower than the limit set by the US (10 μg/kg) and EU (10 μg/kg) [21,22].

4. Conclusions

A method was developed and validated for the analysis of seven PYRs in six aquatic organisms using a modified SPE pretreatment method and detection by GC-ECD. The LOD and LOQ values of this method were 2.0–5.0 μg/kg and 5.0–10.0 μg/kg, respectively. The average recoveries were between 77.0% and 117.2% and the RSD values were less than 11.1%. Compared with previous findings [38], a combination of acetonitrile and n-hexane saturated with acetonitrile, rather than 40 mL acetone and 20 mL ethyl acetate–cyclohexane (50 + 50, *v*/*v*), was used for sample extraction, thus, demonstrating reduced solvent and cost-effectiveness. The extracts were cleaned up on a Cleanert® Alumina N column rather than a Florisil cartridge as it was more effective and with the advantage of a short cleanup period [47]. The method exhibited satisfactory LOQs, specificity, accuracy, and precision for six aquatic products commonly consumed in Shanghai (China) and could be used as an analytical tool by regulatory agencies for monitoring the presence of pyrethroid residues in aquatic organisms. Considering the results from the method application, further investigations could be conducted to determine residues of pyrethroids in environmental samples of aquatic organisms from Shanghai.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9030079/s1, Figure S1. The chromatogram of FEN and t-FLU standards solution using HP-35 capillary column, Figure S2. The effect of three SPE columns cartridges on the removal of matrix interference of 7 PYRs, Figure S3. The chromatograms of PER in a scallop sample (a) and seven PYRs in standards solution (b), Table S1. Real sample analysis of six different aquatic products (n = 3).

Author Contributions: C.F.: Investigation, Data curation, Formal analysis, Writing—original draft. X.L.: Validation, Data curation. X.Z.: Data curation, Investigation. S.L.: Data curation. Y.T.: Investigation, Data curation. Y.S.: Funding acquisition, Supervision. D.H.: Funding acquisition, Supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Central-Public interest Scientific Institution Basal Research Fund (2019T13, 2019T14) and the Central Public-interest Scientific Institution Basal Research Fund, ECSFR, CAFS (NO. 2022TD02, NO. 2021M05).

Institutional Review Board Statement: No ethical parameters required as the experimental materials used were all commercially available edible aquatic products.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Golden, C.D.; Koehn, J.Z.; Shepon, A. Aquatic foods to nourish nations. *Nature* **2021**, *598*, 315–320. [CrossRef] [PubMed]
2. Wongmaneepratip, W.; Leong, M.; Yang, H.S. Quantification and risk assessment of pyrethroid residues in seafood based on nanoparticle-extraction approach. *Food Control* **2022**, *133*, 108612. [CrossRef]
3. Carvalho, F.P. Pesticides, environment, and food safety. *Food Energy Secur.* **2017**, *6*, 48–60. [CrossRef]
4. Tang, T.; Wu, R.X.; Zhang, L.; Wang, Y.H.; Ling, J.; Du, W.; Shen, G.F.; Chen, Y.C.; Zhao, M.R. Distribution and partitioning of pyrethroid insecticides in agricultural lands: Critical influencing factors. *Environ. Int.* **2021**, *15*, 106736. [CrossRef] [PubMed]
5. de Oliveira, L.G.; Kurz, M.H.S.; Guimarães, M.C.M.; Martins, M.L.; Prestes, O.D.; Zanella, R.; da Silva Ribeiro, J.N.; Gonçalves, F.F. Development and validation of a method for the analysis of pyrethroid residues in fish using GC–MS. *Food Chem.* **2019**, *297*, 124944. [CrossRef]
6. Zhang, Q.; Ying, Z.T.; Tang, T.; Guo, B.; Gu, S.J.; Fu, L.L.; Dai, W.; Lin, S. Residual characteristics and potential integrated risk assessment of synthetic pyrethroids in leafy vegetables from Zhejiang in China-Based on a 3-year investigation. *Food Chem.* **2021**, *365*, 130389. [CrossRef]
7. Tang, W.X.; Wang, D.; Wang, J.Q.; Wu, Z.W.; Li, L.Y.; Huang, M.L.; Xu, S.H.; Yan, D.Y. Pyrethroid pesticide residues in the global environment: An overview. *Chemosphere* **2018**, *191*, 990–1007. [CrossRef]
8. Bille, L.; Binato, G.; Gabrieli, C.; Manfrin, A.; Pascoli, F.; Pretto, T.; Toffan, A.; Pozza, M.D.; Angeletti, R.; Arcangeli, G. First report of a fish kill episode caused by pyrethroids in Italian freshwater. *Forensic Sci. Int.* **2017**, *281*, 176–182. [CrossRef]
9. Chen, S.; Gu, S.; Wang, Y.; Yao, Y.; Wang, G.; Jin, Y.; Wu, Y. Exposure to pyrethroid pesticides and the risk of childhood brain tumors in East China. *Environ. Pollut.* **2016**, *218*, 1128–1134. [CrossRef]
10. Li, H.Z.; Cheng, F.; Wei, Y.L.; Lydy, M.J.; You, J. Global occurrence of pyrethroid insecticides in sediment and the associated toxicological effects on benthic invertebrates: An overview. *J. Hazard. Mater.* **2017**, *324*, 258–271. [CrossRef]
11. Aznar-Alemany, O.; Eljarrat, E.; Barcelo, D. Effect of pyrethroid treatment against sea lice in salmon farming regarding consumers' health. *Food Chem. Toxicol.* **2017**, *105*, 347–354. [CrossRef] [PubMed]
12. Langford, K.H.; Oxnevad, S.; Schoyen, M.; Thomas, K.V. Do antiparasitic medicines used in aquaculture pose a risk to the Norwegian aquatic environment? *Environ. Sci. Technol.* **2014**, *48*, 7774–7780. [CrossRef] [PubMed]
13. Brander, S.; Gabler, M.; Fowler, N.; Connon, R.; Schlenk, D. Pyrethroid pesticides as endocrine disruptors: Molecular mechanisms in vertebrates with a focus on fishes. *Environ. Sci. Technol.* **2016**, *50*, 8977–8992. [CrossRef] [PubMed]
14. Alonso, M.B.; Feo, M.L.; Corcellas, C.; Vidal, L.G.; Bertozzi, C.P.; Marigo, J.; Barceló, D. Pyrethroids: A new threat to marine mammals? *Environ. Int.* **2012**, *47*, 99–106. [CrossRef] [PubMed]
15. Arnot, J.A.; Gobas, F.A. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ. Toxicol. Chem.* **2004**, *23*, 2343–2355. [CrossRef] [PubMed]
16. Tyler, C.; Beresford, N.; Van der Woning, M.; Sumpter, J.; Thorpe, K. Metabolism and environmental degradation of pyrethroid insecticides produce compounds with endocrine activities. *Environ. Toxicol. Chem.* **2010**, *19*, 801–809. [CrossRef]
17. Hu, Y.; Zhang, Y.; Vinturache, A.; Wang, Y.; Shi, R.; Chen, L.; Qin, K.; Tian, Y.; Gao, Y. Effects of environmental pyrethroids exposure on semen quality in reproductive-age men in Shanghai, China. *Chemosphere* **2020**, *245*, 125580. [CrossRef]
18. Cremonese, C.; Piccoli, C.; Pasqualotto, F.; Clapauch, R.; Koifman, R.; Koifman, S.; Freire, C. Occupational exposure to pesticides, reproductive hormone levels and sperm quality in young Brazilian men. *Reprod. Toxicol.* **2017**, *67*, 174–185. [CrossRef]

19. Li, H.Z.; Ma, H.Z.; Lydy, M.J.; You, J. Occurrence, seasonal variation and inhalation exposure of atmospheric organophosphate and pyrethroid pesticides in an urban community in South China. *Chemosphere* **2014**, *95*, 363–369. [CrossRef]
20. Wang, Q.; Shen, J.; Zhang, R.; Hong, J.; Li, Z.; Ding, Z.; Wang, H.; Zhang, J.; Zhang, M.; Xu, L. Effects and mechanisms of pyrethroids on male reproductive system. *Toxicology* **2020**, *438*, 152460. [CrossRef]
21. The U.S. Environmental Protection Agency (EPA). *EPA (2013) Electronic Code of Federal Regulations, Title 40: Protection of Environment. PART 180 Tolerances and Exemptions for Pesticide Chemical Residues in Food. Subpart C: Specific Tolerances*; Environmental Protection Agency: Washington, DC, USA, 2013.
22. Commission Regulation (EU) No 37/2010 of 22 December 2009 on Pharmacologically Active Substances and Their Classification Regarding Maximum Residue Limits in Foodstuffs of Animal Origin, Official Journal of the European Communities, L15 (2010). Available online: http://data.europa.eu/eli/reg/2010/37(1)/oj (accessed on 15 February 2024).
23. *GB 2763-2021*; National Food Safety Standard Maximum Residue Limits for Pesticides in Food. Ministry of Agriculture of the People's Republic of China (MOA): Beijing, China, 2021.
24. *GB 31650-2019*; National Food Safety Standard Maximum Residue Limits for Veterinary Drugs in Foods. Ministry of Agriculture of the People's Republic of China (MOA): Beijing, China, 2019.
25. Xie, W.; Zhao, J.; Zhu, X.; Chen, S.; Yang, X. Pyrethroid bioaccumulation in wild fish linked to geographic distribution and feeding habit. *J. Hazard. Mater.* **2022**, *430*, 128470. [CrossRef] [PubMed]
26. Yang, C.; Lim, W.; Song, G. Mediation of oxidative stress toxicity induced by pyrethroid pesticides in fish. *Comp. Biochem. Physiol. C* **2020**, *234*, 108758. [CrossRef]
27. Mekebri, A.; Crane, D.B.; Blondina, G.J.; Oros, D.R.; Rocca, J.L. Extraction and analysis methods for the determination of pyrethroid insecticides in surface water, sediments and biological tissues at environmentally relevant concentrations. *Bull. Environ. Contam. Toxicol.* **2008**, *80*, 455–460. [CrossRef] [PubMed]
28. Jia, F.Y.; Wang, W.W.; Wang, J.; Yin, J.A.; Liu, Y.M.; Liu, Z.B. New strategy to enhance the extraction efficiency of pyrethroid pesticides in fish samples using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. *Anal. Methods* **2012**, *4*, 449–453. [CrossRef]
29. *GB29705-2013*; Determination of Cypermethrin, Fenvalerate and Deltamethrin Residues in Aquatic Products by Gas Chromatographic Method. Ministry of Agriculture of the People's Republic of China (MOA): Beijing, China, 2013.
30. Wu, M.; Zhang, W.; Miao, J.; Sun, C.; Wang, Q.; Pan, L. Pyrethroids contamination and health risk assessment in seafood collected from the coast of Shandong, China. *Mar. Pollut. Bull.* **2022**, *186*, 114442. [CrossRef] [PubMed]
31. Ray, S.; Shaju, S.T. Bioaccumulation of pesticides in fish resulting toxicities in humans through food chain and forensic aspects. *Environ. Anal. Health Toxicol.* **2023**, *38*, e2023017. [CrossRef] [PubMed]
32. Meng, T.; Wang, C.; Florkowski, W.J.; Yang, Z. Determinants of urban consumer expenditure on aquatic products in Shanghai, China. *Aquacult. Econ. Manag.* **2023**, *27*, 1–24. [CrossRef]
33. *GB/T 30891-2014*; Practice of Sampling Plans for Aquatic Products. Ministry of Agriculture of the People's Republic of China (MOA): Beijing, China, 2014.
34. Sannino, A.; Bandini, M.; Bolzoni, L. Determination of pyrethroid pesticide residues in processed fruits and vegetables by gas chromatography with electron capture and mass spectrometric detection. *J. AOAC Int.* **2003**, *86*, 101–108.
35. Xue, J.; Zhu, X.; Liu, Z.; Hua, R.; Wu, X. Using silicone rubber and polyvinylchloride as equilibrium passive samplers for rapid and sensitive monitoring of pyrethroid insecticides in aquatic environments. *Sci. Total Environ.* **2020**, *728*, 138797. [CrossRef]
36. Sun, T.; Huang, Q.; Zhang, W.; Chen, R.N.; Li, W.; Chen, H.P.; Hu, S.Q.; Cai, Z.Q. Performance and selectivity of amphiphilic pillar[5]arene as stationary phase for capillary gas chromatography. *J. Chromatogr. A* **2022**, *1671*, 463008. [CrossRef]
37. Zhang, X.; Fang, C.; Huang, D.; Yang, G.; Tang, Y.; Shi, Y.; Kong, C.; Cai, Y. Determination of 8 biogenic amines in aquatic products and their derived products by high-performance liquid chromatography-tandem mass spectrometry without derivatization. *Food Chem.* **2021**, *361*, 130044. [CrossRef]
38. Saraiva, M.; Cavalheiro, J.; Lanceleur, L.; Monperrus, M. Synthetic musk in seafood products from south Europe using a quick, easy, cheap, effective, rugged and safe extraction method. *Food Chem.* **2020**, *200*, 330–335. [CrossRef] [PubMed]
39. Mao, X.; Wan, Y.; Li, Z.; Chen, L.; Lew, H.; Yang, H. Analysis of organophosphorus and pyrethroid pesticides in organic and conventional vegetables using QuEChERS combined with dispersive liquid-liquid microextraction based on the solidification of floating organic droplet. *Food Chem.* **2020**, *309*, 125755. [CrossRef] [PubMed]
40. Gould, N.S.; Li, S.; Cho, H.; Landfield, H.; Caratzoulas, S.; Vlachos, D.; Bai, P.; Xu, B. Understanding solvent effects on adsorption and protonation in porous catalysts. *Nat. Commun.* **2020**, *11*, 1060.
41. Nilsson, U.J. Solid-phase extraction for combinatorial libraries. *J. Chromatogr. A* **2000**, *885*, 305–319. [CrossRef] [PubMed]
42. Badawy, M.E.I.; El-Nouby, M.A.M.; Kimani, P.K.; Lim, L.W.; Rabea, E.I. A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. *Anal. Sci.* **2022**, *38*, 1457–1487. [CrossRef] [PubMed]
43. Yang, Q.; Ai, X.; Dong, J.; Liu, Y.; Zhou, S.; Yang, Y.; Xu, N. A QuEChERS-HPLC-MS/MS Method with Matrix Matching Calibration Strategy for Determination of Imidacloprid and Its Metabolites in *Procambarus clarkii* (Crayfish) Tissues. *Molecules* **2021**, *26*, 274. [CrossRef]
44. Shelepchikov, A.A.; Ovcharenko, V.V.; Kozhushkevich, A.I.; Brodskii, E.S.; Komarov, A.A.; Turbabina, K.A.; Kalantaenko, A.M. A new method for purifying fat-containing extracts in the determination of polybrominated diphenyl ethers. *J. Anal. Chem.* **2019**, *74*, 574–583. [CrossRef]

45. dos Anjos, J.P.; de Andrade, J.B. Determination of nineteen pesticides residues (organophosphates, organochlorine, pyrethroids, carbamate, thiocarbamate and strobilurin) in coconut water by SDME/GC–MS. *Microchem. J.* **2014**, *112*, 119–126. [CrossRef]
46. Zhao, C.; Jin, H.; Xu, F.; Zheng, G.; Li, L.; Lin, J.; Shi, X.; Shan, Q.; Zhou, H.; Wang, B.; et al. Determination of teicoplanin and ramoplanin residues in aquaculture products by a modified QuEChERS method combined with UPLC-MS/MS. *Microchem. J.* **2024**, *199*, 109942. [CrossRef]
47. Esteve-Turrillas, F.A.; Pastor, A.; de la Guardia, M. Determination of pyrethroid insecticide residues in vegetable oils by using combined solid-phases extraction and tandem mass spectrometry detection. *Anal. Chim. Acta* **2005**, *553*, 50–57. [CrossRef]